Chapter 2
Cardiovascular Risk Factors and Markers

Quamdiu vivimus, necesse habemus semper quærere
[As long as we live, we must always investigate]
(H. de Lubac, [1896–1991]) [223]

Cardiovascular risk is assessed for the prediction and appropriate management of patients using collections of identified risk markers obtained from clinical questionnaire information, concentrations of certain blood molecules (e.g., N-terminal proB-type natriuretic peptide fragment and soluble receptors of tumor-necrosis factor-α and interleukin-2 [IL-2]), imaging data using various modalities, and electrocardiographic variables, in addition to traditional risk factors. These risk markers and factors can be combined to form standard cardiovascular risk scores (e.g., the Framingham risk score). These risk markers and factors can also be ranked according to their efficiency in predicting each cardiovascular outcome.

The term *biomarker* has been defined as an indicator of physiological or pathophysiological processes and hence used for diagnostic, prognostic,[1] and/or therapeutic purposes. Biomarker is defined by the National Institutes of Health as an entity "that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacological responses to a therapeutic intervention." In a pathophysiological context, the useless prefix *bio* is omitted.

Markers of redox stress encompass molecules modified by interactions with reactive oxygen species (ROS) (i.e., proteins, lipids, carbohydrates, and DNA) and molecules of the antioxidant defense [90]. Examples of circulating markers that have a value in addition to traditional cardiovascular risk factors include glycated hemoglobin (HbA1c) for glycemic control in diabetes, high-sensitivity troponin-I

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[1]Prognosis, in general, refers to the expected course of a disease without treatment (natural prognosis) or after treatment (clinical prognosis). Prognosis is determined by certain endpoints (e.g., absence of complication recurrence). Prognosis is ameliorated by the technical improvement of medical and surgical interventions.

(hsTnnI) and C-reactive protein (hsCRP) for cardiovascular risk prediction, and N-terminal proB-type natriuretic peptide for heart failure (HF).

A *risk marker* is not necessarily involved in the cause or set of causes of a cardiovascular disease (CVD), like a *risk factor*.

Markers of pathological conditions serve to:

1. Detect and sort individuals at risk for CVDs
2. Monitor disease progression
3. Select therapy and assess treatment efficacy

Hence, features that determine the clinical utility of a marker include (1) the ease and cost of measurement, (2) its sensitivity and specificity, and (3) evidence for guiding management and improving patient outcome.

A *competing risk or cause* precludes the occurrence of another risk or cause. For example, an adverse event is a competing cause with respect to death attributable to any cardiovascular event.

The health of individuals can be defined, at least partly, by low levels of adverse markers of CVD. Cardiovascular health also relies on sets of risk factors that determine long-term survival and quality of life (Vol. 7, Chap. 2. Context of Cardiac Diseases). Clinical markers and factors, in particular, those related to inflammation, adverse remodeling, and thrombosis, assess the risk for CVD, which is refined using biological constants and imaging data.

The risk factors comprise behavioral habits, such as smoking, physical inactivity, eating pattern (food quantity, meal composition and frequency, and use of processed, canned, and frozen food), which have an impact on the body weight, along with blood concentrations of cholesterol and glucose, and secondarily on arterial pressure [224].

In addition to clinical markers that are used for early detection, prevention of acute cardiovascular events targets modifiable risk factors, especially changes in habits (e.g., smoking cessation and supervised exercise) and therapy.

Most CVD is indeed related to risk factors that can be controlled or treated, such as hypertension (systolic and diastolic arterial pressure greater than 18.6 kPa [SBP >140 mmHg] and 12.0 kPa [DBP >90 mmHg], respectively), dyslipoproteinemia, obesity, tobacco smoking, physical inactivity, and diabetes. The leading CVD risk factors comprise hypertension (∼13% deaths), followed by tobacco use (∼9%), hyperglycemia (∼6%), lack of physical activity (∼6%), and obesity (∼5%) [30].

Cardiovascular risk factors are similar for men and women.

- The *total risk* of a person can be estimated by summing the risk imparted by each of the major risk factors.
- The *absolute risk* is defined as the probability of developing CVD over a given time period.
- The *relative risk* is the ratio of the absolute risk of a given patient to that of a low-risk group, that is:

- Systolic and diastolic blood pressure lower than 130 and 80 mmHg
- Total cholesterol concentration ranging from 160 to 199 mg/dl
- Low-density lipoprotein (LDL)^{CS} ranging from 100 to 129 mg/dl[2] and a 1-mmol reduction in LDL^{CS} in middle-aged individuals for 5 years being estimated to yield a 20% decrease in CVD risk [121]
- High-density lipoproteins (HDLs)^{CS} greater than or equal to 45 mg/dl for men or 55 mg/dl for women
- Nonsmoker
- Absence of diabetes mellitus [226]

The major *independent cardiovascular risk factors* include cigarette smoking, physical inactivity, aging, hypertension, obesity, diabetes, and dyslipoproteinemia with hypercholesterolemia and elevated LDL^{CS} concentration but decreased HDL^{CS} concentration [226].

Behavioral risk factors are shared by atherosclerosis complications (myocardial infarction [MI] and stroke) and diabetes, cancer, and respiratory disease [30].

Hypertension is a primary risk factor for stroke, especially long-term large variations of both systolic and diastolic arterial pressure observed in a series of medical examinations (either measured or recorded by ambulatory blood pressure monitoring), such as early morning (arousal) arterial pressure surges [227]. Large variations in arterial pressure can result from abnormal baroreceptor functioning, excessive sympathetic activity, elevated sensitivity to the renin–angiotensin axis, transient high-salt diet, acute fluctuations in body weight, sleep disorders, and ingestion of nonsteroidal anti-inflammatory drugs [228]. In addition, excessive arterial pressure reductions during treatment (SBP <16.0 kPa [<120 mmHg]; DBP <9.3 kPa [<70 mmHg) can increase the cardiovascular event rate.

Coronary arterial calcium score is the most important predictor of adverse coronary events, whereas fasting glycemia and carotid ultrasonographic measures are salient predictors of stroke [229].

Predisposing risk factors, such as a family history of premature coronary artery disease, ethnic characteristics, and psychosocial factors (hostility, depression, and social isolation), worsen the independent risk factors.

Conditional risk factors encompass hypertriglyceridemia; a high proportion of small, dense LDLs; elevated plasmatic concentrations of homocysteine and lipoprotein-A; and the presence of prothrombotic factors (e.g., fibrinogen) and inflammatory markers (e.g., C-reactive protein [CRP]).

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[2]Plasmatic LDL concentration is generally not measured directly; it is instead estimated from its cholesterol concentration (LDL^{CS}), a measure of the total amount of cholesterol contained in LDLs [225]. In general, LDL^{CS} concentration and LDL number are highly correlated. However, in the metabolic syndrome, diabetes, and hypertriglyceridemia, plasmatic concentrations of LDL and LDL^{CS} become discordant because of the predominance of small, dense, cholesterol-poor LDLs. Therefore, plasmatic LDL^{CS} concentration does not accurately reflect LDL concentration, requiring measurement of LDL number and apolipoprotein B (ApoB) concentration.

Risk factors can be also categorized as modifiable and nonmodifiable. *Modifiable risk factors* include behavioral, metabolic, and other controllable risk factors.

Behavioral risk factors include:

1. Tobacco smoking
2. Physical inactivity
3. Unhealthy hypercaloric diet rich in lipids and salt
4. Abuse of illicit substances (e.g., amphetamines, cocaine, ecstasy, and heroin), which is associated with an increased risk of ischemic and hemorrhagic strokes
5. Harmful use of alcohol

Metabolic risk factors comprise:

1. Hypertension
2. Hyperglycemia and diabetes
3. Hyperlipidemia and hypercholesterolemia
4. Obesity

Other modifiable risk factors consist of:

1. Poverty and low educational status
2. Psychological factors (e.g., stressful life and depression)
3. Hyperhomocysteinemia
4. Air pollution, particularly in populated agglomerations, which, in general, do not meet the ambient air quality standards

Some major CVD risk factors cannot be controlled:

- Aging, with augmented frequency of CVD
- Gender, a man being at a greater risk for CVD than a premenopausal woman
- Family history, that is, inherited genetic predisposition
- Ethnicity[3]

Additional risk factors encompass inflammatory disorders, infection, pollution, and cardiac atrial disorders, independently of atrial fibrillation [230]. Almost all patients with atherosclerosis have lesions in many arterial territories and risk factors. Major risk factors for limb and visceral ischemia (i.e., kidney, bowel, liver, or spleen), which are usually late-stage manifestations of atherosclerosis, include hypertension, smoking, and diabetes [231].

Because atherosclerosis is involved in both abdominal aortic aneurysms (AAAs) and coronary stenoses, these two types of vascular lesions share primary risk factors (i.e., age, smoking, high LDL^{CS} or total cholesterol concentration, and male sex).

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[3]The risk for stroke is twice as high in Black American people than in the White American population [230]. In North America, Hispanic and Latino Americans are at an increased risk for stroke. American Indians have an increased incidence of stroke compared with non-Hispanic Whites.

On the other hand, the role of hypertension in AAA genesis is less well established than in chronic obstructive airways disease (CoAD), and White race is more relevant to AAA than to CoAD [232].

In a sample of 4232 men and women aged 60 years, the plasmatic concentration of PCSK9[4] is associated with the future risk for CVD (e.g., myocardial infarction and ischemic stroke), even after adjustments for established CVD risk factors (LDL^{CS}, HDL^{CS}, LP$_A$, TGs, hypertension, diabetes, smoking, overweight, obesity, and physical inactivity), and statin use [234].

The American Heart Association cardiovascular health score is inversely related to CVD. According to the Framingham Study (mean age 58 years, 55% women), circulation markers of subclinical CVD comprise [235]:

1. Structural and functional criteria:

   - Reduced ankle–brachial index (ABI)
   - Carotid intima–media thickness and possible stenosis
   - Left ventricular hypertrophy
   - Left ventricular systolic dysfunction
   - Microalbuminuria

2. In addition to lifestyle:

   - Smoking status[5]
   - Body mass index
   - Physical activity
   - Diet[6]

3. In addition to mechanical parameter values:

   - Blood pressure

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[4]PCSK, proprotein convertase subtilisin/kexin. The secretory proprotein convertase (PC) category comprises nine members (PCSK1–PCSK9). The first member, PCSK1, is also called neuroendocrine convertase NEC1 and prohormone and PC1 and PC3; PCSK3 corresponds to furin (FUR: FeS upstream region) and PCSK8 to membrane-bound transcription factor site-1 peptidase (MBTPS1), or simply site peptidase S1P, and subtilisin–kexin isozyme SKI1, which cleaves membrane-bound transcription factors at nonbasic residues [233]. PCSK9 cleaves itself once, and the secreted inactive peptidase transfers low- (LDLR) and very-low-density lipoprotein receptors (VLDLRs) to the endosomal compartment for lysosomal degradation. It reduces inflammation in a LDLR-dependent manner [233].

[5]Although some smokers neither experience an infarct nor develop a cancer, smoking represents a strong risk factor for atherosclerosis and aneurysms.

[6]Nutrient restriction and changes in dietary composition improve health during aging. In people (age 20–70 years) who consume a fasting mimicking diet (FMD; low in sugars and proteins but high in unsaturated lipids) for 5 days per month for 3 months and maintain their normal diet during the other periods in addition to their lifestyle and in control subjects who have an unrestricted diet for 3 months and who then switch to the fasting mode, FMD can reduce body weight and lipid-based depot, blood pressure, concentrations of insulin-like growth factor (IGF1), fasting glucose, triglycerides (TGs), total cholesterol, LDL^{CS}, and CRP, especially in individuals at risk for CVD [236].

Table 2.1 Examples of risk factors related to lipid metabolism (Source: [238]; *lpPLA2* lipoprotein-associated phospholipase-A2, *PCSK* proprotein convertase subtilisin/kexin, $_s$*PLA2* secretory PLA2)

Marker	Features
Oxidized Phospholipids	Released by redox stress
	Binds to ApoB100+ lipoproteins
	Transport by lipoprotein-A
PCSK9	Released by statins
	Degrades hepatocytic LDLR
LpPLA2	Released by inflammation
	Depletion of oxPLs from lipoproteins
$_s$PLA2	Released by inflammation
	Processes LDLs into smaller and denser LDLs

In diabetic patients, cholesterol esters and sphingo-, phospho-, and glycerolipids are linked to an elevated risk for adverse cardiovascular events

4. Blood concentrations of agents related to lipid and glucose metabolism, inflammation, and thrombosis (Tables 2.1, 2.2, 2.3, and 2.4):

- Cholesterol, in particular, LDP–cholesterol (LDL^{CS}) and its regulator proprotein convertase subtilisin/kexin PCSK9
- Glucose
- Natriuretic peptides (Table 2.5) in addition to N-terminal fragments of A- (atrial; ANP) and B-type (brain; BNP) natriuretic peptide precursors ($_{NT}$proANP and $_{NT}$proBNP)[7]
- Plasminogen activator inhibitor-1
- Aldosterone
- C-reactive protein (or pentraxin-1)
- ^{D}Dimer[8]
- Fibrinogen
- Homocysteine
- Troponins[9]
- Growth differentiation factor (GDF15)

Hence, an overlap exists between risk factors and markers.

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[7]The $_{NT}$proBNP fragment is a HF marker used to evaluate its severity.

[8]^{D}Dimer signals the presence of a thrombus. Plasmatic ^{D}dimer measurement is used when deep vein thrombosis and pulmonary embolism are suspected (concentration ≥ 250 ng/ml) [237]. ^{D}Dimer is the degradation product of crosslinked fibrin by coagulation factor-*XIII* and hence reflects activated hemostasis. Venous thrombosis usually results from venous stasis due to immobility or obstruction, a hypercoagulable state linked to triggers of the blood coagulation cascade, and possible wall injury.

[9]Cardiac troponins cTnnT and cTnnI in addition to creatine kinase (CK) are widely used markers when myocardial infarction is suspected owing to their sensitivity and specificity for cardiomyocyte (CMC) necrosis.

Table 2.2 Examples of risk markers related to inflammation and thrombosis (Source: [238])

Marker	Features
Endothelial microvesicles	Released in endothelial dysfunction and vascular injury
Platelet-derived microvesicles	Deposition of inflammatory and clotting mediators
	Interaction with endotheliocyte
Red blood capsule (RBC)-derived microvesicles	Released from entrapped RBCs in the thrombus
	Increases thrombin generation
C-reactive protein	Produced by hepatocytes exposed to interleukin-6
^{D}Dimer	Marker of thrombosis
	Released by fibrin degradation
Interleukin-6	Primed production of fibrinogen, pentraxin-1, and other acute phase reactants
Myeloperoxidase	Stored in leukocyte granules
	Converts H_2O_2 to other ROS that suppress nitric oxide and oxidize proteins
Monocyte	Mo1 (classical CD14++ FCγ R3−)
	Mo2 (intermediate CD14++ FCγ R3+)
	Mo3 (alternative CD14+ FCγ R3+)
Circulating progenitor cells	Involved in endothelial repair and angiogenesis

In inflammatory sites, neutrophils phagocytose necrotic regions and release their granule content, such as myeloperoxidase, which contributes to redox stress. The subsequent proliferative phase involves many different cellular populations, such as monocytes, fibroblasts, and endothelial progenitor cells. Three types of monocytes, classical (Mo1), intermediate (Mo2), and alternative monocytes (Mo3), are defined according to expression of the lipopolysaccharide coreceptors CD14 and FCγ R3

Lipoproteins (i.e., water-soluble protein–lipid complexes) mediate the transport of cholesterol and lipids in blood. Linkage of cholesterol to lipoproteins (HDLs and LDLs) enables its back and forth motion in blood between the liver and other organs. Relatively high concentration of cholesterol tethered to ApoB-containing (ApoB+) LDLs is an atherosclerosis risk marker. Cholesterol ester links to ApoA1+ HDLs. Therefore, concentrations of low- (LDL^{CS}) and HDL –cholesterol (HDL^{CS}) and TG (or triacylglycerol [TAG]) are measured to identify patients with a high risk for CVD.

The measure non-HDL^{CS} combines the cholesterol content of LDLs, lipoprotein-A (LP_A), an LDL-like particle linked to ApoA, and TG-rich lipoproteins (remnants, i.e., intermediate-density lipoproteins (IDLs), VLDLs, and chylomicron remnants), TGs being mainly carried by remnants. In treated patients, non-HDL^{CS} level is more closely associated with coronary atherosclerosis progression than LDL^{CS} concentration [239].

Table 2.3 Examples of risk markers related to injury, healing, and fibrosis (**Part 1**; Source: [238]; *DAMP* damage-associated molecular pattern, *GDF* growth differentiation factor, *IL* interleukin, *MMP* matrix metallopeptidase)

Marker	Features
Copeptin	Marker of vasopressin secretion
	Released by hemodynamic stress and inflammation
Cystatin-C	Marker of glomerular filtration rate
Galectin-3	Mediates aldosterone-induced fibrosis and transforming growth factorβ-primed myofibroblast activation
	Released by inflammation and fibrosis
GDF15	Acts via Tβ R1/2
	Reduces neutrophil recruitment
	Favors macrophage chemotaxis
	Released by inflammation and redox stress
IL1RL1^S	Upon IL33 binding, primes T_{H2} cytokine response
	Blocks activation of IL1RL1
	Favors myocardial hypertrophy and fibrosis
	Released by cellular stresses
MMP9	Initiates transforming growth factorβ signaling
	Degrades matrix components, cytokines, and DAMPs
	Released by inflammation and redox stress

Copeptin is the C-terminal fragment of vasopressin (or antidiuretic hormone)

Table 2.4 Examples of risk markers related to injury, healing, and fibrosis (**Part 2**; Source: [238]; $_{MR}$*proAdm* midregional fragment of adrenomedullin precursor, $_{MR}$*proANP* midregional fragment of type-A natriuretic peptide [ANP] precursor, $_{NT}$*proBNP* inactive N-terminal fragment of BNP precursor, *TGF* transforming growth factor)

Marker	Features
$_{MR}$proAdm	Triggers vasodilation
	Released by myocardial stretch
$_{MR}$proANP	Inhibits the renin–angiotensin–aldosterone axis
	Provokes diuresis and natriuresis
	Released by myocardial stretch
$_{NT}$proBNP	Inhibits the renin–angiotensin–aldosterone axis and the sympathetic nervous system
	Induces vasodilation
	Provokes diuresis and natriuresis
	Released by myocardial stretch
TGFβ	Suppresses inflammation
	Promotes repair, but also fibrosis
	Released by inflammation
Troponin-I/T	Released by proteolysis upon CMC death
	Released by myocardial infarction

Table 2.5 Several cardiokines can serve as CVD markers (Source: [71])

Cardiokine	Expression
ANP	Highly expressed in hypertensive rats
BNP	Weakly expressed in fasting mice
GDF8	Highly expressed when skeletal muscle mass rises
GDF15	Highly expressed in patients with cardiac hypertrophy and chronic heart failure
CTRP9	Weakly expressed in diabetic patients and after acute myocardial infarction

Cardiokines are proteins secreted from the heart

Values of certain markers differ significantly according to gender, such as markers of lipid metabolism, adiposity, inflammation, endothelial and renal dysfunction, and cardiac stress [240].

- Women have higher concentrations of HDL and HDL^{CS}, leptin, ^{D}dimer, homoarginine, and ^{NT}proBNP.
- Women have lower concentrations of LDL^{CS}, adiponectin, the chemokine CCL2, troponin-T (TNNT), cystatin-C, symmetrical and asymmetrical dimethylarginine, lipoprotein-associated phospholipase-A2, and soluble endothelial cell adhesion molecules.

The benefit of drugs administered in addition to a recommended healthy lifestyle generally exceeds the therapeutic risk. However, cholesterol-lowering therapy in patients with LDL^{CS} concentrations below a certain threshold is unfounded. A treatment threshold is aimed at avoiding treating patients at a low risk for atherosclerosis-associated complications. The LDL^{CS} concentration is monitored to adjust therapy according to its effects.

Measurements of alternative cardiovascular markers (e.g., coronary artery calcium) in addition to concentrations of LDL^{CS} and high-sensitivity C-reactive protein (hsCRP), PWV index (e.g., ABI), etc., used in therapy decisions (e.g., statins), provide additional information when a treatment decision is uncertain.[10] These markers are aimed at identifying individuals a priori defined in the lower risk population who nevertheless can experience an adverse event (MI and stroke) if left untreated, as, in fact, they can belong to a higher risk population when these alternative markers are incorporated in scoring.

Dying parenchymal cells and leukocytes release small DNA fragments into the bloodstream. Blood concentrations of fragmented DNA increase in CVD, among other maladies. Because each cell type has a unique DNA methylation pattern, a source of plasmatic DNA can be identified using cell-specific DNA methylation markers [241].

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[10]Intensive statin therapy to lower LDL concentration has severe side effects.

Single-stranded DNA aptamers are oligonucleotides that contain approximately 50 bases. They can bind proteins with high specificity and affinity and can be used to identify early CVD markers.

Dickkopf-related protein Dkk4, a Wnt inhibitor, and cryptic, a transforming growth factor (TGF) inhibitor, are relevant targets in the context of myocardial injury [242].

Dietary habits are modifiable risk factors. Intestinal microbiota and bacterial metabolites are connected to dietary composition. Gut flora-derived metabolite trimethylamine ^{N}oxide (TMAO) is a small organic compound generated from betaine, carnitine, and choline [243].[11]   It accumulates in fishes, especially elasmobranchs,[12] and protects against the protein-destabilizing effect[13] and ligand-binding inhibition of urea at high concentrations in addition to adverse effects of temperature, salinity, and hydrostatic pressure. Its blood concentration, which depends on diet composition in addition to the activity of gut flora and liver FMO, increases after ingesting trimethylammonium-containing nutrients (^{L}carnitine and phosphatidylcholine-rich food), such as red meat, eggs, and fish [244]. Trimethylamine oxide is a risk factor for major adverse cardio- and cerebrovascular events (ACaVCeVE), especially in patients with chronic kidney disease. Plasmatic TMAO concentration correlates positively with ACaVCeVE and mortality [244]. Trimethylamine oxide, which impairs cholesterol and bile acid metabolism and the activity of sterol transporters in the liver and intestine, activates inflammation,

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[11]TMAO is synthesized from trimethylamine (TMA), which is generated by the action of gut microbiota from dietary choline and phosphatidylcholine (lecithin). The gut microbial metabolism of trimethylammonium-containing nutrients (e.g., choline, phosphatidylcholine, and ^{L}carnitine) forms TMA. In the liver, flavin-containing monooxygenase (FMO) converts TMA into TMAO. TMA is a gas that is oxygenated within living animals to form TMAO by FMO1 and FMO3. It can also be produced from ^{L}carnitine that is first converted into the intermediate metabolite γ-butyrobetaine and then into TMA, which is subsequently processed into TMAO by host hepatic FMO3 [243]. Trimethylamine oxide accumulates in organs as an osmolyte or is eliminated by the kidney. Several bacterial categories produce TMA and TMAO, Anaeroplasmataceae, Deferribacteraceae, Enterobacteriaceae, and Prevotellaceae. In the human intestine, eight species from two different phyla (Firmicutes and Proteobacteria) and six genera (Anaerococcus hydrogenalis, Clostridium asparagiforme, hathewayi, and sporogenes, Escherichia fergusonii, Proteus penneri, Providencia rettgeri, and Edwardsiella tarda) and the choline-degrading sulfate-reducing bacterium Desulfovibrio desulfuricans synthesize TMA from choline. Trimethylamine oxide can be metabolized to small methylated amines, such as tri-, di-, and monomethylamine, which are precursors of marine aerosols and the greenhouse gas nitrous oxide in the marine atmosphere [243].

[12]The term *elasmobranch* refers to cartilaginous fishes, such as sharks, sawfish, rays, and skates, which are endowed with rows of replaceable teeth and the five to seven gill slits on each side of their body. Selachians (group Selachii) consist of the sharks and dogfishes. Fishes of the class *Chondrichthyes* possess a cartilaginous skeleton, whereas bony fishes of the class *Osteichthyes* have a bony skeleton. The subclass Elasmobranchii contains about 1150 species of saltwater- and freshwater-dwelling fishes, which have various sizes and shapes, from the giant whale shark and huge manta ray to the dwarf lanternshark and tiny short-nosed electric ray, and from the hammerhead shark to sawfish.

[13]In organisms that concentrate urea as an osmolyte and buoyancy factor, TMAO restores protein structure and their enzyme function [243].

and markedly reduces reverse cholesterol transport, may favor the formation of macrophage scavenger receptors and foam cells in the arterial wall [243, 244]. In mice, the inhibition of hepatic FMO3, which is controlled by the bile acid-activated farnesoid X receptor (FXR), and microbial TMAO production counters atherogenesis.

Current clinical risk stratification methods (e.g., Framingham score) do not suffice to predict CVD. Many events happen in patients identified to be at a low or moderate risk; a large fraction of patients at risk are identified by a major event. Therefore, better risk stratification tools are needed.

2.1 Environmental Stressors

Environmental stressors, such as air pollution and noise, are cardiovascular risk factors. In particular, Asian windblown sand dust (Asian dust [kosa]) originating in the deserts of China and East Asia that mixes with air pollutants during atmospheric transport and contains microbes correlates not only with respiratory diseases but also with acute MI at least in men older than 75 years, living in southwestern Japan and having hypertension, diabetes mellitus, and, above all, chronic kidney disease [245].

2.1.1 Air Pollution

Air pollution is a major environmental risk factor. Air pollutants include *particulate matter*, *gaseous pollutants*, and *volatile organic compounds* (VOCs).

Particulate matter is classified into three categories according to the size of airborne microparticles:

1. Coarse ($2.5 \leq$ aerodynamic caliber $<10\,\mu\text{m}$ [10^{-5} m]; PM_{10})
2. Fine ($0.1 \leq$ aerodynamic caliber $<2.5\,\mu\text{m}$; $PM_{2.5}$)
3. Ultrafine particles (aerodynamic caliber $<0.1\,\mu\text{m}$ [10^{-7} m]; $PM_{0.1}$), also called nanoparticles

Particles are represented by concentrations of $PM_{2.5}$ and PM_{10} particles, the latter comprising ultrafine, fine, and coarse particles.[14] Nanoparticles suspended in air (nanoaerosol; size $\mathcal{O}[10^{-9}$ m]) contribute to indoor air pollution exposure.

Among PM, inhalable particles of aerodynamic caliber less than $10\,\mu\text{m}$ can travel deep into the bronchial tree inside lungs. Air pollution facilitates and exacerbates respiratory infections.

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[14]Typical background concentrations of PM_{10} in North America or Western Europe range from 20 to $50\,\mu\text{g/m}^3$ [246].

In fact, environmental toxins cause numerous acute and chronic illnesses, particularly cardiovascular maladies, especially in individuals with diabetes during episodes of high air pollution exposure [247]. Exposure to air polluted with fine PM ($PM_{2.5}$) augments the risk for cardiovascular morbidity and mortality via endothelial dysfunction and systemic inflammation. Air pollution exacerbates existing maladies and can be involved in the development of CVDs (atherosclerosis, cardiac arrhythmias, HF, stroke, and venous thromboembolism) [248]. In mice, concentrated ambient $PM_{2.5}$ potentiates atherosclerotic plaque vulnerability.

In Canada, Europe, and the USA, a $10\,\mu g/m^3$ increase in PM_{10} is associated with a 0.2–0.6% increase in all-cause mortality [248]. In Europe, a $10\,\mu g/m^3$ elevation of NO_2 causes a 0.4% augmentation in the cardiovascular mortality rate. In China, outdoor air pollution ranks fourth among preventable causes of diseases, current $PM_{2.5}$ concentrations ($61\,\mu g/m^3$) having increased from those during the Beijing Olympic games ($55\,\mu g/m^3$), which were already much higher than World Health Organization standard ($10\,\mu g/m^3$) [249].

According to the World Health Organization, outdoor air pollution in both cities and rural areas in 2012 is estimated to cause 3.7 million premature deaths worldwide per year, 88% of those premature deaths occurring in low- and middle-income countries [250]. Reducing outdoor emissions from household coal and biomass energy systems, agricultural waste incineration, forest fires, and certain agroforestry activities (e.g., charcoal production) may reduce rural air pollution sources in developing regions.

According to the Global Burden of Disease study in 2010, air pollution may be responsible for about 6% of all-cause and all-age deaths, in addition to development and exacerbation of respiratory diseases (e.g., asthma, chronic obstructive pulmonary disease, and pulmonary cancers) [248].

Air pollution ranks as the ninth contributor among modifiable disease risk factors, that is, above low physical activity, high-sodium diet, high cholesterol, and drug use [248].

Acute exposure to environmental toxicants can lead to angina, arrhythmia, MI, and HF. Repeated exposure to air pollution can cause vascular inflammation and redox stress, thereby promoting atherosclerotic plaque expansion and rupture [246]. Long-term exposure increases the risk for CoAD-caused death.

Inhalation of combustion-derived nanoparticles that incorporate soluble reactive organic compounds, polycyclic aromatic hydrocarbons, and oxidized transition metals causes pulmonary inflammation with secondary or direct cardiovascular effects after passage from airways and pulmonary alveoli into the blood circulation.

Combustion-derived nanoparticulate matter induces cardiovascular effects via classical and alternative pathways (Table 2.6).

- The *classical pathway* is defined by indirect effects that result from pulmonary inflammation caused by inhaled particulate matter. Lung inflammation provokes heightened plasma concentrations of cytokines, such as IL1β and IL6 and colony-stimulating factor CSF2, in addition to the release of bone marrow-derived neutrophils and monocytes into blood circulation [246].

Table 2.6 Direct and indirect vascular effects of combustion-derived nanoparticulate matter (Source: [246])

Process	Mechanism
Inflammation and redox stress	Cytokine release
	Release of bone marrow-derived neutrophils and monocytes
	Increased formation of ROS
Thrombosis	Elevation of fibrinogen concentration
	Promotion of platelet activation and aggregation
	Reduction in tissue plasminogen activator level
Hypertension	Vasoconstriction
Atheroma destabilization	Inflammation induced-plaque progression and rupture
Heart rhythm	Stimulation of irritant receptors in the airways and reflex activation of the autonomous nervous system

- The *alternative pathway* is related to the passage from the lung into the blood circulation of inhaled fine PM and nanoparticles, directly across the alveolocapillary membrane or after ingestion by alveolar macrophages. Particles can cross the lung–blood barrier according to particle size, charge, and chemical composition. Once in the blood, particles can interact with the vascular endothelium and provoke endothelium dysfunction, redox stress, and inflammation.

Short-duration exposure (hours to weeks) to particulate matter $PM_{2.5}$ can provoke the occurrence of an event (fatal or not) related to a cardiovascular malady [247].

In young, healthy, nonsmoking adults, episodic exposures to elevated $PM_{2.5}$ levels raise the endotheliocyte (EC) apoptosis rate as well as densities of circulating monocytes and T lymphocytes (but not B lymphocytes) and concentrations of Anx5+ Itgα2b− pecam1+ endothelial microvesicles containing arterial-, venous-, and lung-specific markers (but not selectin+ microvesicles)[15] and cause an antiangiogenic profile [251]. These microparticles carry metallopeptidases, which can damage endothelium, erode eventual atherosclerotic plaques, and cleave soluble adhesion molecules. Elevated blood concentrations of endothelium-derived microvesicles shed from activated and apoptotic cells demonstrate endothelial damage, even in young, healthy nonsmokers.

On the one hand, blood concentrations of proangiogenic factors (EGF, PDGF, VEGF, soluble TNFSF5, CCL5, and CXCL1) decrease. Exposure to $PM_{2.5}$ provokes loss of trophic and angiogenic factors, thereby increasing the apoptosis rate of ECs and endothelial injury [251]. On the other hand, blood concentrations of antiangiogenic factors (TNFSF1 and CXCL10) and other proinflammatory cytokines (IL1β and IL6) and chemokines (CCL2 to CCL4 and CXCL8), in addition to

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[15]Release by activated endothelium of CD62+ (selectin+) microparticles is linked to a high inflammatory state that accompanies acute cardiovascular events.

soluble endothelial adhesion molecules ($icam1^S$ and $vcam1^S$) rise. Proinflammatory cytokines and chemokines (e.g., TNFSF1, IL6, and CXCL8) can be released by alveolar macrophages or bronchial epitheliocytes exposed to airborne particles. Both TNFSF1 and CXCL10 are secreted by activated T lymphocytes, monocytes, and ECs. The mild inflammatory state is characterized by augmented densities of circulating CD4+ CD8+ T lymphocytes, CD14+ monocytes, and $Fc\gamma$ R3+ natural killer cells, but not CD19+ cells. Moreover, upon $PM_{2.5}$ exposure, platelet–monocyte aggregates increase, establishing a procoagulation state [251]. Systemic inflammation due to $PM_{2.5}$ inhalation can thus favor athero- and thrombogenesis.

The $PM_{2.5}$-induced pattern of released cytokines, chemokines, and endothelial microparticles differs from that resulting from inhalation of other toxins, such as tobacco smoke. Although smoking is also associated with EC apoptosis and a decrease in plasmatic concentrations of EGF, TNFSF5, and CXCL1, it is generally characterized by elevated blood concentrations of VEGF, owing to the stimulatory effect of nicotine and hypoxia induced by CO in cigarette smoke and not by augmented plasmatic concentrations of TNFSF1 and CCL2 [251].

In long-term $PM_{2.5}$ exposure, an approximately 10% increase in all-cause mortality is observed per 10-$\mu g/m^3$ average elevation. Longer-term exposure (years) reduces life expectancy (months or a few years). Long-term exposure to air pollution correlates with cardiovascular mortality. Air pollution is linked to cardiovascular morbidity via redox stress, inflammation, endothelial dysfunction, platelet hyperreactivity, altered fibrinolysis, and hence thrombogenicity and arrhythmogenesis.

Exposure to ultrafine carbon particles alters cardiac frequency and its variability (i.e., perturbation of the autonomic function in addition to that caused by diabetes) in type 2 diabetes mellitus (T2DM) patients [252]. These disturbances persist many hours after the end of exposure.

Recently emitted ultrafine particles and aged fine PM are both associated with changes in cardiac function in T2DM patients and individuals with impaired glucose tolerance in urban zones [253].

Environmental risk factors include indoor and outdoor air pollution. Most of the pollutant exposure commonly occurs indoors (i.e., at home, school, working place, and other community sites), as, along with indoor pollutants, outdoor air pollutants infiltrate buildings.

In addition to outdoor air pollution, indoor smoke resulting from cooking and home heating with biomass fuels and coal is a health risk for about 3 billion people [250].

Air pollution in accommodation, particularly due to the use of solid fuels, was a leading risk factor of altered health in sub-Saharan Africa and southern Asia in 2010 [254]. Mitigation of air pollution can reduce the burden of CVDs [250].

Approximately 80% of outdoor air pollution-related premature deaths are caused by cardiac ischemia and stroke, 14% by chronic obstructive pulmonary disease or acute lower respiratory infections, and 6% by pulmonary cancer.[16]

Temporal variation of daily average air pollution concentrations is more related to weather (i.e., wind direction and speed and atmospheric stability) that affects pollutant dispersion than the intensity of pollutant sources [248]. In addition, temperature and sunlight affect chemical reaction rates (e.g., ozone formation rate).

2.1.1.1   Airborne Pollutants

Air components encompass airborne *particulate matter* (or toxic aerosol; i.e., particles from multiple sources and various sizes and compositions; Vol. 6, Chap. 4. Physiology of Ventilation) and *gaseous pollutants* (ozone [O_3], nitrogen dioxide [NO_2], carbon monoxide [CO], sulfur dioxide [SO_2], and volatile organic compounds [e.g., benzene]).

Environmental toxins exist in air, water, and in the food supply. They include volatile organic compounds, polychlorinated biphenyls, phthalates, pesticides, dioxins, asbestos, heavy metals (aluminum, arsenic, cadmium, lead, and mercury), chlorine, and mycotoxins, among others. Although many phytochemicals are normally toxins at high doses, their consumption at relatively low doses activates an adaptive cellular stress response that protects cells.

Among pollutants, transition metals (e.g., copper, iron, nickel, vanadium, and zinc), organic compounds, semiquinones, and endotoxin (e.g., lipopolysaccharide) are relevant to cardiovascular pathological conditions, as these PM-related components are able to prime redox stress and inflammation. Certain features of ultrafine particles (e.g., high surface area, particle number, metal and organic carbon content) may augment cardiovascular risk after short-term exposure [247].

Semivolatile organic compounds found in both the gas and particle phases constitute with volatile organic compounds (VOCs) a large category of pollutants (e.g., benzene, (1,3)-butadiene, toluene, xylene, and polycyclic aromatic hydrocarbons) [247]. They emanate from both combustion and fugitive emissions and with secondary formation. Volatile organic compounds can be converted into less or more toxic substances. They can be oxidized in the atmosphere, becoming semivolatile organic compounds, and then contribute to the composition of $PM_{2.5}$.

Concentrations of PM_{10} and $PM_{2.5}$ are typically measured in mass per volume of air ($\mu g/m^3$), whereas ultrafine particles are often measured by their number per cubic centimeter (Table 2.7) [247].

Aerosol, a suspension of liquid or solid particles of organic and inorganic substances in air, is thus constituted of particles that have various sizes, shapes, chemical and physical properties, and chemical components. Major components include sulfate, nitrate, ammonia, sodium chloride, black carbon, mineral dust, and water.

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[16]Outdoor air pollution is carcinogenic to humans. The PM component of air pollution is associated with increased cancer incidence, especially cancer of the lung [255].

Table 2.7 Typical concentration peaks of various types of ambient air pollutants (Source: [247] *ppb* parts per billion, *ppm* parts per million)

Pollutant	Typical peak (average hourly concentration)
O_3	200 ppb
NO_2	200 ppb
NO	200 ppb
SO_2	150 ppb
CO	20 ppm
NH_3	100 ppb
HNO_3	10 ppb
Methane	5 ppm
Formaldehyde	40 ppb
Acetaldehyde	20 ppb
	(24-h average concentration)
PM_{10}	300 $\mu g/m^3$
$PM_{2.5}$	100 $\mu g/m^3$
$PM_{0.1}$	100,000/cm^3
Nonmethane hydrocarbons (volatile organic carbons)	250 $\mu g/m^3$
Propane	500 $\mu g/m^3$
Benzene	100 $\mu g/m^3$
(1,3)-Butadiene	10 $\mu g/m^3$
Sulfate	30 $\mu g/m^3$
Nitrate	20 $\mu g/m^3$
Organic carbon	30 $\mu g/m^3$
Elemental carbon	10 $\mu g/m^3$
Polycyclic aromatic hydrocarbon	200 ng/m^3

More than 98% of the air pollutant mass in urban zones is composed of gases or vapor-phase compounds that have independent, synergistic, or antagonistic effects with each other and with PM. Nitrogen oxides, carbon monoxide, sulfur dioxide, $PM_{2.5}$, and carbon dioxide originate mainly from the combustion of fuel or other high-temperature industrial processes. Ammonia, methane, pesticides, reduced sulfur compounds, resuspended dust, and natural coarse particles derive chiefly from noncombustion surface or fugitive releases that arise from human (e.g., agriculture) and natural (e.g., erosion) activities. Secondary pollutants emanate primarily from chemical reactions in the atmosphere among directly emitted pollutants (e.g., ozone; hydroxyl radical; peroxyacetyl nitrate; nitric, formic, and acetic acid; formaldehyde; acrolein; PM-associated sulfate, nitrate, and ammonium; many of the organic compounds in $PM_{2.5}$; inorganic and organic acids; and volatile organic carbons)

Environmental tobacco smoke, organic compounds (polycyclic aromatic hydro-carbons, volatile organic substances, formaldehyde, naphthalene), carbon monox-ide, and benzo[a]pyrene have a detrimental effect on health [248].

However, PM affects health more than any other pollutant [250]. In addition, it plays a major role in global climate change, which alters ecosystems, along with food production and the water supply.

Analysis of mixtures of pollutants emitted by given sources (e.g., traffic-related pollution, power plants, steel mills, and wood smoke) can be more pertinent to investigations of health outcomes and to the development of effective air quality strategies [247].

Primary pollutants, such as soot particles and oxides of nitrogen and sulfur, are emitted from the combustion of fossil fuels. Major sources of nitrogen dioxide are motorized vehicles, industrial sources, and residential heating.

Secondary pollutants are formed in the atmosphere from other components. In particular, ozone is formed from photochemical reactions of nitrogen oxides and volatile organic components.

Both *acute* and *chronic exposure* to high levels of air pollution disturb the endothelial function [256]. Expression of redox stress markers (e.g., NOS2 and nitrotyrosine) increases after exposure to diesel gas exhaust.

Oxidized lipids can mediate adverse vascular effects of air pollutants, especially particulate matter $PM_{2.5}$. In mice, $PM_{2.5}$ increases 7-*ketocholesterol*, an oxidated form of cholesterol and a major component of oxysterols, in plasma IDLs and LDLs and in aortic atherosclerotic plaques concomitantly with increased ScaRb3 expression in plaque macrophages [256]. 7-Ketocholesterol can be continuously formed on the surface of cholesterol-loaded lipoproteins.

In addition, air pollution favors uptake of 7-ketocholesterol through ScaRb3 by macrophages. Exposure to engine emissions increases not only the scav-enger receptor ScaRb3 but also ScaRe1, thereby dysregulating the metabolism of oxidized lipids [256]. On the other hand, 7-ketocholesterol can be exported from cells to lipoproteins. For example, ABCg1 carries 7-ketocholesterol and 7β-hydroxycholesterol to HDLs, hence protecting cells. Once they are subjected to 7-ketocholesterol, cholesterol ingress increases in ECs and cholesterol egress decreases in macrophages [256].

Chronic exposure to $PM_{2.5}$ increases the generation of derivatives of oxidized phospholipids. In airways, derivatives (oxPAPC) of 1-palmitoyl 2-arachidonyl sn-glycero 3-phosphorylcholine (PAPC), a dominant phospholipid of surfactant, com-prise 1-palmitoyl 2-(5-oxovaleroyl) sn-glycero 3-phosphocholine and 1-palmitoyl 2-glutaroyl sn-glycero 3-phosphocholine). These derivatives have elevated concen-trations in circulating lipoproteins (LDLs and HDLs). The generation of derivatives of oxidized phospholipids, such as oxPAPCs, stimulates NFκB and then expression of proinflammatory genes (e.g., the chemokine CCL2) via toll-like receptor TLR4 and NADPH oxidase (TLR4–NOx2 pathway), hence mobilizing bone marrow-derived mononuclear cells [256].

2.1.1.2 Determinants of Air Pollution Exposure

Pollutant emission or formation rate, atmospheric lifetime, weather pattern, and diurnal and seasonal cycles in solar radiation and temperature impact on pollutant concentrations.

The gaseous pollutants nitrogen oxides and carbon monoxide, in addition to the particle constituents elemental carbon and volatile organic compounds, are emitted during combustion, and their concentrations peak during rush hour [247]. Photochemical oxidants (e.g., ozone and secondary $PM_{2.5}$ and VOCs) peak in the afternoon.

Meteorological conditions affect pollutant concentrations. A stationary atmosphere, that is, a period of low horizontal and vertical mixing in the lower atmosphere, gradually accumulates regionally multiple pollutant types.

The microenvironment and individual activities (e.g., trafficking duration, indoor sources, tobacco smoke and occupational exposure, and degree of indoor penetration of background pollution) cause between-subject variability of pollutant exposure.

2.1.1.3 Aerosol Classification

Aerosol classifiers select a monodisperse aerosol. Aerosol particles can be classified according to electrostatic forces and drag using a *differential mobility analyzer*. Charged particles move in an electric field with a velocity that depends on the particle size and number of elementary charges on the particle. However, particles can have the same electrical mobility, but different numbers of charges and sizes.

Aerosol particle mass analyzer that comprises two coaxial electrodes rotating at an equal angular velocity categorizes aerosol particles according to their mass-to-charge ratio using centrifugal and electrostatic forces applied on charged particles in opposite directions. Particles with mass-to-charge ratios greater and smaller than that set by the force balance adhere to the outer and inner cylinder, respectively. In fact, the *Couette centrifugal particle mass analyzer*, in which the inner cylinder rotates slightly faster than the outer cylinder, has better performance [257].

In addition, equivalent aerodynamic diameter can be derived from particle relaxation time.[17] The *aerodynamic aerosol classifier* uses a centrifugal force and sheath flow between two concentric cylinders rotating at the same angular velocity to produce a monodisperse aerosol [258]. Different particle sizes are distinguished by changing the rotational speed. Particles with short relaxation times exit the

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[17]Aerosol particle equivalent diameter describes the size of nonspherical particles defined according to a specific physical property or measurement technique (aerodynamic, mobility, volume, mass, envelop, and projected area equivalent diameter). The aerodynamic equivalent diameter is defined as the diameter of a spherical particle with unit density that has the same terminal settling velocity as the actual particle.

classifier through a gap in the outer cylinder with the exhaust flow, those with long relaxation times impact and adhere to the outer cylinder.

Coarse particles result mainly from the resuspension of soil and road dust by wind or moving vehicles along with construction work and industrial emissions. Fine particles are engendered by combustion. They are composed of carbon, transition metals, and complex organic molecules, in addition to sulfate and nitrate formed in the atmosphere from volatile organic compounds, SO_2, and NO_2. They can travel large distances (>100 km). Ultrafine particles are generated by motorized vehicles.

Air pollution-linked mortality is due to exposure to suspended PM of $10\,\mu m$ or less in diameter (PM_{10}), which contributes to cardiovascular (Vol 7, Chap. 2. Context of Cardiac Diseases) and respiratory diseases and cancers [250]. Small particulate pollution has an impact on health, even at very low concentrations. No health-damage threshold has been identified. However, guideline values are proposed ($PM_{2.5}$: annual mean $10\,\mu g/m^3$ and 24-h mean $25\,\mu g/m^3$; PM_{10}: annual mean $20\,\mu g/m^3$ and 24-h mean $50\,\mu g/m^3$).

2.1.1.4 Air Filtration

The effects of PM can be attenuated by technological advancements and regulations. For example, enhanced urban public transport associated with proper traveling of cyclists and pedestrians can lower urban air pollution, encourage physical activity, lessen traffic injuries, and reduce the mobility cost [254]. Improved home insulation in addition to ameliorated heating and cooking systems and indoor ventilation can reduce vulnerability to diseases.

Air filtration is the most effective method for air sampling and cleaning. Nanofiber filtration has a good filtration efficiency and low resistance to air flow. However, nanoparticles with their given physical properties (surface area, shape, density, diffusivity, rigidity, electric charge, and aerodynamics) behave differently than microparticles. Yet, conventional filters are replaced by nanofibrous filters. The interfacial behavior between nanoparticles and nanofibers (caliber 10–100 nm) remains to be investigated.

Factors that influence the efficiency of a fiber filter in capturing particles include:

- Particle characteristics (size, physical state, chemical composition, density, charge distribution, and concentration)
- Filter characteristics (material type, fiber caliber, filter thickness, porosity, packing fiber density, filter fiber orientation, and electrical property)
- Collection (mechanical and electrostatic) mechanisms
- Operating conditions (temperature, filtrated air flow rate [face velocity], pressure, and viscosity)
- Thermal rebound associated with the Brownian motion

Nanoparticles (size $\leq 1\,\text{nm}$) may have a sufficiently high impact velocity to rebound from the surface upon collision (*thermal rebound*) [259]. Rebound of nanoparticles depends on the relative humidity of the air. However, the influence of thermal rebound on the collection efficiency may be low, as the filter is a fibrous medium consisting of entangled fibers; hence, the probability of rebound throughout the finite filter thickness is low.

Particle removal relies on four particle collection mechanisms.

- *Inertial impaction* occurs when the particle near a filter fiber changes its direction of travel and collides with the filter fiber.
- *Interception* appears when a particle is carried by air that flows very close to a filter fiber.
- *Diffusion* due to Brownian motion causes the particle to impact the filter fiber.
- *Electrostatic attraction* between particles and filter fibers is caused by Coulombic and dielectrophoretic attraction forces. In a heterogeneous electric field, a dielectric particle becomes electrically polarized due to charge separation that creates a dipole and bears a net dielectrophoretic force. The magnitude of this force, which can be exerted on neutral particles, depends on the medium and particle properties (shape, size, and electrical features) and on the frequency of the electric field. If a suspended particle has a polarizability higher than the medium, the dielectrophoretic force attracts the particle toward regions of higher electric field (positive dielectrophoretic force) and conversely (negative dielectrophoretic force).

The combined effect of these mechanisms governs the penetration of particles through the filter. Interception and inertial impaction are dominant collection processes for microparticles and diffusion for nanoparticles. At low air flow rates (face velocities) and hence high residence times, diffusion and electrostatic forces can contribute to the capture efficiency. For an order of magnitude of the particle size ranging from 10 to 100 nm and face velocity from 10 to 100 mm/s, the filtration efficiency augments at a low face velocity, whatever the particles' shape [260].

Humidity influences the filtration performance, at least for a particle size greater than 100 nm. Using mechanical filters, elevated relative humidity raises capillary force and filtration [260]. On the other hand, using charged filters, the filtration decreases as the humidity increases, as water molecules can remove charges from filter fibers.

Two major classes of filters comprise fibrous and membrane filters. The important filter characteristics are efficiency (capacity to arrest the passage of and hold airborne particles), dust holding capacity, face velocity, and pressure drop. The quality factor (QF) evaluates the filter performance:

$$\text{QF} = \frac{\ln(1/P)}{\Delta p}, \tag{2.1}$$

where P is the penetration and Δp the pressure drop across the filter. The latter increases for a given face velocity when the filter becomes dirty.

In conventional aerosol filtration theory, diffusion is supposed to be the dominant transport means of submicron particles, and impaction of these aerosol particles is followed by adhesion. The classical filtration theory also assumes that nanoparticles[18] adhere when they strike a filter with a Brownian motion. However, nanoparticles (size $<10\,nm$) may have a sufficiently high impact velocity to rebound from the surface upon collision (thermal rebound) [259]. Rebound of nanoparticles depends on the relative humidity of the air. Membrane-coated filters, that is, filters coated with a polytetrafluoroethylene membrane, can be considered as two combined layers, a fibrous and membrane layer. They may control nanoparticle spreading.

Investigations quantify atmospheric pollution, measure immersed particle properties, particle size distributions, and particle filtration efficiency; and determine nanoparticle toxicology and aerosol pulmonary dose.

The relative particle velocity with respect to the air flow velocity is the migration velocity. An external force, such as gravity and electrostatic force, is required to separate particles and select a given particle size. *Impactors* endowed with a nozzle and an impaction plate or a receiving tube enable particle classification according to inertia. Large particles with high inertia tend to cross gas flow streamlines, whereas small particles with low inertia remain in the gas flow.

2.1.1.5 Biological Mechanisms

Inhalation of PM can operate via [247] (1) the release of proinflammatory mediators and cells (e.g., cytokines along with activated immunocytes and platelets), vasculoactive molecules (e.g., angiotensin, endothelin, and histamine), or microparticles from pulmonary cells, (2) perturbation of the autonomic nervous system via stimulation of lung receptors and irritant and afferent nerves, and (3) passage of ultrafine particles or particle constituents (e.g., organic compounds and metals) into the blood.

In addition to the role of copollutants, pollutant features (size, charge, solubility, surface chemistry [e.g., the ROS-producing potential of metals, organic compounds, and semiquinones], and aggregation) are involved in their clearance (or retention rate) and interaction with pulmonary epitheliocytes and immunocytes, and within cells with organelles (e.g., mitochondrion) or molecules (e.g., cytochrome P450 and NADPH oxidase), and hence in pulmonary inflammation and redox stress [247].

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[18]Nanoparticles have at least 1 dimension smaller than 100 nm. Airborne nanoparticles are referred to as nano-aerosols and ultrafine PMs. They are produced mainly by combustion (transportation, indoor fumes, smoking, heating biomass, burning) and nanotechnology (e.g., use of injectable and inhalable drugs and tracers). Other sources include polymers, laser printers, and photocopiers, along with cleaning, agriculture, and welding. Nanoparticles have a large surface area-to-volume ratio, hence a higher surface reactivity. Their deposition in the pulmonary acinus is high ($>90\%$), hence facilitating subsequent entry into the bloodstream.

Blood concentrations of stress hormones (corticotropin-releasing and adrenocorticotropic hormone, cortisol, cortisone, and catecholamines, adrenaline and noradrenaline), metabolites (amino acids, glucose, fatty acids, and other lipid types), and markers of redox stress and inflammation were investigated in response to PM exposure in 55 healthy Shanghai college students. Exposure to $PM_{2.5}$ increases significantly concentrations of stress hormones and markers of insulin resistance, along with blood pressure, and provokes redox stress and inflammation [261]. Exposure to $PM_{2.5}$ can thus activate the hypothalamus–pituitary–adrenal and sympathetic–adrenal axes, contributing to adverse metabolic and cardiovascular effects. The hypothalamus releases corticotropin-releasing hormone, which stimulates the anterior pituitary gland, which secretes adrenocorticotropic hormone (ACTH) into the blood, reaching the adrenal cortex, where it stimulates the synthesis and release of glucocorticoids, mainly cortisol and cortisone. The synthesis and secretion of catecholamines in the adrenal medulla is regulated by preganglionic sympathetic neurons. Glucocorticoids can also modulate catecholamine synthesis via phenylethanolamine methyltransferase. Conversely, adrenaline can stimulate ACTH secretion from the pituitary gland. Glucocorticoids provoke vasoconstriction. In addition, they increase metabolic rate and energy expenditure, glycemia, as well as lipolysis, lipid oxidation, and proteolysis, and they favor insulin resistance. Catecholamines augment glycolysis and lipolysis, cause vasoconstriction, and raise cardiac output and formation of proinflammatory cytokines. Exposure to $PM_{2.5}$ may also stimulate the secretion in the pineal gland via β-adrenoceptors of melatonin, a circadian cycle regulator, which has an anti-inflammatory effect and is a ROS scavenger. In addition, greater levels of lysolipids (e.g., lysophosphatidylcholine and lysophosphatidylethanolamine) accompany higher PM exposure. Indoor air purification for 48 h not only reduces personal exposure to fine PM but also yields cardiopulmonary benefits following consecutive exposures [261].

2.1.1.6 Cardiovascular Events

Some individuals can be more susceptible to air pollution than the general population at a given pollutant concentration, especially the elderly, those who are obese, and patients with diabetes, chronic lung disease, coronary atherosclerosis, or HF, in addition to humans with low socioeconomic status. On the other hand, vulnerability refers to subjects at greater risk, as they are exposed more frequently to high pollutant concentrations.

Infraclinical changes can occur in individuals in response to $PM_{2.5}$ exposure. Short-term exposure to $PM_{2.5}$ (hours to weeks) can contribute to arrhythmias, myocardial ischemia, and strokes. Long-term $PM_{2.5}$ exposure increases the risk for cardiovascular mortality.

Inflammation with elevated circulating markers (CRP, fibrinogen, TNFSF1, IL1β, IL6, and soluble adhesion molecules [e.g., icam1]) can be detected after PM inhalation [247]. Pollutant exposure raises plasma concentrations of CRP and fibrinogen that alter expression by leukocytes of adhesion molecules. Artery wall inflammation destabilizes atheromatous plaques.

Moreover, pollutant nanoparticles that alter the features of arterial and venous ECs and enhance platelet activation and aggregation may act as foci for thrombus formation [246]. Exposure to PM_{10} can be associated with elevated platelet aggregation and concentrations of fibrinogen, von Willebrand factor, and plasminogen activator inhibitor PAI1 [247]. In addition, release of tissue plasminogen activator that participates in fibrinolysis is reduced after diesel exhaust inhalation.

Furthermore, prolonged exposure to PM generates vasoconstriction, hence favoring hypertension. In particular, exposure to black carbon, a component of fine PM derived from vehicular pollution, is associated with hypertension. In older adults, short- to moderate-term ambient black carbon concentrations are correlated with blood mitochondrial abundance, a compensatory response that attenuates the cardiac effects of pollution [262]. A 2-h exposure to diesel exhaust alters the pulmonary vasomotor tone, thereby increasing pulmonary vascular resistance, under stress (but not rest) conditions (i.e., at high cardiac output) [263]. On the other hand, diesel exhaust exposure does not affect concentration of plasmatic endothelin-1 or exhaled nitric oxide (NO). Air pollution can thus potentiate acquired pulmonary hypertension.

Redox stress is linked to inflammation, as it is often induced by and elicits inflammation. It can remain confined to the cell level and hence not be directly observable by circulating markers. However, some studies show that ambient PM can increase the plasmatic concentration of homocysteine, a mediator of redox stress [247].

Lead can be absorbed via the respiratory and gastrointestinal tracts (inhalation of lead-containing dust and gas exhaust) and through the skin. Ninety-nine per cent of blood lead content is bound to RBCs. Bones serve as the main lead repository in the body. The kidney is a principal organ of lead excretion.

Chronic lead exposure causes hypertension and cardiovascular disease. It favors redox stress. It activates NFκB, promoting inflammation [264]. In addition, it downregulates expression of soluble guanylate cyclase β subunit and limits NO availability, hence reducing endothelium-dependent vasorelaxation; augments activity of adrenoceptors, protein kinase-C, and plasma angiotensin convertase, as well as endothelin production; upregulates PGhS2 expression, raising concentration of vasoconstrictory thromboxane and lowering that of vasodilatory prostacyclin; attenuates atrial natriuretic peptide production; and disturbs calcium signaling in vSMCs, thereby inducing vasoconstriction [264]. Moreover, lead induces endothelial injury, impedes endothelial repair and angiogenesis, diminishes endotheliocyte growth, but stimulates vSMC proliferation. It also reduces production of proteoglycans and tissue plasminogen activator, and raises that of plasminogen activator inhibitor (PAI1).

In two large European cohorts (144,082 adult participants), greater road traffic noise exposure is associated with a higher level of high-sensitivity CRP, TGs, and HDL. Greater air pollution exposure (PM_{10} and NO_2) is linked to higher TG and CRP concentrations [265]. Moreover, exposure to road traffic noise and air pollution raises fasting glycemia.

Heart Ischemia and Stroke

Ischemic cardiac events are related to long-term exposure to elevated $PM_{2.5}$ levels [247]. The risk for an adverse post-MI outcome (death, subsequent infarction, or congestive HF) rises with greater exposure to PM_{10}. Fatal coronary events can be statistically correlated with $PM_{2.5}$ exposure.

Nonfatal and fatal cerebrovascular diseases increase with prolonged exposure to $PM_{2.5}$, with a small statistically significant correlation [247].

Cardiac Arrhythmias and Heart Failure

Cardiac arrhythmias can appear in the context of exposure to fine PM and related pollutants. Death due to arrhythmias and HF is associated with prolonged exposure to $PM_{2.5}$ [247]. Hospitalization for heart failure can be linked to short-term changes in PM exposure.

Arterial and Venous Diseases

A nearly statistically significant positive association can be found between daily changes in PM pollution and hospitalizations for peripheral vascular diseases [247]. The risk for deep vein thrombosis augments with long-term PM_{10} exposure.

2.1.2 Noise

Whereas certain types of sounds and music have beneficial effects on cardiovascular physiology, tempo, melodic structure, and periods of silence being crucial factors that have an impact on blood flow parameters and baroreflex, chronic low-intensity noise disturbs sleep and activity. Disturbed sleep and repetitive exposure to noise are risk factors [266]. Noise engendered by road, railway, and air traffic augments the incidence of hypertension and atherosclerosis complications (MI and stroke). Night-time noise alters endothelial function and sleep quality and tends to increase arterial pressure [267]. Vitamin C significantly improves flow-mediated dilation, suggesting the occurrence of redox stress upon noise exposure.

Noise does indeed cause redox stress and endothelial dysfunction. Although noise increases NOS3 production, it decreases NO concentrations due to NOS3 uncoupling. It raises not only vascular ROS production but also inflammation marker levels along with circulating concentrations of stress hormones (adrenaline and cortisol). Monocytes, macrophages, natural killer cells, and neutrophils infiltrate vascular walls. Reactive oxygen species are linked to various pathways (PI3K–PKB, FoxO, TGFβ1, NFκB, and ET1). Noise increases vascular expression not only of endothelin-1 but also of NOx2 mainly via the Agt2–AT_1 axis [267].

Moreover, noise activates the sympathetic nervous system. In mice exposed to intermittent noise mimicking aircraft noise delivered in short episodes (peak sound A-weighting intensity 85 dBA; mean sound intensity 72 dBA) for 4 days at the most increases systolic blood pressure, cardiac frequency, and plasmatic concentrations of IL-6, noradrenaline, and angiotensin-2 (Agt2) [267].[19]

2.2 Transcripts

Precision medicine relies on identification of additional risk markers linked to pathogenic genetic variations (Chap. 7). Many gene variants affect blood pressure and lipid concentrations.

Transcription, the first stage of gene expression, consists of translating information from a DNA segment, that is, a protein-coding gene (among $>22,000$ protein-coding genes), to a pre-messenger RNA molecule, which is spliced and exported into the cytosol. Translation converts information contained in a mature mRNA into a polypeptide that engenders a protein properly folded and located within the cell.

Ribonucleic acids are classified into protein-coding messenger RNAs (mRNAs) and nonprotein-coding RNAs (ncRNAs). In addition, the superclass of RNAs include many nucleotide-derived coenzymes, self-processing ribozymes, metabolite-binding riboswitches, and even ribosomes [268]. Many of the most common signaling molecules are also formed from RNA nucleotides or their precursors. The most widespread second messengers include the cyclic ribonucleotides cAMP and cGMP, in addition to pyrimidine nucleotides such as cytidine and uridine triphosphate. Other signaling effectors comprise cyclic adenosine diphosphate–ribose and nicotinic acid adenine dinucleotide phosphate, in addition to the dimeric signaling mediator diadenosine tetraphosphate (AP_4A).

Messenger RNA elements, riboswitches, bind to small molecules to regulate gene expression. Among numerous riboswitch classes, many regulate gene transcription in response to the selective binding of coenzymes and signaling mediators derived from RNA monomers or their precursors.

Nonprotein-coding RNAs (ncRNAs) comprise functional RNAs that are not translated into proteins. They include spliceosomal (small nuclear) RNAs, transfer (tRNAs), ribosomal (rRNAs), and other types of small (sncRNAs; <200 nucleotides [nts]) and long ncRNAs (lncRNAs; >200 nts).

Aminoacyl–tRNA synthetases (aaRSs) act during the first stage of ribosome-dependent protein synthesis. These enzymes attach the appropriate amino acid (aminoacylation) onto cognate tRNA isoacceptors (AA–tRNA^{AA}; AA: a given type of amino acid). They are categorized into two groups according to their chemical

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[19]Angiotensin-2 (II) is also abbreviated as AngII.

Table 2.8 Activities of free aminoacyl–tRNA synthetases (aaRSs) and aaRS-interacting multi-functional proteins (AIMPs) released from the human aaRS–AIMP complex (Source: [269])

Proteins	Activities
Group-II	Extracellular inflammatory cytokine
LysRS	Plasmalemmal viral assembly agent
	Nuclear transcriptional controller
Glu–ProRS	Translational silencing
GlnRS	Antiapoptosis
MetRS	rRNA transcription
IleRS	Autoimmunity
AIMP1	Cytokine activation of macrophages
AIMP2	Degradation of FBP, a transcriptional activator of MyC
AIMP3	P53 induction and DNA repair via nuclear ATMK and ATRK activation

Lysyl–tRNA synthetases are unique among the aminoacyl–tRNA synthetases, as they belong to category I or II aaRSs

properties, catalytic domain architecture, and consensus sequences[20] in addition to subgroups based on their mechanistic properties, anticodon-binding domain characteristics, and structural motif organization [269].

Mammalian aaRSs differ from those of prokaryotes and lower eukaryotes, as they associate as multienzyme complexes. Nine aaRSs (ArgRS, GlnRS, IleRS, LeuRS, LysRS, and MetRS, along with aspartyl– [AspRS or DaRS] and glutamyl–prolyl–tRNA synthetases [Glu–ProRS or EPRS]) and three auxiliary aminoacyl–tRNA synthetase-interacting multifunctional proteins (AIMP1–AIMP3) assemble into a multi-aaRS complex that operates in alternating activities of its members [269]. Under some circumstances, one of the aaRSs or AIMPs is released to trigger cell signaling (Table 2.8).[21]

In addition to their canonical role in translation, aaRSs have auxiliary functions in nutritional stress response, apoptosis, and cell survival along with the regulation of gene transcription and transcript translation via linkage to transcription factors or

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[20]A consensus sequence is an amino acid sequence of proteins that is generally used for intra- or intermolecular interactions, which enables the classification of functional sites [270]. Phosphorylation sites recognized by kinases represent a first type of consensus sequence. Another consensus sequence type is the *leucine zipper* in DNA-binding proteins, every seventh amino acid residue being a leucine.

[21]In humans, LysRS generates diadenosine tetraphosphate (AP_4A) to activate mastocytes. This signaling effector binds to histidine triad nucleotide-binding protein HinT (HinT1), also called adenosine 5′-monophosphoramidase and protein kinase-C inhibitor PKCI1 (PKCNH1), thereby lowering its interaction with the transcription factor MiTF (bHLHe32) that then dissociates from LysRS and HinT, which transactivates its responsive genes [269]. Once it is released by TNFSF1 (tumor-necrosis factor-α, one member of the TNF superfamily consisting of 19 ligands and 29 receptors), LysRS is secreted and can then also act as a proinflammatory cytokine that binds to macrophages, thereby priming TNFSF1 production and migration (positive feedback loop). TNFSF1 activates NFκB and the MAPK module (ERKs, P38MAPKs, and JNKs).

Table 2.9 Aminoacyl–tRNA synthetases (aaRSs) and aaRS-associated anti- and pro-angiogenic and angiostatic proteins (Source: [271])

Molecule	Effect on angiogenesis
AaRS acting outside the cell	
ThrRS	Proangiogenic agent
(TaRS)	Stimulates endotheliocyte migration
TrpRS	Angiostatic agent; interacts with cadherin upon loss of its
(WaRS)	WHEP domain by alternative splicing or after secretion by ElaNE
	Truncated TrpRS (miniWRS) disrupts between-endotheliocyte contacts and inhibits ERK1/2, NOS3, PKB
TyrRS	Proangiogenic agent secreted and cleaved into
(YaRS)	N- and C-terminal fragments by ElaNE
	N-terminal fragment (miniYRS) activates ERK1/2, NOS3, PKB, Src
	C-terminal fragment elicits immunocyte migration
	MiniYRS interacts with CXCR1
AaRS acting inside the cell	
Glu–ProRS	Cytoplasmic angiostatic agent; phosphorylated Glu–ProRS binds to mitochondrial ribosomal protein MRPL13, glyceraldehyde 3-phosphate dehydrogenase, and synaptotagmin-binding, cytoplasmic RNA-interacting protein (SynCRIP or hnRNPq) to form the interferon-γ-activated inhibitor of translation (GAIT) complex
	Interferon-γ liberates Glu–ProRS from the multisynthetase complex and GAIT complex
SerRS	Nuclear angiostatic agent; it binds to VEGFA promoter and
(SaRS)	prevents MyC-primed VEGFA transcription
AaRS-interacting multifunctional proteins	
AIMP1,	Caspase-7 cleaves the C-terminus from AIMP1 that forms EMAP2 endotheliocyte–monocyte-activating polypeptide EMAP2, a chemoattractant released in response to apoptotic signals Extracellular pro- and antiangiogenic agents at low and high concentrations via TNFSF1-mediated angiogenesis, and Apoptosis that is linked to upregulated TNFRSF1 formation, respectively
	EMAP2 regulates angiogenesis via CXCR3

They are classified according to the location of angiogenic action, outside or inside the cell (ElaNE: neutrophil expressed elastase)

tRNA-like molecule, RNA splicing,[22] inflammation via recruitment of immunocytes, and angiogenesis, as some aaRSs and/or their secreted cleavage products contribute to controlling EC survival, proliferation, and migration via activation of ERK1, ERK2, P38MAPK, and PKB (Table 2.9) [271].

Loss-of-function mutations in TARS and IARS genes that encode threonyl– and isoleucyl–tRNA synthetases (premature stop codon and point mutation, respectively) upregulate synthesis of proteins implicated in the unfolded protein

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[22]Mitochondrial TyrRS and LeuRS possess a splicing factor-like activity; they assist in the excision of group-*I* introns [271].

response (UPR; e.g., ATF4, ATF6, and XBP1)[23] in addition to vascular endothelial growth factor (VEGFa) via UPR-stimulated activating transcription factor ATF4 and increase branching angiogenesis [273]. The ATF4–VEGF pathway does indeed prime angiogenesis,[24] whereas secreted TaRS signaling supports angiogenesis independently of its synthetase activity. In fact, many aaRSs can promote angiogenesis upon nutrient depletion, which launches UPR and VEGF production, or via extracellular mechanisms [272].

In murine embryonic ECs, formation of JmjD8, which belongs to the JmjC category[25] and promotes TNFSF1-primed NFκB signaling, is upregulated during their differentiation. It regulates both endothelial sprouting and metabolism, as it interacts with pyruvate kinase-M2 [274].[26]

Small nonprotein-coding RNAs include microRNAs (miRs), P-element-induced wimpy testis-interacting-associated RNAs (piRNAs), and endogenous small interfering RNAs (endo-siRNAs).

Long nonprotein-coding RNAs operate as signals,[27] decoys,[28] guides,[29] and scaffolds.[30] They control the chromatin structure and hence can repress gene

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[23]The UPR comprises a set of signaling cascades that are activated in response to accumulation of unfolded proteins in the endoplasmic reticulum (ER). These pathways slow translation and raise protein degradation but augment production of UPR mediators. When it fails, continued UPR activation triggers apoptosis. Loss-of-function mutations in aaRSs and molecules that bind to aaRSs (e.g., inhibitors of glutamyl–prolyl– and Thr–tRNA synthetase halofuginone and borrelidin, respectively) along with nutrient depletion can provoke ER stress and elicit the UPR [272].

[24]Increased concentrations of unfolded proteins activate eIF2α K3 in the endoplasmic reticulum membrane, which phosphorylates eukaryotic initiation factor eIF2α and activates ATF4, an inducer of the transcription of genes involved in protein degradation, translation, and autophagy.

[25]Most of the JmjC domain-containing proteins function as demethylases, a subgroup serving as histone demethylases, affecting chromatin accessibility and subsequently gene transcription. Many members of the JmjC category are involved in cellular differentiation and proliferation. JmjD8 synthesis is upregulated during endothelial differentiation of murine embryonic stem cells (ESCs). JmjD8 is exclusively detected in the cytoplasm [274].

[26]Members of the JmjC category regulate angiogenesis, such as JmjC domain-containing histone demethylase JHDM1b (KDM2b), which elicits endothelial network formation in vitro via fibroblast growth factor (FGF2); bifunctional arginine demethylase and lysyl hydroxylase JMJD6, which acts in endothelial sprouting via Vegfr1 splicing control; and Jumonji and ARID domain-containing demethylase JARID1b (KDM5b), which represses transcription of the proangiogenic genes HOXA5 and Ccl14 [274]. Hypoxia induces the production of several JmjC domain-containing proteins, such as JHDM2a (KDM3a) and JmjD2b (KDM4b) to JmjD2d (KDM4c).

[27]LncRNAs are transcribed by RNA polymerase-2 upon stimulation in a cell type-specific manner. They control the spatiotemporal pattern of gene transcription and determine the developmental stage [275].

[28]LncRNAs can act as molecular sponges. In particular, they can function as sinks for RNA-binding proteins, such as transcription factors, chromatin modifiers, or other regulators, in addition to miRs and splicing factors [275].

[29]LncRNAs can serve not only as scaffolds but also as guides for epigenetic and transcription factors. They can bind to proteins and hence determine the localization of ribonucleoproteic complexes. They can guide in cis (on neighboring genes) or trans (on distant genes) [275].

[30]LncRNAs can serve as platforms for relevant molecules, simultaneously tethering multiple effectory partners, which may then act as transcriptional activators or repressors [275].

transcription. They also participate in post-transcriptional regulation, in particular in cell metabolism and differentiation [275].

The nucleoplasm and cytoplasm contain numerous small ribonucleoprotein complexes, the small nuclear (snRNPs) and cytoplasmic RNPs (scRNPs), respectively. They are composed of at least one protein and a small stable RNA ($<$ ∼300 nt). SnRNPs and scRNPs regulate nuclear and cytosolic RNA processing (i.e., pre-mRNA splicing and mRNA translation). Hence, other subsets of ncRNAs include (1) Components of the Ro (Ro60) ribonucleoprotein, ^{Y}RNAs,[31] (2) small cytoplasmic RNAs (scRNAs or ^{7SL}RNAs), and (3) constituents of large cytoplasmic ribonucleoproteins, the *vaults*.

^{Y}RNAs are small ncRNAs (∼100 nt) transcribed by RNA polymerase-3 that form stem–loop structures. Ro small cytoplasmic ribonucleoproteins are a subgroup of La ribonucleoproteins.[32] The Ro RNPs are stable and cytoplasmic, whereas the majority of the La RNPs turn over rapidly and reside in the nucleus [277]. These components of Ro RNPs are bound to 60-kDa Ro protein, which can be detected in the blood of patients with autoimmune diseases, such as systemic lupus erythematosus (or simply lupus)[33] and Sjögren's syndrome.[34] Binding to Ro60 is a prerequisite for the export of ^{Y}RNAs from the nucleus to the cytoplasm [276].

Ubiquitous small cytoplasmic RNA component 7SL of the signal recognition particle (SRP) serves as a scaffold for the six SRP proteins (SRP9, SRP14, SRP19, SRP54, SRP68, and SRP72) [278]. It links to the ribosome and assists translocation of nascent presecretory proteins to the endoplasmic reticulum[35] and then to secretory pathway, sorting them for membrane insertion or secretion from the cell.

Vaults participate in nucleocytoplasmic transfer. They include the major [36] and two minor vault proteins, PARP4[37] and TEP1,[38] in addition to various small

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[31]The letter Y refers to the cytoplasmic location and the term Ro to the patient name in whom it was first identified.

[32]The term La also refers to the name of the patient in whom it was first identified. The protein La is required for the accurate and efficient termination of transcription by RNA polymerase-3 and binds the 3′-polyuridine tail of newly synthesized RNAs in the nucleus [276]. In addition to Ro60 and La, several other proteins interact with a subset of ^{Y}RNAs such as nucleolin.

[33]The most common type of autoantibody in lupus is an antinuclear antibody.

[34]Sjögren's syndrome affects glands such as those that produce tears and saliva. In fact, it alters the entire body, most commonly in older women.

[35]The SRP connects to the nascent peptide that emerges from the large subunit of the ribosome and then targets the nascent polypeptide–ribosome complex to the SRP receptor in the rough endoplasmic reticulum.

[36]Also known as vault1.

[37]Poly^{ADP}ribose polymerase PARP4 is also termed vault poly^{ADP}ribose polymerase (VPARP) and vault3.

[38]Telomerase-associated protein TEP1 is also named vault2.

untranslated RNAs (i.e., vault RNA components vaultrc1–vaultrc3 [vtRNA1-1–vtRNA1-3] and vtRNA2-1 [or pre-miR886]).[39]

However, within the transcriptome, bifunctional RNAs have both coding and noncoding functions and can serve as both messenger and nonprotein-coding RNAs [279], although bifunctional RNAs comprise RNAs that have two different ncRNA functions.[40] They include [282]:

1. mRNAs (e.g., TNFRSF17as, Dmpk, H2B, Mdfic,[41] fortilin,[42] Sra) either with an intrinsic noncoding function (base pairing, scaffolding, and quenching) or with a nonprotein-coding isoform due to alternative splicing or allele-specific transcripts with variants that destroy coding potential
2. lncRNAs (e.g., ac074183.4, cgat1, ctd2651B20.6, dancr, gas5, malat1, snhg8, rp5-875o13.1, rp11-21n3.1, rp11-220i1.1, rp11-354p17.9, and rpph1) containing a small open reading frame that are strongly associated with ribosomes and thus encoding small peptides (<100 amino acids)

Nonprotein-coding RNAs can be identified within exosomes, which are intercellular communication shuttles formed from multivesicular bodies and then secreted. They contain miRs, rRNAs, tRNAs, lncRNAs, piRNAs, and small nuclear (snRNAs) and nucleolar RNAs (snoRNAs). Among them, miRs and lncRNAs, which regulate cell signaling, have an altered expression in pathogenesis and hence are additional markers.

2.2.1 MicroRNAs

Aberrantly regulated nonprotein-coding RNAs, such as short microRNAs and long nonprotein-coding RNAs that are released into the bloodstream, where their con-

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[39]Vault RNA2-1 is also called cord blood lymphocyte-derived ncRNA CBL3.

[40]For example, steroid receptor activator (SRA) is an ncRNA that, dependently and independently of ligands, acts as a transcriptional coactivator supporting transactivation mediated by steroid hormone nuclear receptors and encodes steroid receptor RNA activator SRA1 (or SRAP) that antagonizes SRA ncRNA at steroid hormone receptors [279–281]. The ncRNA SRA, DDX5, and DDX17 are coactivators of myogenic differentiation factor MyoD1 (a.k.a., myogenic factor MyF3 and bHLHc1) [194].

[41]The transcript that encodes MyoD family inhibitor domain-containing protein MDFIC binds to and translationally activates positive transcription elongation factor PTEFb, as it removes [7SK]RNA from the inhibitory complex formed by hexamethylene bis-acetamide-inducible protein (HEXIMs) and [7SK]RNA [283]. The PTEFb factor is also a protein kinase that phosphorylates RNA polymerase-2 in addition to other transcription factors. It is composed of CDK9 and cyclin CcnT1, CcnT2, or CcnK. Two genes encode small cytoplasmic [7S]RNA species K and L, which are transcribed by RNA polymerase-3.

[42]Also known as tumor protein that is translationally controlled TPT1 (or TCPT), histamine-releasing factor, and p23.

centrations differ thus from those in healthy subjects, contribute to cardiovascular pathophysiology.

MicroRNAs are linked to CVD risk factors, such as hyperlipidemia, hypertension, obesity, diabetes, lack of physical activity, and smoking. They participate in CVD, especially atherosclerosis and aneurysms.

MicroRNAs (content 18–22 nucleotides; Vol. 11, Chap. 3. Small Nonprotein-Coding RNAs—MicroRNAs) are transcribed from genomic mir loci by RNA polymerase-2 assisted by epigenetic regulators, enhancers, and transcription factors into transcripts, the primary microRNAs, which are capped, polyadenylated, and spliced. These mir transcripts contain either a single miR or a miR cluster. The primary mir transcript is processed by the nuclear microprocessor complex that contains the ribonuclease-3 drosha and the double-stranded RNA-binding protein DGCR8 (or pasha).[43] The precursor microRNA is then exported into the cytoplasm using exportin-5 in conjunction with Ran. The cytosolic ribonuclease-3 dicer generates the mature miR–miR* duplex. Then, dicer, in cooperation with the RNA-induced silencing complex (RISC)-loading complex subunit transactivation-responsive RNA-binding protein TARBP2, recruits argonaute-2 to the bound miR. Argonaute proteins, which unwind the double strand and incorporate one of the two miR strands into the RISC, lead to mRNA translation repression or degradation.[44] Therefore, argonaute proteins are key elements of gene silencing and microRNAs regulate amounts of proteins produced from target mRNAs.

For example, miR504, which resides in an intron of the Fgf13 gene, targets p53 mRNA [285]. The FGF subtype, FGF13, does not function like a regular growth factor. In the nucleolus, it represses transcription of ribosomal RNA and hence protein synthesis. On the other hand, P53 prevents transcription of the Fgf13 locus and thus synthesis of both FGF13 and miR504 (negative feedback loop).

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[43]DGCR: DiGeorge syndrome critical region gene product.

[44]Argonaute proteins (Ago1–Ago4) form the core of the RISC. They serve as binding modules for small RNAs, including microRNAs. Only one strand of the short double-stranded precursor, the guide strand, is linked to the Ago protein. During a loading step, the small dsRNA dicer product binds to Ago. Then, the passenger strand is displaced and the guide strand is stably linked to Ago. Only Ago2 is catalytically active, whereas Ago1, Ago3, and Ago4 operate on miRs independently of cleavage. Ago2 cleaves the passenger strand and displaces it. The bound small RNA strand guides Ago to complementary target sites on mRNAs. On the mRNA, Agos recruit a member of the TNRC6 family (TNRC6: trinucleotide repeat-containing glycine–tryptophan-enriched proteins [TNRC6a–TNRC6c]). These scaffolds recruit de-adenylation enzymes that remove the polyadenylated tail of the mRNA [284]. Afterward, decapping enzymes are recruited and mRNA is degraded.

Argonautes are targets of regulatory post-translational modifications. When miR genesis is impaired, Agos are ubiquitinated and degraded in the proteasome. On the other hand, hydroxylation of Pro700 increases Ago stability [284]. Their parylation impedes their repression in stress granules. Ago2 is phosphorylated at Ser387 by the MAPK module, thereby affecting localization of Agos into processing bodies, and by PKB3, shifting Ago2 activity from cleavage to translational repression. A C-terminal Ser–Thr cluster is phosphorylated at five residues. In humans, hyperphosphorylation does not affect microRNA binding, localization, or cleavage activity of Ago2, but mRNA binding [284].

MiR33 targets autophagy regulators and effectors in macrophages, diminishing lipid droplet catabolism and free cholesterol generation and subsequent efflux [286]. Concentration of oxysterol-binding protein-like protein OSBPL6 and hence cholesterol transfer is also regulated by microRNAs. MiR548p reduces ApoB secretion and lipid synthesis via HMGCoAR and long-chain fatty acylCoA synthase ACSL4 [286].

Some microRNAs, such as miR1, miR126, miR138, miR143, and miR145, participate in cardiogenesis via angiogenesis, establishment of cardiac cell polarity, development of the cardiac conduction system, cardiac patterning, or smooth myocyte differentiation. Often, these miRs regulate the core cardiac transcriptional network [287].

Two miRs are highly expressed in vascular smooth myocytes and ECs, miR221 and miR222, which preclude EC migration and proliferation and hence angiogenesis, but favor vSMC proliferation and migration and counter apoptosis [288]. MiR221 and miR222 reduce NOS3 expression and can cause endothelial dysfunction.

In patients, circulating miR15b-5p allows the differentiation of individuals with well and poorly developed collateral vessels, as miR15b-5p produced in vascular ECs regulates angiogenesis [286]. Proangiogenic miR126a is implicated in lymphangiogenesis via VEGFR3 signaling. Suppression of angiopoietin-2 formation by miR150 enables repair of vascular injury.

- MiR182-3p modulates a vascular smooth myocyte phenotype.
- MiR22 plays a role in smooth myocyte differentiation.
- MiR155 supports the migration of smooth myocyte progenitors via CCL2.
- MiR590-3p increases vSMC contractility and represents a risk factor for hypertension in the presence of genetic defects [286].

MiR125b is associated with vascular calcification [286]. MiR146a targets the transcript encoding polo-like kinase PLK2 to induce bone marrow cell apoptosis and senescence [286].

Redox stress due to excessive ROS production and insufficient scavenging is linked to aging, hypertension, hypercholesterolemia, diabetic vasculopathy, atherosclerosis, and ischemia. Reactive oxygen species increase expression of MIR200 family members (miR141, miR200a–miR200c, and miR429) [289].[45]

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[45]Members of the MIR200 family form two clusters in humans, genes encoding miR200b, miR200a, and miR429 residing in chromosome 1 (1p36 locus) and miR200c and miR141 in chromosome 12 (12p13 locus).

Both ROS and NO induce expression of MIR200 family members. Nitric oxide promotes differentiation of ESCs to the mesendoderm and then cardiovascular lineages directly and via MIR200 family members. In ECs subjected to hydrogen peroxide (200 μmol/l; exposure period range 8–24 h), miR200c and miR141 are the most upregulated miRs, their synthesis rising more than eightfold [290]. In addition, miR200c targets the apoptosis inhibitor PTPn13, thereby sensitizing tumoral cells to apoptosis.

In addition, hypoxia favors formation of miR429 that targets HIF1α.

2.2.1.1 Endothelial Mechanical Stress-Responsive MicroRNAs

The vascular endothelium forms a barrier that regulates its permeability. Moreover, this mechanosensor controls leukocyte adhesion, blood coagulation, the vasomotor tone, and vascular cell proliferation, especially that of smooth myocytes, in addition to angiogenesis.

MicroRNAs and histone deacetylases (HDACs) control gene expression and hence endothelial function. MiR10a pertains to the endothelial mechanical stress-responsive microRNAs.[46] It targets Gata6, which elicits vcam1 synthesis (an anti-inflammatory effect). Mechano-miR10a expression is regulated byKLF2 via RARα–RARE binding, which heterodimerizes with RXRα to bind RARE in the enhancer region of target genes [294].[47] According to the local flow pattern, HDAC3, HDAC5, and HDAC7, which link to myocyte enhancer factor MEF2, thereby hampering production of anti-inflammatory KLF2, or NR2b1, operate as mechanosensitive repressors and enhancers, respectively.

Vascular ECs in strongly curved segments with low wall shear stress (WSS) induce a quiescent-to-activated phenotypic transition of adjacent smooth myocytes

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Furthermore, MiR200 family members inhibit epithelial–mesenchymal transition (EMT), as they target the transcriptional repressors of the CDH1 gene that encodes E-cadherin, zinc finger-containing E-box-binding homeobox-derived (homeodomain-containing) proteins ZEB1 and ZEB2 [291].

Epithelial–mesenchymal (EMT) and mesenchymal–epithelial transitions (METs) occur in tumorigenesis. E-cadherin maintains the epithelial phenotype. On the other hand, EMT is characterized by the loss of E-cadherin, cell polarity, cell–cell and cell–matrix contact, in addition to gain in cell motility. Various transcription factors (FoxC1, Snai1, Twist, ZEB1, and ZEB2) induce EMT transition.

MiRs favor tumor development (miR21, miR155, and members of the miR17-92 cluster) or suppress it (Let7, miR15a, miR16, and miR34a) [291]. Several miRs support metastasis (miR10b, miR21, miR373, and miR520c) or impede it (miR126, miR200, and miR335). Conversely, ZEB1 can repress production of miR141 and miR200c. In addition, ZEB1 and ZEB2 prevent the production of crumbs homolog Crb3 and lethal giant larvae homolog LLGL2, which are involved in the establishment of epitheliocyte polarity.

Nanog, an important ESC agent, represses transcription of the MIR200B and MIR200B genes, at least in colorectal cancerous cells [292].

MiR200 promotes METs, as it suppresses multiple members of the ZEB2 and Snai1 transcriptional repressor complexes [293]. MiR200c targets cartilage-associated protein, FHOD1 (formin homology-2 domain-containing protein), Map3k1, Mapk12, Smad2, Smad5, Snai1, TOB1 (transducer of ERBB2/HER2), Xbp1, YWHAB (14-3-3β), YWHAG (14-3-3γ), and Zfp36, Smad2 and Smad5 binding to ZEB2, and 14-3-3β and 14-3-3γ to Snai1 [293]. MiR200 suppresses TGFβ and bone morphogenetic protein (BMP) signaling, promoting epithelial gene expression and suppressing cell invasion. Conversely, TGFβ downregulates expression of MIR200 family members.

[46]*Mechano-miRs* include miR19a, miR21, miR23b, miR92a, miR101, miR126, miR145, miR148a, miR155, miR221, and miR663 [294].

[47]RARα: retinoid acid receptor-α (NR1b1); RARE: retinoid acid-responsive element; RXRα: retinoid X receptor-α (NR2b1). RARs recruit RXRs to activate gene expression and HDACs to inhibit gene transcription.

and hyperplasia. Endothelial microRNAs, such as miR126-3p and miR200a-3p, are released in low WSS regions via activation of the SNAREs, vesicle-associated membrane protein VAMP3[48] and synaptosomal-associated protein SnAP23 [295]. These miRs then favor hyperplasia of recipient vSMCs.

2.2.1.2   Regulators of Vascular Smooth Myocyte Phenotype

Vascular smooth myocytes (vSMCs) are not irreversibly differentiated, because they can modify their phenotype according to environmental conditions, whereas fully differentiated smooth myocytes (SMCs) are refractive to PDGFbb stimulation.

In large and medium-sized blood vessels, they constitute the main cellular population of the media. They are organized in a variable number of monolayers of circumferentially oriented vSMCs.

The *arterial media* contains quiescent and contractile vSMCS surrounded by their own basement membrane, immersed in an extracellular matrix, and a small quantity of macrophages and fibroblasts.

Heparan sulfate proteoglycans (HSPGs) are major components of the vSMC *glycocalyx*. The glycocalyx contains heparan and chondroitin sulfate proteoglycans and hyaluronic acid in addition to glycoproteins with terminal sialic acids.

The *basement membrane* surrounding vSMCs contains predominantly collagen-4, laminin, and HSPGs, such as perlecan and syndecan. HSPGs are low-affinity receptors that sequester growth factors such as FGF2, which can modify vSMC phenotype, hence priming proliferation.

Proteoglycans are proteins bearing glycosaminoglycans (GAGs). Glycosaminoglycans are long linear (unbranched) polysaccharide chains, which are also termed mucopolysaccharides. They encompass chondroitin (ChS), dermatan (DeS), heparan (HeS), and keratan sulfate (KeS), as well as heparin and hyaluronan, DeS being stereoisomeric variants of ChS. ChS and DeS interact with various types of extracellular molecules, such as growth factors, cytokines, chemokines, and morphogens, and are therefore involved in cell adhesion, proliferation, and migration.

The *medial interstitial matrix* contains collagen-1 and -3, various types of glycoproteins (e.g., fibronectin, tenascin, thrombospondin, and vitronectin), and chondroitin sulfate proteoglycans such as versican [296].

Under normal conditions, vascular SMC differentiation is controlled by messengers such as TGFβ; plasma membrane components, such as adhesion molecules; matrix constituents, such as collagen and elastin; mechanical forces; neuronal signals; and intercellular interactions.

On the other hand, dedifferentiated vSMCs synthesize matrix metallopeptidases (MMPs), tissue inhibitors of metallopeptidases (TIMPs), and matrix components.

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[48] Also known as cellubrevin and synaptobrevin-3.

Lysosomal heparin lyases (or heparinase)[49] released by macrophages remove HS chains and favor vSMC proliferation.

Heparanase is an endoglycosidase that cleaves HS from the cell surfaces and matrix constituents, hence facilitating the release of exosomes and subsequent intercellular communication in addition to remodeling the extracellular matrix.

The dedifferentiated vSMC can activate programmed cell death pathways and elaborate proinflammatory cytokines [298]. Migration, proliferation, and death of vSMCs are events regulated by HSPGs and hence cell surface (extracellular) HS sulfataseheparan sulfate sulfatase-1 [299][50]

Furthermore, activated vSMCs can be involved in efferocytosis and phagocytosis, ossification, reverse cholesterol transport, and foam cell formation.

Phenotype switch and abnormal vSMC proliferation and migration constitute a major process in the genesis of arterial wall diseases, especially neointima formation, a hallmark of atherosclerosis and post-stenting restenosis.

Once they are exposed to stressors, vSMCs change their phenotype, that is, they dedifferentiate from their quiescent contractile phenotype to a state characterized by abnormal proliferation, migration, matrix synthesis, and inflammation. This dedifferentiation contributes to maladaptive remodeling of the vascular wall (e.g., atherogenesis, post-angioplasty intimal hyperplasia, aneurysmal degeneration, and medial calcinosis).

Cell motility relies on cycles of cell protrusion and retraction and of formation and disruption of cell–matrix adhesion sites, on polarized vesicular transfer and exocytosis, and, at the nanoscale, extracellular signal-regulated kinase (ERK). The MAPK module component ERK and its effector kinase, p90 ribosomal

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[49]Glycosidases hydrolyze carbohydrate chains. *Exoglycosidases* release monosaccharides from nonreducing ends of oligosaccharides and sugar chains of glycoproteins and glycolipids (e.g., α-fucosidase and β-galactosidase). *Endoglycosidases*, which were originally detected in bacteria and plants, and now identified in mammals and hence humans, hydrolyze the inner part of the sugar chains. They process N- and O-linked sugar chains of glycoproteins. Polysaccharide lyases cleave specific glycosidic linkages in acidic polysaccharides and depolymerize GAGs. This category of enzymes includes heparin and HS lyases (heparinases and heparitinases), chondroitin lyases (chondroitinases), and hyaluronate lyases (hyaluronidases). Heparin lyase-1 (heparinase) degrades heparin and very weakly HS, whereas heparin lyase-3 (heparitinase) degrades HS, but not other GAGs.

Macrophages cocultured with vascular smooth myocytes degrade HSPGs of the vSMC surface, decrease the myofilament number within vSMCs, and induce vSMC phenotypic change [297].

[50]Heparan sulfate (HS) chains of HSPGs determine ligand binding and hence function. They contain N-sulfated, acetylated, and unmodified glucosamine and O-sulfate groups at characteristic positions.

Heparan sulfate 6-O-endosulfatases, Sulf1 and Sulf2, selectively remove sulfate from the C6 position of glucosamine in the HS chain, desulfating HSPG and preventing signaling due to decayed interaction between growth factors and their coreceptors on the cell surface and, hence, diminishing the cell proliferation rate but facilitating apoptosis in response to stimuli.

S6 kinase (RSK), regulate cell migration mainly via phosphorylation of various substrates [300].

The Ras–Raf–MAP2K–ERK–RSK pathway is activated by liganded receptor protein Tyr kinases (i.e., growth factors) and stimulated integrins. Activated ERK translocates into the nucleus, where it phosphorylates some transcription factors, thereby regulating gene expression. It also phosphorylates (activates) various types of kinases (RSKs, MSKs, and MNKs). Cytosolic MKnKs regulate mRNA translation, as they phosphorylate eukaryotic translation initiation factor eIF4e. Nuclear MSKs also regulate gene transcription, as they phosphorylate transcription factors, high-mobility group nucleosome-binding domain-containing protein HMGN1, and histone H3 [300]. Nuclear and cytosolic RSKs phosphorylate various transcription factors in the nucleus and the 40S ribosomal subunit protein S6 and eIF4b in the cytoplasm.

Therefore, ERK regulates cell motility via genomic and nongenomic effects. It engenders motile mesenchymal cells from polarized epitheliocytes. Epithelial–mesenchymal transition is triggered by various transcription factors (e.g., Snai1 and Snai2, Twist1, and ZEB1 and ZEB2) [300]. Both ERK and RSK phosphorylate regulators of cell protrusion and retraction, cell–matrix adhesion, and exocytosis (Table 2.10).

Cell movement is induced by activation of the small GTPase Ras, which in turn stimulates both PI3K and ERK axes. The PI3K pathway stimulates actin polymerization and the formation of a protrusion (a lamellipodium) at the leading edge of the moving cell via activation of the small GTPase Rac1 and protein kinase PKB.

The ERK kinase phosphorylates MLC kinase, cortactin, WASP family protein member WASF2 (wave2), and FAK, among other substrates, thereby promoting cell migration.[51]

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[51]Two types of actin nucleation factors, the actin-related protein ARP2–ARP3 complex and formins, nucleate branched actin meshwork and linear filamentous actin, respectively, in lamellipodia and filopodia. Activity of the ARP2–ARP3 complex, which is weak, increases upon its interaction with nucleation-promoting factors. Nucleation-promoting factors (NPFs) are classified into two sets. Set-*I* NPFs are further categorized into five subsets [300]: (1) Wiskott–Aldrich syndrome protein (WAS or WASP) and WAS-like (WASL or neural WASP [nWASP]); (2) WASP family verprolin homolog (wave); (3) WASP and SCAR homolog (WASH); (4) WASP homolog associated with actin, membranes, and microtubules (WHAMM); and (5) junction-mediating and regulatory protein, which acts both as a nuclear P53 cofactor and as a cytoplasmic regulator of actin dynamics. Set-*II* NPFs contains a major member, cortactin, which interacts with the ARP2–ARP3 complex and enhances its actin nucleation activity. Cortactin also interacts with WAS and WASL, synergistically activating the ARP2–ARP3 complex by WAS, WASL, and cortactin. In addition, actin elongation factors, such as proteins of the Ena/VASP (enabled homolog and vasodilator-stimulated phosphoprotein) family, regulate the extension of lamellipodia and filopodia. Formins also function as actin elongation factors.

The wave proteins (wave1–wave3) promote actin nucleation by the ARP2–ARP3 complex via the wave regulatory complex (WRC), which scaffolds actin monomers and the ARP2–ARP3 complex. ERK phosphorylates wave2 and Abl interactor AbI1, a WRC component, enabling interaction of WRC with the ARP2–ARP3 complex and actin [300].

Table 2.10 Components of the cell motility machinery phosphorylated by ERK or RSK (Source: [300]; *AbI* Abelson kinase interactor, *CKI* cyclin-dependent kinase inhibitor, *EPH* erythropoietin-producing hepatocyte receptor protein Tyr kinase, *Exo* exocyst subunit, *FAK* focal adhesion kinase, *MLCK* myosin light chain kinase, *SH3P* SH3 domain-containing protein, *SorbS3* sorbin and SH3 domain-containing protein-3 [vinexin], *vasp* vasodilator-stimulated phosphoprotein, *WAVe* WASP family verprolin homolog)

Substrate	Effect
ERK	
AbI1	Actin polymerization, cell protrusion
Calpain-2	Focal adhesion dynamics
Cortactin	Actin polymerization, cell protrusion
Exo70	Exocyst assembly, exocytosis
FAK	Focal adhesion dynamics
MLCK	Myosin-2 activation
Paxillin	Focal adhesion dynamics
Rac1	Actin polymerization, cell protrusion
RhoGAP31	Inactivation of Rac and CDC42
RhoGEF2	Rho activation, cell retraction
Sorbs3	Cell spreading (SorbS3α)
	Actin stress fiber formation (SorbS3β)
Wave2	Actin polymerization, cell protrusion
RSK	
CKI1b	Rho inhibition (cell retraction)
EPHa2	Phosphorylation of EPHa2 (cell motility)
Filamin-A	Filamentous actin crosslinking
Plastin-2	Filamentous actin bundling
SH3P2	Cytoplasmic anchor for myosin-1E
Vasp	Actin polymerization, cell protrusion

Cell protrusions are formed by the extension of lamellipodia and filopodia (i.e., sheet- and needle-like structures) at the leading edge of cells in response to motility stimuli

In addition, ERK phosphorylates (activates) MLCK, which phosphorylates myosin light chain and activates myosin-2 [300]. Its effector RSK phosphorylates (inactivates) SH3P2,[52] an inhibitor of the nanomotor myosin-1E,[53] disrupting

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The monomeric GTPases, CDC42, Rac, and Rho, regulate the activity and localization of effectors, activating actin nucleation factors and P21-activated kinase (PAK). The latter phosphorylates (activates) LIMK, which phosphorylates (inhibits) actin-severing cofilin, and (activates) filamin-A, a ^{F}actin crosslinker [300]. Guanine nucleotide-exchange factors (GEFs) activate small GTPases, whereas GTPase-activating proteins (GAPs) inactivate them. Extracellular-signal-regulated kinase (ERK) phosphorylates (inhibits) RhoGAP31, a GAP for Rac and CDC42, Furthermore, among the three Rac isozymes (Rac1–Rac3), ERK phosphorylates (inhibits) Rac1 in the cytosol, but launches Rac1 translocation to the nucleus, promoting cell proliferation rather than motion.

[52]SH3 domain-containing protein SH3P2 is also called SH3 domain-containing protein SH3D3 and mainly osteoclast-stimulating factor OSF1, an intracellular protein produced by osteoclasts that indirectly promotes osteoclast formation and bone resorption.

[53]Myosin-1E interacts with both cell membranes and actin filaments. Class-*I* myosins can serve as crosslinkers that connect and generate force between actin filaments and membranes, thereby regulating plasma membrane tension. The Myo1e SH3 domain acts as a recruiter of effectors involved in myosin-based and actin nucleation-based force generation at the plasma

SH3P2–Myo1e tethering and enabling Myo1e to move to the lamellipodium tip, where it facilitates cell motion [301].

Among the four isozymes (RSK1–RSK4), RSK1 impedes actin elongation, as it phosphorylates (inactivates) VASP [300]. On the other hand, VASP is also phosphorylated by PKA and then re-localizes to lamellipodia.

Ribosomal s6 kinase (RSK) phosphorylates filamin-A, a dimeric actin crosslinker, at the same site phosphorylated by PAK, and, among plastins (Pls1–Pls3), Pls2 that then form actin bundles.

Focal cell–matrix adhesions are mediated by heterodimeric transmembrane integrins, which interact with matrix components (e.g., collagen, fibronectin, and vitronectin) and cytosolic constituents, transducing bidirectional signals between the extra- and intracellular media (outside–in and inside–out signaling).

Focal adhesion kinase (FAK) and SRC family kinases (SFKs) participate in proteic complex formation at the cytoplasmic tail of integrins. Autophosphorylated FAK recruits various SFKs, which further phosphorylate FAK and process FAK-associated proteins such as the docker paxillin, thereby accelerating formation of signaling complexes at focal adhesion sites. Extracellular-signal-regulated kinase (ERK) phosphorylates both FAK and paxillin in focal adhesion sites, increasing FAK–Pax binding and eliciting cell adhesions and protrusions [300].

Sorbin and SH3 domain-containing protein Sorbs3 (or vinexin) are other regulators of cell spreading and growth factor signaling at focal adhesion sites [300]. Among several alternative forms (Sorbs3α–Sorbs3γ), Sorbs3β is phosphorylated by ERK at the leading edge of moving cells, decreasing cell spreading and motility.

Calpain-2 cleaves proteins involved in cell–matrix adhesion and cell protrusion, such as FAK, SFKs, cortactin, integrins, and paxillin [300]. ERK phosphorylates (activates) calpain-2. Calpain-2 can complex with ERK and FAK at focal adhesion sites. Calpain-2 also cleaves linkers of focal adhesions to the cytoskeleton, such as spectrin and talin, thereby eliciting the turnover of adhesion complexes.

In the retracting tail of migrating cells, RhoA, RhoB, and RhoC are implicated in the detachment of cell–matrix adhesions and myosin-2-mediated cell retraction. These small Rho GTPases activate rock that phosphorylates (activates) LIMK, which phosphorylates (inhibits) cofilin, precluding further actin polymerization. Rock also phosphorylates (inhibits) myosin phosphatase, supporting myosin-based contractile force.

Extracellular-signal-regulated kinase (ERK) phosphorylates (activates) RhoGEF2 in focal adhesions in response to stretch. On the other hand, ERK phosphorylates (activates) RhoGAP7 in focal adhesions, thereby enabling cycles of Rho deactivation–reactivation. Therefore, ERK functions as a spatiotemporal regulator of cell retraction. In addition, ERK phosphorylates RhoA, priming its ubiquitination and subsequent proteasomal degradation [300]. Furthermore, RSK phosphorylates cyclin-dependent kinase inhibitor CKI1b, which is then sequestered in the cytoplasm, where it binds to Rho, hampering its activity.

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membrane, such as dynamin, synaptojanin-1, and the WASL–WIPF1 (nWASP–WIP) complex at the membrane–cytoskeleton interface [301].

Polarized endo- and exocytosis during cell migration is defined by internalization of membrane proteins, such as integrins and RTKs, at the cell trailing edge to be recycled to the leading edge and displacement of secretory vesicles carrying newly synthesized proteins from the Golgi body to the leading edge. These processes are controlled by the small GTPases of the ARF, Rab, Ral, and Rho superfamilies. The octameric proteic complex exocyst tethers secretory vesicles to the plasma membrane. ERK phosphorylates the exocyst component Exo70, promoting its binding to other exocyst components and secretion of matrix metallopeptidases [300].

Transcription Factors

Phenotypic switching of vSMCs involves both activators of SMC dedifferentiation and repressors of SMC differentiation, such as ETS-like factor ELk1, once it is phosphorylated by ERK, E26 transformation-specific sequence ETS1, forkhead box-containing FoxO4, four-and-a-half LIM-only FHL2 hairy and enhancer of split-related HRT2, and Krüppel-like transcription factor KLF4, specificity protein SP1, and yin yang transcription factor YY1, which act in a coordinated manner [302].

Serum response factor and its cofactor promote differentiation of vSMCs. Myocardin (Myocd) is the most powerful serum response factor (SRF) cofactor from embryogenesis to adulthood, as myocardin stimulates SRF-initiated gene transcription. The SRF–Myocd couple activates smooth and cardiac muscle gene promoters. Both SRF and Myocd undergo post-translational modification (e.g., phosphorylation), which affects SRF–Myocd activity in SMC differentiation.

The SRF homodimer connects to the $CC(A/T)_6GG$ (CArG) DNA consensus sequence, which is found in promoters and introns of SMC-specific genes. Transforming growth factor-β provokes SRF expression and stimulates SMC differentiation.

Myocardin selectively induces transcription of most of the CArG-containing genes, especially SMC marker genes, such as those encoding smooth muscle α-actin (actα2) and myosin heavy chain (MyH11), transgelin, calponin,[54] and telokin, but not the CArG-containing early response gene FOS [302]. Myocardin mediates inducible expression of CArG-containing SMC marker genes in response to Agt2, TGFβ, PDGFbb, and the Ca_V1–RhoA axis.

On the other hand, the osteogenic Runt-related transcription factor Runx2 is a SRF corepressor, as it tethers to SRF and hinders SRF–Myocd complex formation.

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[54]Filamentous actin-associated proteins, such as caldesmon, calponin, smoothelin, and tropomyosin, regulate actomyosin interaction and thus smooth muscle contraction. Calponin binds to actin in a Ca^{2+}-independent manner in smooth myocytes, thereby lessening ^{Mg}ATPase activity and hence the actomyosin crossbridge cycling rate up to arresting actin filament movement. Calponin–actin interaction is suppressed, and inhibition by calponin of the actomyosin ^{Mg}ATPase is relieved upon phosphorylation of calponin by PKC or CamK2. Actin-linked caldesmon also inhibits actomyosin ^{Mg}ATPase and thus lowers actin sliding velocity; inhibition of actomyosin ^{Mg}ATPase is also reversed by caldesmon phosphorylation.

Olfactomedin-2 (Olfm2)[55] regulates TGFβ-induced SMC differentiation from mesenchymal stem cells, Runx2 being undetectable [307]. Olfm2 promotes the dissociation of SRF from HRT2, a transcriptional repressor for SMC genes.

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[55]The first glycoprotein of the OLFM (olfactomedin) family was discovered in the olfactory neuroepithelium of the bullfrog, Rana catesbeiana. This member is secreted into the nasal lumen, where it homopolymerizes at the lower mucus layer in contact with chemosensory dendritic cilia and was assumed to serve as a differentiation signal for dendritic knobs of chemosensory neurons, which are replaced throughout adult life from stem cells in the base of the olfactory neuroepithelium [303].

The family of olfactomedin domain-containing proteins consists of 13 known members in mammals. Most of these proteins are secreted glycoproteins, other family members being membrane-bound proteins that may serve as receptors [304]. They operate in neurogenesis, neural crest formation, dorsal ventral patterning, intercellular adhesion, cell cycle regulation, and tumorigenesis. They modulate Wnt and BMP signaling.

Olfactomedins possess the canonical olfactomedin sequence. They intervene in the early neurogenesis, functional organization of the nervous system, and synaptic stability, in addition to hematopoiesis. They also play a role in cell adhesion and differentiation. They are categorized into eight subsets in the animal kingdom, the subset II including the family of latrophilins, which are G-protein-coupled receptors with an extracellular olfactomedin domain near the N-terminus [303]. Human olfactomedins and olfactomedin-like proteins (OlfmL) are encoded by different genes (OLFM1–OLFM4, OLFML1, OLFML2A–OLFML2B, and OLFML3, GLDN [gliomedin, or colmedin], LPHN1–LPHN3 [latrophilins], and MYOC [myocilin]) [304].

The neuronal olfactomedin, Olfm1 (a.k.a. noelin-1 and pancortin), which abounds in the hippocampus and cortex, is also expressed in the embryonic heart. Olfm2 (or noelin-2) resides in the pancreas and prostate, Olfm3 (or optimedin) in the cerebellum, and Olfm4 (or tiarin) in the small intestine, colon, and prostate [303]. The secreted glycoprotein OlfmL1 is highly expressed in the heart, lung, small intestine, liver, and spleen (but not in the brain) [304]. OlfmL2a and Olfm2b (photomedin-1 and -2) form homodimers and oligomers, OlfmL2a being predominantly detected in the photoreceptor layer and OlfmL2b in the retina. OlfmL3 is preferentially formed in the placenta. Gliomedin is a type-II transmembrane protein, which can be cleaved by BMP1 and tolloid-like metallopeptidases. It is synthesized in the central and peripheral nervous systems. Latrophilins Lphn1 and Lphn3 (or lectomedin-1 to -3) are preferentially produced in the brain and retina and Lphn2 in other organs [304].

Myocilin belongs to a family of olfactomedin domain-containing proteins. It abounds in various types of muscles (e.g., the ocular ciliary muscle, papillary sphincter, skeletal muscles, and the heart). This OLFM member is also called trabecular meshwork-induced glucocorticoid response protein (TIGR), glaucoma-1A primary open angle (Glc1a, or GPOA), and juvenile-onset primary open-angle glaucoma (JOAG1), as gain-of-function MYOC gene mutations at the GLC1A locus on chromosomal locus 1q21-q31 engender autosomal dominant juvenile- and adult-onset primary open-angle glaucoma, a gradual blindness due to ocular neuropathies involving retinal ganglion cell death and the optic nerve layer. Myocilin and its two proteolytic fragments, N- and C-terminal fragments, are synthesized in the eye, that is, the retina, iris, optic nerve, in addition to the trabecular meshwork and ocular ciliary body, which regulate aqueous humor secretion in the anterior ocular chamber and hence intraocular pressure (IOP), abnormally high IOP levels being a strong risk factor in primary open angle glaucoma. This secreted glycoprotein regulates activation of different pathways in adjacent cells to control cell–matrix and cell–cell adhesion; cytoskeleton organization, which involves activation of Rac1 and JNK; and cell migration using the FAK1–PI3K axis. Myocilin induces stress fiber formation via the Wnt pathway; its action is repressed by secreted frizzled-related protein sFRP1 and sFRP3 (which are not highly produced in the sites of myocilin synthesis) and Wnt inhibitory factor WIF1 [305]. Myocilin is a regulator of muscle hypertrophy through the components of dystrophin-associated protein complex (DAPC). It interacts with α1-syntrophin, a DAPC component, and provokes phosphorylation of several regulators of muscle size [306]. It does indeed support the association of α1-syntrophin, NOS1,

On the other hand, in a different cellular context, Olfm2 also participates in mature vSMC phenotype switching, Runx2 being substantially present in mature SMCs. It promotes their proliferation and migration, hence favoring vascular wall remodeling [307]. Although TGFβ triggers formation of Olfm2 and SMC markers in mesenchymal progenitor cells, it does not significantly induce Olfm2 expression in mature rat aortic SMCs, whereas PDGFbb primes synthesis of Runx2 and Olfm2 in SMCs [307]. Olfm2 facilitates interaction of SRF with Runx2, thereby hampering SRF–Myocd interaction.

The gene promoters contain not only a distal CArG element but also a G/C-rich repressor element located proximal to the CArG element. The G/C element binds to specificity proteins SP1 and SP3, members of the KLF family, and early growth response transcription factor EGR1, which are normally absent in differentiated SMCs, but rapidly induced upon vascular injury [302]. For example, PDGFbb counters SRF attachment to the CArG–G/C–CArG motif within the MYH11 and TAGLN promoters of genes encoding smooth muscle myosin heavy chain and transgelin, respectively.

FHL2 interacts with SRF and binds to the promoter of SMC-specific CArG-containing genes in response to RhoA [302]. It precludes SRF-mediated transcription of SMC-specific genes, as it competes with MRTFa for SRF binding.

HRT2 prevents myocardin-dependent transactivation of the MYH11 and TAGLN gene promoters [302]. In addition, it tethers to SRF and hampers SRF binding to CArG box motifs.

Yin yang-YY1 represses SRF binding and Fos activity, hence skeletal and cardiac muscle α-actin formation. However, its overexpression can transactivate the TAGLN promoter in cultured rat aortic SMCs [302].

Krüppel-Like Factors

Krüppel-like factor KLF4 was identified as a binding factor to the G/C-rich TGFB promoter and also connects to the MYH11 and TAGLN promoters. It suppresses expression of myocardin and activation by myocardin of SMC marker genes; competes for binding of SRF to CArG regions, hence reducing SRF binding to CArG-containing SMC marker genes; and provokes hypoacetylation of histone H4 at CArG regions (transcriptional silencing) [302].

KLF4 along with platelet-derived growth factor (PDGFbb), notch, and TGF-β affects the vSMC differentiation status [298]. Moreover, contractile and synthetic vSMC phenotypes are controlled by numerous microRNAs. MicroRNAs are indeed major regulators in vSMC proliferation and migration.

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and α-dystroglycan with DAPC, thereby promoting binding of laminin to α-dystroglycan and PKB activity. It increases phosphorylation of the muscle size regulators PKB and FoxO3 and decreases expression of the muscle atrophy markers, muscle-specific RING finger protein MuRF1 and atrogin-1.

Among 20 members of the KLF family, anti-inflammatory KLF2, KLF4, proproliferative KLF5, and KLF6 play important roles in the vasculature. KLF1 is essential for erythrocyte maturation.

In the vascular wall, KLF2 is exclusively synthesized in ECs, its synthesis being inhibited by inflammatory IL-1β. It induces NOS3 expression; but impedes cytokine-primed formation of adhesion molecules (e.g., E-selectin and vcam1) [308].

KLF4 operates in endothelial inflammation, macrophage gene expression, and vSMC phenotype [309]. In ECs, KLF4, the production of which is induced by proinflammatory agents, induces expression of multiple anti-inflammatory and anti-thrombotic factors (e.g., NOS3 and thrombomodulin) [310].[56]

KLF4 prevents Jun expression and hence intimal hyperplasia. It can also block the PDGFbb–PDGFR–ERK axis and vSMC proliferation primed by PDGFbb. Furthermore, it induces the expression of P53 and subsequently that of CKI1a and represses that of ID3 and retinoic acid receptor RARα (NR1b1), which suppresses CKI1a synthesis. It also promotes the production of several cell cycle inhibitors (CKI1b, DM2, and RB) and represses that of cell cycle stimulators (cyclin-D1 and ornithine decarboxylase) [309].

On the other hand, KLF4 can be a potent transcriptional repressor of SMC differentiation markers (ACTA2, CNN1, TAGLN, and MYH11 gene encoding smooth muscle α-actin, calponin-1, smooth muscle myosin heavy chain, and transgelin, respectively), functioning as an effector of PDGFbb, which induces phosphorylation of ELk1 via the MAP2K1/2–ERK1/2 axis, re-localizing ELk1 to the nucleus, where it competes with myocardin, thereby blocking SRF–myocardin interaction [309]. In the context of gene repression, KLF4 recruits HDAC2, which deacetylates histone H4, hence causing chromatin compaction. It also downregulates the formation of paired-related homeobox-derived protein PrRx1 and MRTFs.

However, acetylated KLF4 can also promote synthesis of smooth muscle actin-α2, myosin heavy chain-11, and transgelin [309]. In the context of gene activation, KLF4 recruits KAT3b to gene promoters. KAT3b acetylates KLF4 in addition to histones. Moreover, KLF4 phosphorylation enhances its transactivation capacity, hence facilitating vSMC differentiation.

Therefore, KLF4 activity depends on post-translational modifications (acetylation, phosphorylation, and sumoylation) induced by various messengers and effectors (PDGFR, Tβ R, and RAR).

In addition, KLF4 supports the synergistic action of ECs and mural macrophages, which originate from circulating and then resident monocytes. It transactivates liver X receptor (NR1h2/3) and cholesterol 25-hydroxylase, which forms 25-hydroxycholesterol, thereby reducing inflammasome activity in ECs, promoting M1-to-M2 phenotypic transition in macrophages, and protecting against atherosclerosis susceptibility [311].

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[56]Its defective activity enables NFκB activation, which launches synthesis of inflammatory cytokines (e.g., TNFSF1 and IL1β), adhesion molecules (e.g., vcam1), and procoagulant factors (e.g., tissue factor).

Cyclosporine-A (CSa), a PP3 inhibitor, suppresses vSMC proliferation. In vSMC, it upregulates the expression of P53 and KLF4 [312]. KLF4 hinders vSMC proliferation, as it elicits CDKN1A gene transcription. It facilitates migration, as it also promotes Mmp3 gene transcription. In addition, it supports the formation of MMP9 and collagen-8. It represses transcription of MYOCD and vSMC differentiation marker genes (ACTA2, SMTN, and TAGLN). In addition, CSa acutely and transiently represses MyH11 production.

KLF5 activates the PDGFA genes, thereby favoring in-stent neointima formation in rabbits [302]. In response to vascular injury, KLF5 is induced in proliferating vSMCs; it initiates the production of EGR1, PDGFa, PDGFb, PAI1 (serpin-E1), NOS2, and VEGFRs [309]. KLF5 can reverse KLF4-induced suppression of SMC marker gene expression. It can also induce the expression of NMHCB and PDGFA genes, which encode nonmuscle myosin heavy chain NMHCb (myosin-10) and PDGFa genes in vSMCs.

Upon vascular injury, in ECs, KLF6 triggers synthesis of TGFβ and its receptors and urokinase plasminogen activator [309].

Growth Factors and Autacoids

Under normal conditions, vSMCs, endowed with their contractile apparatus, are maintained in a quiescent and contractile state. On the other hand, vSMCs can switch their phenotype from a quiescent to a synthetic state associated with formation of a new extracellular matrix and the capacity of proliferation and migration from the media to the intima.

The pathogenic phenotype can be countered by HSPGs, NO, prostaglandins, and TGFβ.

The vSMC phenotype change is triggered by various growth factors, cytokines, and other stimulators released upon vascular injury, such as epidermal (EGF), fibroblast (particularly FGF1 and FGF2), and IGF1, PDGFbb, vascular endothelial growth factor (VEGF), angiotensin-2, endothelin-1, thrombin, tumor-necrosis factor-α (TNFSF1), and IL-1 [313]. Among these factors, PDGFs and FGFs are the most potent for initiating the phenotypic switch and for controlling vSMC proliferation and migration [316].

Platelet-Derived Growth Factors

Three dimeric PDGF isoforms (PDGFaa, PDGFab, and PDGFbb) tether to two reeceptor protomers (PDGFRα–PDGFRβ). PDGFs stimulate proliferation and migration of SMCs in addition to fibroblasts and gliocytes via PDGFRβ. Among PDGFs, PDGFab and PDGFbb support vSMC migration, whereas PDGFaa inhibits migration induced by PDGFab and PDGFbb via heparin-binding fibronectin [314]. Cell adhesion to fibronectin (but not to collagen-4) suppresses vSMC migration. Vascular SMCs and platelets produce PDGFbb in response to acute vascular injury.

However, administration of an antibody that neutralizes activity of PDGFaa, PDGFbb, and PDGFab reduces proliferation and migration of medial SMCs in a rat carotid artery balloon injury model [302]. Whereas inhibition of PDGFRβ prevents SMC accumulation after vascular injury, that of PDGFRα does not impede SMC accumulation. Vascular SMC fate is thus mainly affected by PDGFbb, which signals via PDGFRβ, rather than PDGFRα.

Platelet-derived growth factor (PDGFbb) functions not only as a growth stimulator but also as a repressor of SMC protein production at both the transcriptional and post-transcriptional levels linked to degradation of SMC marker transcripts and proteins. In addition, PDGFbb cooperates with IL1β to repress vSMC differentiation via the PI3K–PKB–S6K pathway.

The principal plasma membrane-associated HSPGs include transmembrane syndecans and membrane-anchored glypican using a glycosylphosphatidylinositol chain. PDGFaa increases syndecan-1 production and causes HSPG accumulation on vSMCs more efficiently than does PDGFbb, whereas both PDGF isoforms decrease formation of perlecan, another HSPG. PDGFaa thus influences the interaction of SMCs with heparin-binding matrix proteins, thereby suppressing vSMC migration.

Fibroblast Growth Factors

The FGF family comprises 22 members that act via their receptors (FGFR1–FGFR4), triggering assembly of a ternary complex with the adaptors FRS2 and GRB2 and the GEF, SOS, which activates the Ras–MAPK signaling cascade.

Among FGFs, FGF12 expression is elevated in contractile vSMCs but down-regulated in synthetic vSMCs. It induces and maintains the quiescent vSMC phenotype [315]. Moreover, it prevents cell proliferation via P53. It upregulates synthesis of myocardin and serum response factor via P38MAPK. Synthesis of FGF12 is inhibited by PDGFbb [315].

Transforming Growth Factor

TGFβ mediates vSMC phenotype switch from the synthetic to the contractile state. It connects to and activates Tβ R2, which then associates with and activates Tβ R1, which phosphorylates SMAD2 and SMAD3, eventually priming production of contractile proteins in pericytes and smooth myocytes [316].

On the other hand, TGFβ which signals via SMAD3 stimulates rather than inhibits vSMC proliferation via phosphorylation and nuclear export of CKI1b [317]. The TGFβ–SMAD3 axis also launches the synthesis of fibronectin and collagen-1 in vSMCs.

The inhibitor of both vSMC proliferation and migration and the stimulator of vSMC proliferation according to the circumstances, TGFβ, signal via various pathways. It does indeed operate via ERK, JNK, and P38MAPK along with PI3K and most commonly SMADs (receptor-activated SMAD2 and SMAD3, common SMAD4, and inhibitory SMAD6 and SMAD7) [317]. Activated TGFβ receptor phosphorylates (activates) SMAD3, which then complexes with SMAD4. The

SMAD3–SMAD4 complex translocates to the nucleus, where it connects to SMAD-binding elements and regulates transcription of target genes. Inhibitory SMADs antagonize TGFβ signaling, as they either prevent activation of receptor-activated SMADs or prime Tβ R degradation.

The repressor of TGFβ signaling, SKi, precludes vSMC proliferation stimulated by TGFβ in addition to vSMC autophagy induced by oxLDLs and PDGF via hampering of PKB phosphorylation, thereby downregulating production of proliferating cell nuclear antigen (PCNA) [318]. Autophagy inducers PDGF and oxLDLs are also involved in vSMC phenotype transition and aberrant proliferation. Decayed production of SKi is thus implicated in intimal hyperplasia. MiR21 impedes SKi synthesis, thereby suppressing P38MAPK–CKI1a/CKI1b signaling and favoring vSMC proliferation [319].

Upon endothelium and internal elastic lamina injury (after angioplasty, near anastomoses of vascular grafts, and in ruptured atherosclerotic lesions), vSMCs can be exposed to blood flow and hence subjected to shear and stretch engendered by the hemodynamic stress field. They align in the streamwise direction. These mechanical stresses enhance NO production but impede vSMC migration and proliferation predominantly via TGFβ1 in an autocrine manner [320]. After exposure of vSMCs to flow, synthesis of TGFβ1 and tissue-type plasminogen activator, which converts plasminogen to plasmin, an activator of latent inactive TGFβ1 form, rises [321]. In addition, hemodynamic stress can prevent vSMC migration, as it lowers PDGFRβ and MMP2 formation [322]. Hemodynamic stress suppresses SMC migration, as it increases NO production and impedes MMP2 activity [323]. Vascular ECs and SMCs directly exposed to hemodynamic stress (shear) detected by mechanosensors (glycocalyx, GPCRs, adhesion molecules, and ion channels) modify their NO production rate.

Semaphorins

Semaphorin-3E is a regulator of cell migration and proliferation; its expression progressively decays during intimal hyperplasia. Sema3e prevents vSMC migration and proliferation via its receptor plexin-D1 and inactivation of the Rap1–PKB axis [324]. On the other hand, after vascular injury, the mutual PDGFbb–sema3e inhibition favors neointimal formation. On the one hand, PDGFbb downregulates sema3e formation in vSMCs. On the other, sema3e precludes synthesis of the PDGFb protomer in ECs via EGR1, PDGFb being the most potent mitogen for vSMCs. In addition, P53 induces sema3e expression, hence suppressing vSMC proliferation and intimal hyperplasia after vascular injury [324].

Heparin

Heparin is a GAG that prevents cell migration and proliferation, particularly vSMC proliferation via inhibition of PKCα, suppressing phosphorylation of myristoylated alanine-rich PKC substrate, and promotes the contractile phenotype [325]. Heparin impedes the transcription of several genes associated with cell cycle progression

(e.g., MyB, MyC, and Fos), the mitochondrial adenine nucleotide translocator (or ATP–ADP carrier) SLC25a5, and histone H3.

Prostaglandins

The balance between thromboxane and prostacyclin is altered in cardiovascular disease. Prostacyclin counters vSMC dedifferentiation using the PtgIR–Gs–AC–cAMP–PKA axis [326]. Prostacyclin is a vasodilator and a potent inhibitor of platelet activation and vSMC proliferation. This main product of prostaglandin-H synthase PGhS2 (or cyclooxygenase COx2) is derived from arachidonic acid in vascular endotheliocytes. It acts via its cognate PtgIR (IP) receptor. On the other hand, thromboxane TxA_2 is a potent activator of platelets and a vasoconstrictor. It is produced by PGhS1 (COx1) from arachidonic acid and released by activated platelets. Loss of prostacyclin and NO causes EC dysfunction and SMC dedifferentiation.

Lactate

Lactate concentration that increases upon ischemia can shift vSMC behavior to the synthetic phenotype [327]. Vascular smooth myocytes cultured in lactate-enriched medium have a synthetic phenotype and a larger proliferation and migration rate with respect to those grown in glucose-free and standard media. These changes are accompanied by an increased expression of monocarboxylic acid transporters. In pigs, concentrations of lactate, lactate dehydrogenase, and monocarboxylic acid transporter in addition to vSMC proliferation rate are greater in ischemic regions than in other sites.

Intracellular Regulators

Protein kinase-B is activated by hormones such as insulin, growth factors, and cytokines. It regulates cell metabolism, survival, differentiation, proliferation, and migration. Members of the PKB family (PKB1–PKB3) play distinct roles. Multiple factors can explain the distinct role of PKB isoforms, such as cell type, subcellular localization, expression level of the PKB subtypes in addition to their substrates and regulators, and interference with other signaling cascades. For example, PKB1 promotes and PKB2 inhibits migration of mouse embryonic fibroblasts using distinct facets of the Rac–PAK pathway [328].

In vECs, PKB1, the major isoform, is involved in angiogenesis and in inflammation. In vSMCs, the structurally similar (but not identical) PKB1 and PKB2, which are formed at similar concentrations, exert opposing effects on the cell phenotype, although they share many substrates. PKB1 promotes vSMC proliferation and migration and hence the dedifferentiated phenotype, PKB2, supports the differentiated phenotype [328]. In addition, PKB1 can play an antiapoptotic role. Moreover, PKB1 can inhibit PKB2.

Rapamycin, a TORC1 inhibitor stored in a drug-eluting stent, is aimed at avoiding intimal hyperplasia, as it prevents vSMC proliferation and migration. TORC1 represses insulin and IGF1 action and hence PI3K signaling, as it phosphorylates (inhibits) insulin receptor substrate IRS1. Its inhibition thus activates PKB. The latter phosphorylates (inactivates) transcription factors of the FOXO family (FoxO1, FoxO3a, and FoxO4), leading to their nuclear exclusion and cytosolic sequestration by 14-3-3 proteins. Rapamycin activates the PKB2 isoform.

Rapamycin launches vSMC differentiation via myocardin and PKB2 and subsequent nuclear exclusion of forkhead box-derived transcription factor FoxO4 (but neither FoxO1 nor FoxO3), thereby preventing its binding to the MYOCD promoter and subsequent MYOCD gene transcription [328]. FoxO4 interacts with both myocardin and SRF and thus hinders myocardin action. In addition, FoxO4 supports MMP9 formation, thereby assisting vSMC migration. On the other hand, myocardin promotes synthesis of smooth muscle contractile proteins. In addition, the transcription factor GATA6 is another PKB2 substrate that promotes SMC differentiation [328].

MicroRNAs

Let7g, miR21, miR29, miR126, miR132, miR145, miR146a, miR155, miR221, and miR222 are implicated in vSMC dysfunction associated with aberrant vSMC proliferation and migration and adverse intimal remodeling in atherosclerosis and restenosis. Vascular injury downregulates formation of miR143 and miR145, which cause vSMC differentiation and repress vSMC proliferation, but upregulates miR21, miR146a, and miR222, eliciting synthetic vSMC phenotype. Other important miRs include antiproliferative miR1, miR133, and miR195 and proproliferative miR21, miR221, miR222, and miR146a.

Let7g controls autophagy and apoptosis of vSMCs exposed to oxidized LDLs [329]. Oxidized LDLs (10–40 μg/ml) elicit autophagy via beclin-1 (Atg6), MAP1LC3 (Atg8), and Atg5 in addition to apoptosis via BCL2 and BCL2L1 on the one hand and BAX and caspase-3 on the other. Let7g impedes autophagy, as lipoxygenase LOx1 expression and intracellular ROS generation decline. In airway SMCs, Let7g also hampers synthesis of LOx1 and Oct1.

Myocardin is a cardiac and smooth myocyte-specific transcription cofactor that promotes their differentiation, but impedes proliferation, partly via HDAC4 and HAND2. Myocardin induces expression of miR1 in CMCs and in SMCs [330]. Transcription of the MIR1 gene depends on SRF in the myocardium and transcription factors of the MyoD family in skeletal muscles. Spindle-shaped SMCs possess higher amounts of both myocardin and miR1 than epithelioid SMCs. MicroRNA-1 targets PIM1 and prevents vSMC proliferation.

MiR21 favors vSMC proliferation and intimal hyperplasia after vascular injury via downregulation of the expression of proapoptotic proteins BCL2 and PTen [298]. It also prevents SKi action, hence priming vSMC proliferation and migration induced by oxLDLs [319]. It also contributes to TGFβ-regulated endothelial-to-mesenchymal transition. Differentiation of pulmonary SMCs in-

duced by BMPs is mediated by miR21, partly via suppression of programmed cell death protein PdCD4 [331]. On the other hand, differentiation of aortic SMCs can reduce miR21 expression.

Like miR21, miR31 regulates aberrant vSMC proliferation [332]. In proliferative vSMCs, proproliferative miR31 expression is upregulated by ERK. It then targets large tumor suppressor homolog LATS2.

Formation of miR24 and miR29a is primed by myocardin, the former being implicated in atherosclerosis retardation and the latter in matrix remodeling and fibrosis [298].

MiR26a hampers vSMC differentiation, thereby favoring their proliferation and migration via the TGFβ pathway, as it hinders production of SMAD1 and SMAD4 [298].

MiR29 expression is lost in calcified vSMCs in vitro and in calcified rat arteries in vivo. MiR29 targets ADAMTS7, which represses vSMC calcification [298].

MicroRNAs contribute to sexual differentiation. Female-specific miR29b targets DNA methyltransferases DNMT3a and DNMT3b [333]. Oxidized LDL raises aortic SMC migration, as it upregulates synthesis of MMP2 and MMP9 via diminished DNA methylation by DNMT3b [334]. Upon oxLDL exposure, miR29b targets DNMT3b and promotes migration of arterial SMCs.

Apoptosis of vSMCs favored by redox stress, partly via JNK, intervenes in vascular remodeling and atherosclerotic plaque instability. JNK is a substrate of miR92a in macrophages. In quiescent vSMCs, miR92a is produced at a low level. Its synthesis is hampered by H_2O_2 [335]. Conversely, miR92a represses H_2O_2-primed vSMC apoptosis, as it inhibits MAP2K4–JNK1 signaling.

MicroRNA-124 expression is downregulated in proliferative vascular diseases. MiR124 targets specificity protein SP1 and attenuates PDGFbb-induced vSMC proliferation and differentiation [336].

Histone H3 Lys9 methyltransferase KMT1a and associated repressive epigenetic mark $H_3K_9me^3$ prevents sustained inflammatory gene expression (heterochromatic silencing and euchromatic transcriptional repression). MiR125b targets KMT1a, thereby favoring production of inflammatory cytokines and chemokines, such as IL6 and CCL2 [337]. MiR125b promotes the proinflammatory response in diabetic vSMCs that possess a preactivated phenotype with augmented inflammatory gene expression. Hence, miR125b favors diabetic vSMC phenotype. Expression of miR125b and MIR200 family members is upregulated in vSMCs of T2DM db/db mice with respect to control db/+ mice.

MicroRNA-126, one of the most abundant endothelial miRs, the transcription of which is regulated by ETS1 (a substrate of miR155, miR221, and miR222), ETS2, and flow-dependent KLF2, attenuates the rate of EC proliferation and supports VEGF-primed apoptosis decay and migration [331]. Thus, endothelial miR126 modulates angiogenesis. Activated and apoptotic ECs release miR126 in microvesicles and transfer it to adjoining cells, regulating SMC turnover. Argonaute-2, a component of the miR-induced silencing complex, is transmitted with miR126 via extracellular vesicles (EVs) and facilitates miR126 transfer from ECs to SMCs. Endothelial miR126 represses transcripts encoding FoxO3, BCL2, and IRS1 [338].

Angiotensin-2 induces miR132–miR212 cluster synthesis in vSMCs and increases inflammatory gene transcription [339]. MiR132 targets the PTEN transcript. The miR132–miR212 cluster mediates Agt2-induced CCL2 production in vSMCs.

MiR133 is synthesized in vSMCs, whereas miR1 concentration is negligible. It represses vSMC phenotype switch. The miR133a1–miR1-2, miR133a2–miR1-1, and miR206–miR133b clusters are bicistronic, the latter being produced in skeletal muscles, but not in the heart. MiR133 production is regulated by ERK1 and ERK2 [340]. MiR133 suppresses formation of the transcription factor SP1, which induces synthesis of cyclin-dependent kinase inhibitor CKI1b and subsequently cell cycle arrest.

In diabetes, miR138 also regulates vSMC proliferation and migration [286].

Cardiovascular-specific miR143 and miR145 encoded by a bicistronic gene cluster abound in vSMCs. Transcription of the miR143–miR145 cluster is primed by SRF and its coactivators, myocardin and myocardin-related transcription factors. They are atheroprotective and prevent intimal hyperplasia induced by vascular injury, which launches vSMC dedifferentiation and cytoskeletal remodeling. MiR143 and miR145 are also transferred from ECs to vSMCs by extracellular vesicles; KLF2 increases their amount in ECs and triggers their release via microvesicles [331]. They play a crucial role in cooperation with other miRNAs, such as miR1 and miR24, in the differentiation and proliferation of vSMCs. MiR1, the formation of which is triggered by myocardin in vSMCs, precludes cell proliferation but inhibits myocardin-induced vSMC contractility. The Jag1–notch axis can also induce transcription of the miR143–miR145 cluster. MiR143 and miR145 repress Krüppel-like factors KLF4 and KLF5 that inhibit myocardin, thereby supporting vSMC differentiation and repressing their proliferation. TGFβ and BMP4 are potent inducers of contractile vSMC phenotype; they prevent vSMC proliferation and migration. KLF4 hampers activation of the CArG box by SRF and its coactivators. TGFβ and BMP4 downregulate KLF4 synthesis via miR143 and miR145 [341]. In addition, miR143 also reduces the production of ETS-like factor ELk1, hence promoting synthetic phenotype. Furthermore, miR145 downregulates ACE. Angiotensin-2 is a vasoconstrictor that also exerts a proinflammatory effect via IL6 and CCL2 and elicits vSMC proliferation and hypertrophy, mostly via AT_1, the MAPK module, NFκB, and CREB [339]. Conversely, miR143 and miR145 are repressed by ACE, KLF4, and KLF5, which then favor neointima formation [298]. In addition, miR145 targets slit-robo GTPase-activating protein SRGAP1 (RhoGAP13) and SRGAP2 (RhoGAP34), adducin-3, slingshot-2 phosphatase, and calmodulin kinase CamK2δ, which are involved in the regulation of SRF activity and actin dynamics [331]. Genetic deficiency in miR143 and miR145 in mice shifts the vSMC phenotype from a contractile to a synthetic state.

MiR146a targets Krüppel-like factor KLF4, thereby promoting vSMC proliferation and intimal hyperplasia [342]. KLF4 competes with KLF5 for MIR146A promoter binding; KLF4 and KLF5 exert opposing effects on the MIR146A promoter, KLF4 impeding MIR146A gene transcription. MiR146a and KLF4 form a feedback loop to regulate each other's expression and vSMC proliferation.

MiR155 supports vSMC proliferation [343]. STK3 (MST2), a component of MAPK modules and the hippo pathway,[57] mediates miR155-primed inflammation and redox stress, which lead to vSMC proliferation and vascular remodeling. Inflammation and redox stress, which occur after vascular injury, are coordinated by the ERK1/2 pathway. STK3 interacts with cRaf, thereby competing with MAP2K and regulating ERK1/2 signaling. Downregulation of STK3 expression by proproliferative miR155 activates ERK1/2 signaling, as it facilitates the interaction between cRaf and MAP2K, and then boosts vSMC proliferation. In addition, miR155 supports migration of smooth myocyte progenitors via CCL2 [286].

MiR155, which is synthesized and secreted upon KLF5 stimulation in vSMCs, is a potent regulator of the endothelial barrier, as it regulates production of endothelial tight junction proteins [344]. Exosomes originating from vSMCs transfer miR155 from SMCs to ECs, which then alter tight junction renewal and hence the integrity of the endothelial barrier, increasing endothelial permeability. Moreover, miR155 in ECs impedes their proliferation and migration and hence re-endothelialization.

MiR182 expression is markedly downregulated during vSMC dedifferentiation [345]. MiR182 increases synthesis of SMC-specific contractile proteins and prevents SMC proliferation and migration in basal conditions and upon PDGFbb exposure. It targets the Fgf9 transcript. FGF9 is a potent mitogen secreted from bone marrow-derived cells that drives neovascular maturation via PDGFRβ, which resides particularly on vSMCs and pericytes, the production of which is upregulated by sonic hedgehog [346].

MiR182-3p counters vSMC proliferation and migration via ERK [347]. This microRNA targets myeloid-associated differentiation marker (MyADM), hence repressing intimal hyperplasia. The NOS inhibitor, asymmetrical dimethylarginine (ADMA), downregulates miR182-3p production, thereby supporting intimal hyperplasia.

The subunit of the AP1 transcription factor complex, JunB, promotes blood vessel development and maintenance but precludes cell proliferation and inflammation. Under hypoxia, JunB regulates angiogenesis, as it upregulates synthesis of VEGFa, core-binding factor CBFβ, which with Runx forms the heterodimeric core-binding transcription complex CBF, and MMP13, a CBF target. Components of the AP1 complex regulate expression of various microRNAs (miR21, miR101, miR144, miR155, and miR203) [348]. MiR182 is a JunB target implicated in lymphangiogenesis, at least in zebrafish [348]. MiR182 attenuates FoxO1 formation to avoid its overproduction, hence driving proper lymphangiogenesis.

MicroRNA-195 abounds in vSMCs [349]. It impedes vSMC proliferation, migration, and synthesis of IL1β and IL6 and the CXCL8 chemokine. It represses translation from Cdc42, CCND1, and Fgf1 transcripts.

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[57]The mammalian hippo pathway is composed of core kinases (STK3/4 and LaTS1/2), adaptors (WW45 for STK3/4 and MOBKLs for LaTS1/2), effectors (YAP1/2 and WWTR1 [or Taz]), and transcription factors (TEAD1–TEAD4). The STK3 and STK4 kinases phosphorylate (activate) the LaTS1 and LaTS2 kinases, which then phosphorylate YAP (Ser127), thereby yielding a docking site for 14-3-3 that sequesters YAP in the cytoplasm. Otherwise, YAP translocates into the nucleus, associates with TEADs, and promotes transcription of genes involved in cell proliferation and survival.

MiR200 downregulates expression of the zinc finger E-box-binding homeobox-containing transcription factor ZEB1, hence raising production of proinflammatory PGhS2 (COx2) and CCL2 [298].

Estrogens such as estradiol (E_2) protect vascular walls via miR203 production, which is upregulated in vSMCs via NR3a1 (ERα) [350]. The transcription factors ZEB1 and AP1 mediate E_2-induced upregulation of miR203 transcription. MiR203 represses Abl1 and P63 production and vSMC (but not vEC) proliferation.

MiR221 and miR222 are expressed by a gene cluster of the X chromosome in vSMCs and ECs [331]. They favor neointima growth. Therefore, downregulation of miR143 and miR145 expression and upregulation of miR221 and miR222 after vascular injury support intimal hyperplasia. Proliferation of vSMCs results from the elimination of cyclin-dependent kinase inhibitors CKI1b and CKI1c by miR221 and miR222. On the other hand, they impede EC proliferation. Whereas miR221 and miR222 lower the vSMC apoptosis rate and support the latter's migration, they hinder EC migration and favor apoptosis. Moreover, miR221 and miR222 prevent angiotensin-2-induced inflammation, as they suppress the formation of VEGFR1, vcam1, and CCL2, thereby reducing leukocyte recruitment and adhesion [331]. In addition, miR221 suppresses the synthesis of SCFR in SMCs, which initiates myocardin production.

Angiotensin-2 regulates the long nonprotein-coding RNA lncAng362, which is responsible for the production of two microRNAs implicated in vSMC proliferation, miR221 and miR222 [351]. In fact, angiotensin-2 controls the production of many lncRNAs, downregulating that of lncAng219 and lncAng249 and upregulating that of lncAng112, lncAng162, and lncAng362. Production of lncAng362, miR221, and miR222 is upregulated once vSMCs are exposed to Agt2.

Hyperglycemia contributes to abnormal vSMC activation. Hyperglycemia stimulates vSMC proliferation and migration via ERK and ROS. In addition, hyperglycemia hinders vSMC apoptosis, as it upregulates the expression of antiapoptotic proteins BCL2 and BCL2L1. Furthermore, hyperglycemia inactivates AMPK, which can improve endothelial function and suppress vSMC proliferation and migration. On the other hand, family with sequence similarity protein Fam3b (its secretory form named pancreatic-derived factor and abbreviated pander),[58] which is secreted from the endocrine pancreas, can provoke apoptosis of α and β cells. It is cosecreted with insulin from β cells by hyperglycemia [352]. It boosts vSMC proliferation and migration and precludes miR322-5p expression. Conversely, miR322-5p impedes Fam3b-primed vSMC proliferation and migration.

MiR424 (ortholog of rat miR322) concentration decays shortly after induction of vSMC proliferation and then rises. It precludes vSMC proliferation and migration without affecting apoptosis [353]. It targets cyclin-D1 and Ca^{2+}-regulating proteins

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[58]The FAM3B family comprises four members (Fam3a–Fam3d). Fam3b is synthesized in β cells of pancreatic islets and stored in insulin+ granules. Upon glucose stimulation, production of full-length Fam3b and secretion of cleaved Fam3b rise via PKA, PKC, ERK1, ERK2, and CREB, along with PI3K and ROS [352]. Fam3b maintains β cell function and induces hepatic gluconeogenic gene expression and glucose output during starvation.

calumenin (directly) and stromal-interacting molecule STIM1 (indirectly), lowering store-operated Ca^{2+} entry. It is upregulated after vascular injury, thereby protecting against restenosis.

Cyclin-D1 transcript is a substrate of antiproliferative miR365 [354]. It lowers PDGFbb-primed vSMC proliferation, as it blocks G1–S transition. On the other hand, vSMC proliferation triggered by PDGFbb and angiotensin-2 downregulates miR365 expression.

Oxidized LDLs induce vSMC proliferation, downregulate the synthesis of miR490-3p and IGF1, but upregulate that of pappalysin-1 (or PAPPa)[59] and IGF2 in vSMCs [355]. Overproduction of PAPPa and the resulting activation of proinflammatory IGF in vSMCs may promote their proliferation, but IGF1 may have anti-inflammatory and antioxidant effects. MiR490-3p targets PAPPA, countering the effect of oxLDLs and suppressing the PAPPa metallopeptidase effect and hence IGFBP4 cleavage.

In diabetes mellitus, vascular smooth myocytes acquire a proatherogenic and proinflammatory phenotype. Diabetes mellitus is linked to elevated concentrations of glucose, advanced glycation end products, growth factors, and oxidized lipids that favor vSMC dysfunction. In vSMCs of T2DM mice, 135miRs are differentially expressed, among which several regulate vSMC function. MiR504, the production of which is upregulated, targets the adaptor GRB10 and transcription factor EGR2 [356]. It provokes a vSMC phenotype switch, as it downregulates expression of contractile genes and upregulates that of ERK1 and ERK2, thereby promoting vSMC proliferation and migration. High concentrations of glucose and palmitic acid increase miR504 formation and the expression of inflammatory genes.

The PDGFbb stimulant elicits vSMC proliferation and suppresses miR599 synthesis [357]. Conversely, miR599 represses vSMC proliferation and migration in addition to the synthesis of PCNA, an auxiliary protein of DNA polymerase-δ involved in the control of DNA replication, and of cellular proliferation marker protein MKi67, an antigen identified by monoclonal antibody Ki67, which is encoded by the MKI67 gene and linked to the perichromosomal layer during mitosis, acting as a surfactant [358]. In addition, miR599 suppresses production of collagen-1 and -5 and proteoglycan. In vSMCs, the TGFB2 transcript is a substrate of miR599 [357]. TGFβ2 can reverse miR599-primed prevention of vSMC proliferation along with collagen and proteoglycan synthesis. MiR590-3p increases vSMC contractility and represents a risk factor for hypertension in the presence of genetic defects [286].

miR638 is one of the most significantly downregulated in human aortic vSMCs during their proliferation and migration launched by PDGFbb [359]. Conversely, miR638 hinders vSMC proliferation, as it targets the nuclear receptor NR4a3 (NOR1), subsequently impeding cyclin-D1 production.

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[59]PAPPa: pregnancy-associated plasma protein-A. It is also called IGF-dependent IGFBP4 peptidase (IGFBP4ase). Thus, it activates IGF, relieving its inhibition by IGFBP4 upon IGFBP4 cleavage. It was originally identified in the placenta and abounds in vSMCs.

2.2.1.3   Platelet MicroRNAs

Platelets, which contain mRNA and can thus synthesize new proteins (e.g., integrin-α_3, or glycoprotein-3A, and fibrinogen receptor), are a major source of miRs, ^{Y}RNAs, circular RNAs, and lncRNAs.

Newly formed platelets are larger, more reticulated, and more reactive than older platelets; they also contain a higher amount of RNA and can thus produce greater amounts of proteins, especially α granule-stored proteins. On the other hand, platelets may inherit their miR content during shedding from megakaryocytes.

Platelets release microvesicles and exosomes, especially during activation. Inappropriate activation of platelets initiates thrombosis. MicroRNAs can thus be used as markers of platelet activation [360].

Whereas the platelet miR expression pattern remains stable over the lifespan of the platelet in healthy individuals, it is markedly altered in diabetes, after myocardial infarction, and in cancer (Table 2.11) [360]. MiR release from platelets may result from shedding of microvesicles and synthesis of mature miRs from precursor miRs. On the other hand, activation of calpain that targets dicer lowers the concentrations of several platelet miRs.

2.2.1.4   Hepatic MicroRNAs Operating in Metabolism

Because the liver is a major organ in the regulation of lipid, cholesterol, and glucose metabolism,[60] hepatic microRNAs, such as miR24,[61] miR29,[62]

Table 2.11   Platelet microRNA] (Source: [360])

MicroRNA	Target (effect)
MiR96	Vamp8 mRNA (vesicle-associated membrane protein), VAMP8 being involved in platelet degranulation (reduced platelet reactivity)
MiR126	Adam9 mRNA
	Adamlysin-9 attenuates platelet–collagen adhesion (augmented platelet reactivity)
MiR223	P2RY12 mRNA
	Gi2-coupled P2Y$_{12}$ receptor activated by ADP primes platelet aggregation and granule secretion (reduced platelet reactivity)
MiR376c, miR599	Pctp mRNA (phosphatidylcholine transfer protein), (lowered PAR$_4$ [thrombin receptor] reactivity)

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[60]For example, certain microRNAs directly target the transporter ABCa1, which enables the efflux of cholesterol from cells to ApoA1 and nascent HDL, such as miR10b, miR17, miR19b, miR26, miR27a, miR27b, miR33a, miR33b, miR33a*, miR33b*, miR93, miR101, miR106b, miR128, miR144, miR145, miR148a, miR302a, and miR758, whereas miR223 regulates ABCa1 indirectly, hence suppressing HDL synthesis [361].

[61]MiR24 targets insulin-induced gene InsIG1.

[62]In the liver, miR29 impedes lipoprotein lipase synthesis.

miR30c,[63] miR33,[64] miR33*,[65] miR122,[66] miR144,[67] miR148a,[68] and miR223,[69] packaged in microvesicles and proteic complexes,[70] affect hepatic metabolism and hence are linked to metabolic syndrome, non-alcoholic fatty liver disease, T2DM, and atherosclerosis; they can thus serve as markers [361].

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[63] In the liver, miR30c represses microsomal triglyceride transfer protein, which is needed for lipoprotein assembly. Hepatic miR30c reduces lipid synthesis and lipoprotein secretion [361].

[64] MiR33 targets ABCa1 and thus suppresses HDL synthesis. MiR33a and miR33b are encoded in introns of the genes coding for the basic helix–loop–helix and leucine zipper (bHLHLZ) transcription factors sterol regulatory element-binding proteins SREBP2 (bHLHd2) and SREBP1 (bHLHd1), respectively. More precisely, the mir33b gene resides in intron 17 of the Srebp1 gene on chromosome 17 and miR33a in intron 16 of the Srebp2 gene on chromosome 22 [362]. Both miR33a and miR33b are cotranscribed with their host genes and regulate cholesterol and fatty acid metabolism. MiR33a and miR33b target enzymes of fatty acid oxidation, such as carnitine ^{O}octaniltransferase and palmitoyltransferase-1A (CPT1a), hydroxyacyl-CoA dehydrogenase HADHb, sirtuin-6, and AMPKα. MiR33a and miR33b also target insulin receptor substrate IRS2 of the insulin pathway in the liver. Therefore, miR33a and miR33b reduce fatty acid oxidation in addition to insulin signaling. The Srebp1 transcript engenders two major spliced variants (SREBP1a and SREBP1c). Whereas SREBP2 regulates cholesterol synthesis and metabolism (e.g., via HMGCR and transporters LDLR, ABCa1, ABCg1, and endolysosomal NPC1), SREBP1a mostly transactivates both lipogenic and cholesterogenic genes, and SREBP1c controls fatty acid synthesis (e.g., via fatty acid synthase) in the liver and adipose tissue [363]. In addition, miR33 attenuates hepatic secretion of VLDL^{TG}, as it targets ^{N}ethylmaleimide-sensitive factor, a component of exocytosis. Expression of miR33 is upregulated, and those of the products of the mitochondrial regulatory genes PGC1α, SLC25a25, NRF1, and ^{Mt}TFa are downregulated in atherosclerotic plaques [361]. Prevention of miR33 action enhances cellular cholesterol efflux and HDL genesis via ABCa1, thereby attenuating progression and even favoring regression of atherosclerosis in mice [364].

[65] MicroRNAs are generated from primary transcripts processed into precursors and then a duplex of miR strands (5p and 3p strand). Most often, only the guide or mature strand is stable and active, but, in some cases, the passenger or star strand can regulate distinct targets. In addition to the guide strand miR33-5p, the passenger strand miR33*, or miR33-3p, is involved in the regulation of cholesterol, lipid, and glucose metabolism, as its substrates contribute to cholesterol efflux (e.g., ABCA1), fatty acid metabolism, and insulin signaling [361].

[66] MiR122 is the predominant miR in the liver (>70% total hepatic miRs) [361]. It indirectly regulates transcripts linked to cholesterol and fatty acid metabolism in addition to AMPK and glucose 6-phosphatase and direct activators of hepcidin expression.

[67] MiR144 regulates cholesterol metabolism and transport in the liver and macrophages. In the liver, the bile acid receptor FXR upregulates miR144, which eliminates the ABCA1 transcript. In macrophages, activated LXR upregulates miR144 and downregulates ABCa1 [361].

[68] MiR148a-3p is a major regulator of lipoprotein metabolism in hepatocytes and macrophages. Hepatic miR148a-3p hinders LDLR and ABCa1 synthesis, thereby increasing LDL^{CS} level and decreasing HDL^{CS} level in the blood [361].

[69] In hepatocytes, miR223, the formation of which is controlled by cholesterol concentration (low cholesterol levels suppressing miR223 production), reduces cholesterol synthesis, as it represses cytoplasmic hydroxymethylglutaryl-CoA synthase HMGCS1 and sterol C4-methyloxidase-like protein (SC4MOL or methylsterol mono-oxygenase), and precludes HDL^{CS} uptake, as it targets ScaB1 [361]. On the other hand, miR223 promotes cholesterol efflux, as it degrades SP3 that antagonizes SP1-mediated activation of ABCa1 synthesis [365].

[70] Activated circulating cells, in particular platelets, release miRs (e.g., miR21, miR24, miR126, miR191, and miR223). On the other hand, the liver constantly secretes miR122 [361].

Apolipoprotein-B is an essential scaffold in VLDL assembly. MiR548p interacts with the $3'$-untranslated region of the APOB mRNA and promotes its post-transcriptional degradation, thereby reducing ApoB synthesis and secretion from hepatocytes. Furthermore, miR548p significantly decreases lipoprotein production in addition to lipid synthesis in hepatocytes, as it lowers the activity of 3-hydroxy 3-methylglutarylCoA reductase and long-chain acylCoA synthetase ACSL4[71] involved in cholesterol and fatty acid synthesis, but does not affect fatty acid oxidation [366].

2.2.1.5 MicroRNAs and Mitochondrial Function and Dysfunction

MicroRNAs participate in the regulation of mitochondrial metabolism and ATP production via proteins implicated in substrate transport, glycolysis, fatty acid β-oxidation, ketone catabolism, and coupled processing by the tricarboxylic acid cycle (TCAC) and electron transport chain (ETC).

The metabolism evolves from fatty acid oxidation to glycolysis during hypoxia, especially in the context of hypertension-induced HF, as expression of fatty acid oxidation enzymes is downregulated and that of glycolytic enzymes is upregulated [367].

Mitochondria import various species of ncRNAs, such as tRNAs; 5S rRNA, which is the most abundant mitochondrial RNA type; miRs; and lncRNAs [368]. Polynucleotide phosphorylase of the mitochondrial intermembrane space that processes mitochondrial transcripts promotes the import of ncRNAs through the inner mitochondrial membrane into the mitochondrial matrix.

The term *mitomicroRNAs* refers to microRNAs located inside the mitochondrion, *category 1* [Mt]miRs being encoded by the mitochondrial genome and *category 2* [Mt]miRs being transcribed in the nucleus and imported to the mitochondrion (Table 2.12). For example, preLet7b, pre-miR302a, and their corresponding

Table 2.12 MicroRNAs and mitochondrial function (Source: [368]; *CcOx4* cytochrome-C oxidase subunit-4, *CcOx10* cytochrome-C oxidase protoheme-9 farnesyltransferase, *ISCu* iron–sulfur cluster assembly scaffold)

Type	Location	Targets	Effect
MiR1	Cardiac and skeletal myocytes	IGF1	Regulation of mitochondrial protein synthesis, metabolism and apoptosis
MiR23a/b	Ubiquitous	Glutaminase	Tumor suppression
MiR30 group	Heart	P53	Inhibition of mitochondrial fission and apoptosis
MiR210	Ubiquitous	CCOX10, ISCU	Cell adaptation and survival under hypoxia
MiR338	Brain	CCOX4	Regulation of axonal energy metabolism
MiR378	Heart	Caspase-3	Inhibition of apoptosis

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[71] Also known as long-chain fatty acid–CoA ligase LACS4.

mature miRs lodge in the mitochondrion. Most category-2 ^{Mt}miRs originate from loci pertaining to mitochondrial gene clusters or close to mitochondrial genes. MitomicroRNAs may interact with transcripts encoded in the mitochondrion.

Certain mitomicroRNAs can act as translational activators, as they promote translation of mitochondrial transcripts using a specific miR–mRNA base pairing [368]. Under certain conditions, once it is recruited to the 5′-end of target mRNAs and/or internal ribosome entry sites, miR1 can activate translation in the cytosol. MiR1 recruits the ND1 and COX1 mRNAs to mitochondrial ribosomes.

In addition to ^{Mt}miRs, several cytosolic microRNAs modulate mitochondrial function. Brain-specific miR338 lowers the formation of the cytochrome-C oxidase subunit CcOx4 encoded in the nucleus [368]. MiR210, which is induced by HIF1α under hypoxia, can repress oxidative phosphorylation, as it targets transcripts encoding cytochrome-C oxidase assembly protein CcOx10 and iron–sulfur cluster scaffold (ISCu).

Several miRs participate in intrinsic apoptosis. In cardiac and skeletal myocytes, miR1, the formation of which is induced by hyperglycemia, diminishes mitochondrial membrane potential and provokes cytochrome-C release via IGF1 [368]. In the heart, miR378 downregulates caspase-3 expression and hence impedes apoptosis.

Certain miRs such as those of the MIR30 family, which abound in the heart, modulate mitochondrial dynamics. They regulate mitochondrial fission and fusion via the P53–DRP1 axis, as they decrease amounts of P53 and subsequently of DRP1, hence favoring fusion to support energy demand and raising the threshold of apoptotic activation in CMCs [368].

In HF, impaired fatty acid β-oxidation increases circulating free fatty acid (FFA) concentration, which is detected by the liver that uses FFAs as substrates for ketogenesis. Ketones enter the bloodstream, from which they are taken up by CMCs, the rate of oxidation matching the rate of delivery. Cardiac ketolysis carried out by 3-hydroxybutyrate dehydrogenase BDH1, succinylCoA:3-oxoacidCoA transferase (SCOT), and acetoacetyl-CoA thiolase produces acetyl-CoA [367].

In CMCs, some microRNAs (e.g., miR181c and miR378) translocate to mitochondria and affect expression of the mitochondrial transcripts (Table 2.13) [367].

Hypoxia and redox stress affect expression of microRNAs and hence mRNAs. Furthermore, these stressors contribute to oxidation of metabolic enzymes, altering their activity.

Formation of both NOx2 and NOx4 can be induced by HIF1α during hypoxia. In HF, upregulated expression of NOx2 can partly result from downregulated miR17. In addition, the baseline constitutive expression of NOx4 is augmented by hypercholesterolemia that downregulates miR25 production. On the other hand, increased miR25 expression in HF inhibits serca2a production. In HF, downregulated synthesis of serca2 and RyR2 is associated with an altered expression of CK [369].

In most aging-related diseases, mitochondrial ROS are produced by the ETC, which requires NADH and is hence countered by enzymes that need NADPH as cofactor. The nicotinamide nucleotide transhydrogenase (NNT) is an antioxidative enzyme that regenerates NADPH from NADH; it uses the proton gradient to gener-

Table 2.13 MicroRNAs affecting the mitochondrial metabolism, the expression of which is upregulated in heart failure (Source: [367]; *Arl* ADP ribosylation factor-like transcript, *ETC* electron transport chain, *FABP* fatty acid-binding protein, *Hk* hexokinase, *Hsp* heat shock protein, *ISCu* iron–sulfur cluster assembly scaffold homolog, *MTATP6* mitochondrially encoded ATP synthase (^{ETC}complex-*V*) F0 subunit-6, *MTCO1* mitochondrially encoded cytochrome-C oxidase subunit-1 [^{ETC}complex-*IV* component], *PDHB* mitochondrial pyruvate dehydrogenase subunit-β, *PFKM* phosphofructokinase muscle isozyme, *PGC* PPARγ coactivator, *PKM* pyruvate kinase muscle isozyme, *Ppar* peroxisome proliferator-activated receptor, *Vdac* voltage-dependent anion channel)

Type	Targets
MiR21	Pparα (NR1C1 [↓ fatty acid oxidation])
MiR24	Fabp4
MiR27	Pparγ (NR1C3)
MiR26a	PDHB
MiR29a	Vdac1/2, transcripts of proteins involved in fibrosis
MiR103	Pparα
MiR181c	MTCO1 (in mitochondria and BCL2 in the cytosol)
MiR199a	Hk2, PFKM, PKM2, Pparδ (NR1C2 [↓ fatty acid oxidation])
MiR210	ISCU1/2
MiR320	PFKM, Hsp20
MiR378	MTATP6, Pparα
MiR424	Arl2
MiR696	PGC1α (↓ NRF1/2; ↓ mitochondrial amount and activity)

Production of iron–sulfur clusters are used for reduction–oxidation reactions by TCAC enzymes and ^{ETC}complex-*I* to -*III*. The assembly proteins ISCu1 and ISCu2 are chaperones for the assembly of iron–sulfur clusters in addition to transport of these clusters to their functional location in the cell

ate NADPH from NADH and NADP$^+$, hence linking mitochondrial respiration and detoxification by the thioredoxin–peroxiredoxin processor (∼80%) or glutathione peroxidase (∼20%) of H_2O_2, which is produced from mitochondrial superoxide radical by spontaneous dismutation and/or by manganese-containing superoxide dismutase SOD2 and by citric acid cycle enzymes, such as aconitase and α-ketoglutarate dehydrogenase [370].[72]

The enzyme NNT promotes TRdx and TRdx reductase activity. Mitochondrial NADPH can also be regenerated by isocitrate dehydrogenase IDH2 and mitochondrial NADP-dependent malic enzymes (ME2 and ME3).

An adverse metabolic context reverses the NNT processing direction, consuming NADPH to support NADH and ATP production [371]. In HF, changes in the

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[72]Cerebral neuronal mitochondria remove H_2O_2 using malate and glutamate, which feed NADH into ^{ETC}complex-*I* and, to a lesser extent, succinate, which feeds $FADH_2$ to ^{ETC}complex-*II*.

mitochondrial proteome lead to decreased expression of two NADPH-producing proteins, mitochondrial NNT and IDH2 [369]. The latter yields the highest contribution to overall NADPH regenerating capacity in cardiac mitochondria ($\sim$70%; NNT: $\sim$22%; ME: $\sim$8%) [371].

Redox stress in HF is caused by increased expression of prooxidant aconitase, reduced formation of antioxidant superoxide dismutase, in addition to hypersensitivity of the ETC and ATP synthase to glutathionylation [367].

Apoptosis of cardiac cells is caused by upregulated synthesis of apoptotic proteins such as voltage-dependent anion channel, which acts in the mitochondrial permeability transition pore. The major outer mitochondrial membrane importer of ATP constituents and exporter of ATP, VDAC1, has downregulated expression in end-stage heart failure owing to upregulated miR29a [367].

Synthesis of adenine nucleotide transporter ANT1, the inner mitochondrial membrane transporter of ADP and ATP, is inhibited by miR424, which suppresses production of ARL2 [372]. ARL2 complexes with its partner ARL2BP, and, both being predominantly cytosolic, enter the mitochondrion and bind to ANT1 (but not to ANT2).

MiR141, a member of the MIR200 family, targets the inner mitochondrial membrane phosphate transporter, SLC25a3, which provides inorganic phosphate to the mitochondrial matrix for ATP production [288].

Mitochondria constitute the most redox-active cellular compartment; the mitochondrial matrix of CMCs has a highly reduced redox potential with respect to the cytosol [69, 373]. Glutathione peroxidase inactivates H_2O_2 using reduced glutathione. Glutathione is produced in the cytosol and accumulates in the mitochondrial matrix, where it is primarily found in the reduced state, mainly because of NADPH produced by the TCAC. Distinct glutathione nanodomains exist in the *redoxomes* of CMCs, that is, the cytosol, mitochondrion, and other redox-active organelles. Oxidants are also produced in nanodomains inside redoxomes.

Increased ROS generation upon cellular stress provokes a reductive stress in the cytosol and mitochondria and oxidative stress upon reoxygenation [69]. On the other hand, upon prolonged ROS exposure and chronic ischemia, the cytosol and mitochondria are more oxidized media. In addition, ROS scavenging by antioxidants influences redox status. Increased production of reductants such as accumulation of reduced glutathione and NADPH in addition to elevated activity of antioxidant enzymes can result from reductive countering of radicals upon oxidative stress.

Redox stress can involve NFE2L2, HSPs, ROS, and G6PD [69]. Antioxidant flux depends on mitochondrial substrate catabolism along with thioredoxin and glutathione.

Because they modulate mitochondrial metabolism, several miRNAs act as oncogenes or tumor suppressors [368]. Production of miR23a and miR23b, which control expression of mitochondrial glutaminase,[73] is downregulated upon

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[73]Glutaminase (GSL) converts glutamine to glutamate, which is further catabolized in the TCAC for the production of ATP.

oncogenic activation (e.g., MyC overexpression)[74] and accompanied by decreased ROS synthesis, as glutamine is a substrate for glutathione formation, and by increased ATP synthesis via the TCAC.

Expression of both miR23a and miR23b is induced in cardiac hypertrophy. In addition, miR15b, miR16,[75] miR195, and miR424, which preclude formation of ARL2 and lower ATP concentration, affect mitochondrial integrity in CMCs [288]. These members of the MIR15 family undergo upregulated expression in ischemia–reperfusion injury and cardiac hypertrophy.

Ischemia is characterized by hypoxia. The HIF pathway provokes a metabolic shift (*Pasteur effect*) from aerobic mitochondrial oxidative phosphorylation to anaerobic glycolysis, an event linked to repression of mitochondrial genesis and production of glycolytic enzymes (e.g., lactate dehydrogenase, LDHa, which converts pyruvate to lactate) and activation of pyruvate dehydrogenase kinase (PDHK, which decreases glucose utilization and increases fatty acid metabolism and intervenes in ROS generation).[76] During hypoxia, mitochondrial ROS formation rises and repression of the oxidative metabolism by HIF maintains redox homeostasis. During chronic hypoxia and hence ischemia, oxidative metabolism can be maintained, at least in fibroblasts under hypoxia, but generates toxic ROS levels [288].

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[74]MyC promotes glutaminolysis and causes a reprogramming of mitochondrial metabolism according to glutamine catabolism and TCAC anaplerosis [288]. Although miR23a and miR23b target mitochondrial GSL, Myc upregulates GSL synthesis, as it downregulates the expression of miR23a and miR23b. MiR23 production also decreases under redox stress.

Glutamine is a nitrogen donor for de novo purine and pyrimidine synthesis [374]. It is catabolized to aspartate for adenosine nucleotide formation. It is also involved in the production of glucosamine, a communication node between glucose and glutamine metabolism and an agent of glycoprotein synthesis, in addition to proteins for cell growth and proliferation. Glutamine is linked to MyC concentration and transcriptional activity. In some cell types, deregulated expression of MYC, which happens in multiple tumor types, enhances glutamine utilization and renders cell survival dependent on glutamine (glutamine addiction).

The transcription factor MyC binds to gene promoters and enhancers residing within an open chromatin structure. It promotes cell growth and proliferation via its influence on cell metabolism. It raises aerobic glycolysis. It increases uptake of glutamine, as it promotes transcription of genes encoding glutamine transporters, enzymes of glutamine metabolism, and microRNAs that control expression of these genes [375].

The MYC transcript is targeted by microRNAs, such as Let7, miR 17-19b, and members of the miR34 family, among others [375]. Several stress-responsive pathways also suppress MyC formation.

Cancerous cells that produce high MyC levels due to Wnt pathway mutations are not addicted to glutamine but undergo a reversible cell cycle arrest upon glutamine deprivation [375].

Glutamine deprivation rapidly prevents MYC translation, which is associated with diminished transcription, thereby avoiding apoptosis [375]. Glutamine contributes to adenosine synthesis; glutamine controls MYC translation via its metabolite adenosine. Adenosine suffices to rescue MyC expression.

[75]Both miR15 and miR16 impair circulating proangiogenic cell function [288].

[76]PDHK phosphorylates (inactivates) pyruvate dehydrogenase (PDH), which converts pyruvate to acetyl-CoA, which is oxidized in the TCAC.

miR210, which is regulated by NFκB and ELk1, is upregulated by hypoxia. HIF1α represses oxidative phosphorylation and activity of mitochondrial ETC via miR210 in many cell types [288].

MiR210 impedes synthesis of ISCu1 and ISCu2 (which participates in the assembly of iron–sulfur clusters present in several ETC and TCAC components), transferrin receptor, mitochondrial cytochrome-C oxidase assembly protein (protoheme-9 farnesyltransferase) CcOx10 (a component of ^{ETC}complex-I and $-IV$), mitochondrial heme synthase (ferrochelatase [FeCh], the subunit of succinate dehydrogenase SDHd, which oxidizes succinate to fumarate), glycerol 3-phosphate dehydrogenase-1-like protein (GPD1L, which modulates the NAD^+/NADH ratio), and NADH dehydrogenase (ubiquinone)-1α (NADH–ubiquinone oxidoreductase) subunit NDUFα4 [288]. MiR210 may protect ECs and skeletal and cardiac myocytes against redox stress. However, miR210 can raise ROS production at least in some cell types and provokes cellular senescence.

2.2.1.6 MicroRNAs as Risk Markers

MicroRNAs can be released into the extracellular space and blood circulation in vesicles or linked to proteic binding partners, which avoid miR destruction.

Certain microRNAs originating from the heart or other sources can be used as risk markers (Tables 2.14, 2.15, and 2.16) [238, 430]. Concentrations of the cardiac and muscular miRs (miR1, miR133, miR208, and miR499) are elevated in the bloodstream after MI.

2.2.1.7 MicroRNAs Involved in Adverse Cardiac Remodeling

MicroRNAs participate in regulating transcript translation and degradation during cardiac genesis and remodeling (Table 2.17). MiR21, miR29a, miR129, miR210, miR211, miR320, and miR423, along with Let7c, are highly expressed in the fetal heart and in postnatal cardiac development [377]. Members of the MIR1 (miR1-1 and miR1-2) and MIR133 (miR133a1–miR133a2 and miR133b) and miR206 are muscle-specific microRNAs; miR1 and miR133 are also produced in adequate amounts during the early stage of cardiogenesis. MiR1 targets the transcription factor HAND2, which promotes CMC proliferation, in addition to the transcriptional repressor HDAC4. MiR133 acts on the nuclear factor NELFA.[77]

Loss of miR1 and miR133 leads to ventricular septal defects and cardiac chamber dilation. In ventricular septal defects, expression of miR181c rises. In the human heart, miR196a is associated with the HoxB8 (or Hox2D)–sonic hedgehog pathway implicated in cardiac septation, valve formation, and outflow tract morphogenesis.

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[77]NElFa: negative elongation factor-A, which is also termed Wolf–Hirschhorn syndrome candidate protein-2 (WHSC2).

Table 2.14 Examples of microRNAs used as risk markers (Source: [238]; *BNP* brain natriuretic peptide, *vSMC* vascular smooth myocyte)

Type	Features
MiR1	Marker of CMC necrosis
MiR21	Marker of fibrosis
MiR28	Marker of fibrosis
MiR33a	Marker of fibrosis
MiR126	Reduced in diabetes
	Regulates endothelial and vascular integrity
	Modulates formation of adhesion molecules
	High amount in platelet microvesicles
MiR133	Marker of CMC necrosis
	Modulates vSMC phenotype
MiR143/145	Released by mechanical stress
	Modulates vSMC phenotype
MiR150	Inversely linked to post-infarction remodeling
	High amount in platelet microvesicles
	Role in megakaryopoiesis
MiR155	Released by inflammation
	Regulates macrophage activity and lipid uptake
MiR197	Inversely associated with myocardial infarction
MiR208	Marker of CMC necrosis
	(mir208 gene in the intron of the MYH6 gene)
MiR223	Inversely associated with myocardial infarction
	Highly expressed in platelets
MiR499	Marker of CMC necrosis
	(mir499 gene in the intron of the MYH7 gene)
MiR328	Released by atrial fibrillation
MiR622	Released in heart failure
	Its concentration correlates with that of BNP

Tetralogy of Fallot, the most common congenital cyanotic disorder, is linked to an overexpression of miR421 in ventriculomyocytes, whereas miRNA940 is underexpressed [377]. In bicuspid aortic valve, a congenital valvulopathy, miR26a, miR30b, miR141, and miR195 concentrations are weak.

In adverse cardiac remodeling, that is, hypertension-induced hypertrophy, miR1 and miR133 expression is downregulated, whereas that of miR21, miR195, miR208, and miR214 is upregulated [377].

In ventricular arrhythmias, arrhythmogenic miR1 represses expression of the KCNJ2 and GJA1 genes that encode $K_{IR}2.1$ channel and connexin-43 [377]. On the other hand, miR1 concentration is reduced in atrial fibrillation, which is linked to miR328.

Profibrotic miR33a is produced predominantly by cardiofibroblasts, which ensures necessary acute wound healing (*cardiac reactive fibrosis*) [378]. On the

Table 2.15 Cardiac microRNAs as markers of cardiovascular maladies (Source: [376])

MicroRNA	Level of evolution of the disease (target)
MiR1	↑ in coronary atherosclerosis, myocardial infarction
	↓ in tachycardia, atrial fibrillation
	(fatty acid-binding protein FABP3)
MiR133a/b	↑ in coronary atherosclerosis, myocardial infarction, tachycardia, and heart failure
MiR208a/b	↑ in coronary atherosclerosis, myocardial infarction, viral myocarditis, and heart failure
MiR423-5p	↑ in myocardial infarction and dilated cardiomyopathy
MiR499	↑ in myocardial infarction, viral myocarditis, and heart failure

other hand, *cardiac replacement fibrosis* linked to adverse remodeling, which upregulates miR33a expression in the heart, is deleterious. In ^{CFB}mir33$^{-/-}$ (i.e., only in cardiofibroblasts), cardiac fibrosis is attenuated in response to hypertension. Depletion of miR33a impedes PKB activation but favors ABCa1 expression. MiR33 can control cardiofibroblast proliferation, as it can target CDK6, CcnD1, and P53 in some cell types [364], but CDK4 is predominant over CDK6 in fibroblasts [378]. In fact, miR33a preserves the cholesterol content of membrane rafts, which enable PKB activation and cardiofibroblast proliferation.[78] On the other hand, upregulated synthesis of ABCa1, which permits efflux of intracellular free cholesterol to extracellular cholesterol acceptor ApoA1, depletes the cholesterol content of membrane rafts and alters the proliferative capacity of cardiofibroblasts.

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[78]Membrane rafts, which are plasmalemmal nanodomains enriched in cholesterol and glycosphingolipids, and caveolae, a subcategory of rafts characterized by invaginations of the plasma membrane containing caveolin-1, are platforms for signaling effectors, that is, plasmalemmal surface receptors, such as growth factor and T-cell receptors in addition to members of tumor-necrosis factor receptor superfamily (TNFRSF), and intracellular cortical signaling mediators. Cholesterol depletion decreases membrane raft density and BCL2L1 concentration, inactivates PKB, and activates caspase-3, hence provoking anoikis-like apoptosis [379]. The PKB kinase, a major regulator of cell survival and proliferation, especially in cancerous cells, phosphorylates (inactivates) proapoptotic proteins and upregulates transcription of genes encoding antiapoptotic agents, such as BCL2L1 and usurpin (CFLAR). The PI3K–PKB signaling axis can reside in membrane rafts, where it can undergo full activation to promote cell survival. The synthetic antitumoral lipids edelfosine and perifosine displace PKB along with other regulatory kinases such as PI3K, PDK1, and TOR from membrane rafts, at least in mantle cell lymphoma, a B-cell-derived neoplasia characterized by the chromosomal translocation t(11;14)(q13;q32), causing aberrant overexpression of cyclin-D1 and deregulation of several pathways, such as the constitutive activation of the PI3K–PKB axis [380]. PI3K phosphorylates PI(4,5)P$_2$ to generate PIP$_3$, which tethers to PKB and PDK1 and recruits them to the membrane. At the plasma membrane, PKB is phosphorylated at Thr308 and Ser473 by PDK1 and PDK2, the latter being TOR, DNAPK, or ILK, or even PKB (autophosphorylation). This membrane raft re-organization leads to PKB dephosphorylation, whereas proapoptotic TNFRSF6a is recruited into membrane rafts.

Table 2.16 Noncardiac microRNAs as markers of cardiovascular maladies (Source: [376])

MicroRNA	Level of evolution of the disease
MiR21	↑ in coronary atherosclerosis, myocardial infarction hypertrophic cardiomyopathy, and heart failure
	↓ in atrial fibrillation
MiR27a	↑ in myocardial infarction and hypertrophic cardiomyopathy
	↓ in heart failure
MiR30c/d	↓ in coronary atherosclerosis
	↑ in hypertrophic cardiomyopathy
MiR106a-5p	↓ in myocardial infarction and heart failure
MiR122	↑ in coronary atherosclerosis
MiR126	↑ in myocardial infarction
	↓ in heart failure
MiR134	↑ in coronary atherosclerosis, myocardial infarction
MiR145	↓ in coronary atherosclerosis and atrial fibrillation
	↑ in heart failure
MiR146a	↑ in atrial fibrillation
	↓ in heart failure
MiR150	↓ in atrial fibrillation
MiR197	↑ in coronary atherosclerosis
MiR199a	↑ in coronary atherosclerosis and hypertrophic cardiomyopathy
	↓ in heart failure
MiR223	↑ in coronary atherosclerosis
MiR328	↑ in myocardial infarction
	↓ in atrial fibrillation, heart failure
MiR486	↑ in coronary atherosclerosis, myocardial infarction

As myocardial infarction marker, they are as significant as cardiac microRNAs

Some microRNAs are associated with matrix degradation and pathological cardiac remodeling caused by prolonged overactivation of the renin–angiotensin–aldosterone axis and sympathetic nervous system in addition to hypertension, valve stenosis or regurgitation, myocarditis, familial and acquired cardiomyopathies, and MI (e.g., miR100 and miR101) [381]. MiR101 regulates Rab1a formation; miR100 and miR101 (along with miR15b, miR20a, miR92a, and miR132), prime apoptosis, as they target inhibitors of the intrinsic apoptosis pathway.

Many microRNAs favor or counter maladaptive cardiac hypertrophy (Tables 2.18 and 2.19) and fibrosis (Tables 2.20 and 2.21).

Concentrations of circulating microRNAs depend on disease states and can thus serve as markers of CVD evolution. Microvesicles and HDLs can transport miRs in blood and deliver them to cells.

Table 2.17 MicroRNAs in cardiac fate (Source: [377])

Context	Involved microRNAs
Cardiogenesis	MiR1/21/29a/129/133a/210/211/320/423, Let7c
Ventricular septal defect	MiR1/181c
Tetralogy of Fallot	MiR421/940
Bicuspid aortic valve	MiR26a/30b/141/195
Cardiac remodeling	MiR1/21/133/195/208/214
Atrial fibrillation	MiR1/328
Ventricular arrhythmia	MiR1
Hypertension	MiR20a/132/155/181/212/663
Heart failure	MiR1/21–23/29/92b/130/195/199, miR210/320a/423-5p/499/622
Atherosclerosis	MiR33/122/126/145/155/370
Myocardial infarction	MiR1/15/24/133a/208b/499

Table 2.18 Antihypertrophic microRNAs in the heart (Source: [381] *AtG* autophagy-related gene product, *CamK* calcium–calmodulin-dependent kinase, *Ccn* cyclin, *FABP* fatty acid-binding protein, *Fbln* fibulin, *GATA* GATA-binding protein, *HAND* heart and neural crest derivatives expressed protein, *IGF* insulin-like growth factor, *KAT* lysine (K) acetyltransferase, *Ksr* kinase suppressor of Ras, *MAPK* mitogen-activated protein kinase, *NCX* sodium–calcium exchanger, *NFAT* nuclear factor of activated T-cells, *TnnI3K* troponin-I type-3-interacting kinase, *Twf* actin-binding twinfilin homolog [PTK9], *WHSC2* Wolf–Hirschhorn syndrome candidate protein-2, or negative elongation factor NeLFa, *XBP1* X-box-binding protein)

MiRs	Transcript targets (effect)
MiR1	Fabp3, FBLN2, Hand2, Igf1, TWF1 (in addition to Cdk9, FN1, RASGAP, Rheb)
MiR9	Myocardin (↓ NFAT3)
MiR26b	GATA4
MiR30-3p	Xbp1
MiR34a	ATG9A
MiR98	CCND2
MiR101	RAB1A
MiR133	Cdc42, RHOA, WHSC2 (or NELFA)
MiR145	GATA6
MiR150	KAT3B
MiR185	CAMK2D, Ncx1, Nfat3
MiR223	TNNI3K
MiR378	Mapk1, Igf1r, Grb2, Ksr1

MiR208a and miR208b, which are encoded by introns of the MYH6 and MYH7 genes respectively, are also involved in the mutually incompatible regulation of MHC6 and MHC7 expression in adverse cardiac hypertrophy, downregulating MYH6 and upregulating MYH7 expression [383]. They regulate MYH7 expression via the mediator complex subunit Med13 and myostatin [384].

Table 2.19 Prohypertrophic microRNAs in the heart (Source: [381] *BECN1* autophagy-related beclin-1, *CDKN1B* transcript encoding cyclin-dependent kinase inhibitor-1B [CKI1b], *DYRK* dual-specificity Tyr (Y) phosphorylation-regulated kinase, *FBxO32* F-box only protein-32, or atrogin-1, *Fox* forkhead box transcription factor, *GRB* growth factor receptor-bound protein, *GSK* glycogen synthase kinase, *HDAC* histone deacetylase, LPA_1, lysophosphatidic acid receptor-1, *MAPK* mitogen-activated protein kinase, *MED* mediator complex subunit, *MSTN* myostatin, *PPARγ*, peroxisome proliferator-activated receptor-γ, or nuclear receptor NR1c3, *PDLIM5* PDZ and LIM domain-containing protein-5, or enigma homolog [enigma-like PDZ and LIM domain-containing protein EnH1], *PTen* phosphatase and tensin homolog, *serca* sarco(endo)plasmic reticulum Ca^{2+} ATPase, *SIRT* silent information regulator-2, *SorbS* sorbin and SH3 domain-containing protein, *TP53INP1* tumor protein P53-inducible nuclear protein-1, *TriM63* tripartite motif-containing protein-63, or muscle-specific RING finger-containing protein MuRF1)

MiRs	Transcript targets (effect)
MiR19a/b	FBXO32, TRIM63 (↑ NFAT)
MiR21	PTEN (TOR activation)
MiR21-3p	Hdac8, SORBS2, PDLIM5
MiR22	Sirt1, Hdac4, PTEN
MiR23a	FOXO3A, LPAR1
MiR27b	PPARG
MiR30a	BECN1
MiR30a-3p	Xbp1
MiR155	Tp53inp1
MiR199a	GSK3B (TOR activation)
MiR199b	Dyrk1a (NFAT activation)
MiR208a/b	MED13, MSTN
MiR212–miR132 group	FOXO3 (↑ NFAT)
MiR221	CDKN1B
MiR328	SERCA2A (NFAT activation)
MiR350	Mapk8/9/11/14 (NFAT activation)

Table 2.20 Antifibrotic microRNAs in the heart (Source: [381] *Col* collagen, *CTGF* connective tissue growth factor, *Eln* elastin, *Fbn* fibrillin, *Fos* FBJ murine osteosarcoma viral proto-oncogene homolog, *IL* interleukin, *Snai1* zinc finger-containing snail homolog, *TGF* transforming growth factor)

MiRs	Transcript targets (effect)
Let7i	COL1A2, Il6
MiR24	FURIN (Pcsk3; ↓ TGFβ)
MiR26a	COL1A1, Ctgf
MiR29a/b/c	ELN, FBN1, COL1A1/A2, COL3A1
MiR29b	TGFB1
MiR30	Ctgf
MiR101a	FOS, TGFBR1
MiR133	Ctgf
MiR133a	COL1A1, Pp3, SERCA2A, SNAI1

In the vasculature, miR29 expression is upregulated during aging; it represses synthesis of matrix proteins in smooth myocytes and participates in the regulation of aneurysm formation; miR29b-3p counters fibrosis

Table 2.21 Profibrotic microRNAs in the heart (Source: [381] *Apln* apelin, *PTen* phosphatase and tensin homolog, *Spry* sprouty homolog)

MiRs	Transcript targets
miR21	SPRY1 (MAPK activation), PTEN ($\uparrow$ MMP2)
miR125b	APLN

Concentration of profibrotic miR21-5p, which causes cardiac fibrosis, does not correlate with atherosclerotic plaque fibrosis [382]

MicroRNA-146a concentration moderately increases in the left ventricular wall of patients with aortic stenosis. Overexpression of miR146a in CMCs provoked cardiac hypertrophy [385]. MiR146a reduces the formation of dihydrolipoyl succinyltransferase (DLST).[79] MiR181c can act in the mitochondrion of CMCs, where it targets MTCOI mRNA, which encodes cytochrome-C oxidase catalytic subunit-1 (CcOx1) and provokes remodeling of ^{ETC}complex-IV [386].

2.2.1.8 MicroRNAs in Obesity and Diabetes

Increased adiposity, obesity, and diabetes are linked to redox stress and inflammation. Mitochondrial ROS overproduction results from hyperglycemia in the diabetic microvasculature, whereas in the diabetic macrovascular and heart, it is the consequence of increased fatty acid oxidation and insulin resistance [288].

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[79]That is, 2-oxoglutarate dehydrogenase complex component-E2, a rate-controlling enzyme of the TCAC. This enzymatic complex, which catalyzes the conversion of 2-oxoglutarate to succinyl-CoA and CO_2, contains multiple copies of 2-oxoglutarate dehydrogenase (E1), dihydrolipoamide succinyltransferase (E2), and lipoamide dehydrogenase (E3). Its overproduction in wild-type mice protects against cardiac hypertrophy [385].

Conversion of FFAs and glucose accounts for most of the ATP production in the healthy adult heart. At an advanced stage of heart failure, fatty acid oxidation decreases and glycolysis increases.

Glucose is converted to ATP by glycolysis, the TCAC, and the mitochondrial ETC. In glycolysis, glucose is catabolized into two pyruvate, two ATP molecules and two NADH, which can generate five ATPs, assuming 2.5 mol of ATP per mole of NADH.

The TCAC inside the mitochondrial matrix processes acetyl-CoA derived from pyruvate, the product of glycolysis, which first undergoes oxidative decarboxylation by the pyruvate dehydrogenase complex that transfers acetyl groups to coenzyme-A (TCAC step 0). Pyruvate can be formed by amino acid catabolism. In addition, amino acid and fatty acid catabolism also directly provide acetyl-CoA.

The TCAC itself begins and ends with oxaloacetate. AcetylCoA combines with oxaloacetic acid to produce citric acid (hence the other TCAC name, citric acid cycle). A series of oxidations occur from citric acid to oxaloacetic acid. The TCAC consumes and produces metabolites relevant to other processes.

Glycolysis and the TCAC oxidize fuels to CO2 to reduce NAD^+ to NADH and FAD to $FADH_2$. In the ETC, the electron carriers NADH and $FADH_2$ are re-oxidized to oxidatively phosphorylate ADP to ATP; they use their electrons for proton motion across the inner mitochondrial membrane.

The ensemble constituted by three reaction sets (glycolysis, TCAC, and ETC) creates about 30 ATP molecules, assuming 1.5 mol of ATP per mole of $FADH_2$, and GDP and GTP are supposed to be equivalent to ADP and ATP (glycolysis: two ATP and two NADH; TCAC step 0: two NADH; TCAC: six NADH, two $FADH_2$, and two GTP; ETC: 30 ATP).

In obese subjects, redox stress in adipose tissue is associated with chronic low-grade inflammation. Three miRs target antioxidant, anti-inflammatory, and antiapoptotic heme oxygenase HOx1, miR155, miR183, and miR872 [288].

In adipose tissue of obese mice, miR221 and miR222 trigger hypoxia and the formation of miR27, which hampers production of PPARγ and C/EBPα [288].

In diabetes, concentrations of proinflammatory mediators, such as advanced glycation end products, PGhS2, cytokines IL6 and TNFSF1, and chemokines CCL2 and CX$_3$CL1, rise, thereby favoring vSMC proliferation, redox stress, and vascular complications. In vSMCs of diabetic mice, expression of miR125b and MIR200 family members is upregulated, enhancing transcription of proinflammatory genes via an epigenetic process [288].

In diabetic subjects, miR34a and miR504 also play a role in vascular smooth myocyte dysfunction, whereas miR126 targets tissue factor and reduces thrombogenicity and miR191 impedes wound repair, as it hinders migration of fibroblasts and favors apoptosis of dermal fibroblasts [286].

The circular RNA Hipk3 (circHipk3) acts as a miR30a-3p sponge, thereby increasing VEGFc, Fzd4, and Wnt2 formation. Its production is upregulated in diabetic retinal ECs [387]. CircHIPK3 affects survival, proliferation, and migration, and hence the angiogenic capacity of retinal ECs. In vivo, circHIPK3 thus controls diabetic proliferative retinopathy; the silencing of circHIPK3 alleviates retinal vascular dysfunction.

2.2.1.9   MicroRNAs Involved in Atherosclerosis

When atherosclerotic lesions become unstable, miR concentrations increase. MiR29 may contribute to inflammation and plaque destabilization [388]. Other microRNAs, such as miR92, miR126, miR145, and miR155, are implicated in the initiation of endothelial dysfunction and macrophage activity.

MiR155 has both anti- and proatherogenic effects according to the disease stage [286]. MiR150-5p, miR365a-3p, and miR1275 are linked to coronary atherosclerosis. The angiomicroRNAs miR378 and Let7b are upregulated in CD34++ progenitor cells in response to MI.

In patients with atherosclerosis, especially with coronary stenoses and acute coronary syndrome (ACS),[80] miR133a, miR133b, miR208a, miR208b, and miR499, can serve as diagnostic and/or prognostic markers across different progression stages [389]. MiR1[81] and miR145b[82] are also ACS markers, with

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[80]This medical emergency, commonly known as heart attack, is a set of disorders, ranging from unstable angina (i.e., chest pain due to myocardial ischemia) to actual MI.

[81]MiR1 limits endothelial permeability and promotes angiogenesis, as it represses mRNA encoding seryl tRNA synthetase, in addition to CMC differentiation [389]. It shares with miR133 four mRNAs, which encode HCN4, LASP1, PNP, and transgelin-2. MiR1 together with miR155, miR206, and miR613 target liver X receptors, thereby controlling the transcription of the ABCA1 gene.

[82]MiR145 shares with miR133 2 mRNAs, FSCN1 and Igf1r. MiR145 and miR208 target CDKN1A mRNA [389].

miR1 having a higher sensitivity for all types of MI and miR145 for ST segment elevation myocardial infarction (STEMI). In addition, miR1, miR21, miR122, miR134, miR14525, miR37026, and miR49931 correlate positively with CoAD severity. The greatest prognostic or diagnostic value is yielded by the miR132–miR150–miR186 combination. Both miR208a and miR208b, which are exclusively produced in CMCs, are the early detectable specific diagnostic MI markers with respect to cTnIn and cTnnT. MiR320a and miR49922 are also sensitive and specific MI diagnostic markers. For some investigators, only miR126,[83] miR223, and miR19736 provide an MI risk.

The identified miRs regulate endothelial function and angiogenesis (miR1 and miR133), vSMC differentiation (miR133 and miR145), communication between vEC and vSMC (miR145), apoptosis (miR1, miR133, and miR499), and CMC differentiation (miR1, miR133, miR145, miR208, and miR499), and repress cardiac hypertrophy (miR133). Their shared targets include mRNAs encoding CKI1a, ETS1,[84] Fscn1,[85] HCN4,[86] IGF1R,[87] LASP1,[88] PNP,[89] and Tagln2 [389].[90]

Matrix metallopeptidases degrade collagens, elastin, proteoglycans, and glycoproteins, such as fibronectin and laminin. They are counteracted by TIMPs. In unstable atherosclerotic lesions, the myelopoietic growth factor CSF2,[91] which is

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[83]In patients with stable CoAD, miR126 concentration is lowered in circulating microvesicles. However, in patients with unstable plaques, its plasmatic concentration is higher than that of controls [389].

[84]ETS1 is involved in stem cell development and cell senescence and death in addition to regulation of PDGFRA gene transcription in vSMCs [389].

[85]Fscn1: fascin. The actin-bundling protein organizes filamentous actin into actin filament bundles. It acts in the formation of microspikes, membrane ruffles, and stress fibers and hence in cell adhesion and migration in addition to cellular interactions [389].

[86]HCN4: potassium- and sodium-permeable hyperpolarization-activated cyclic nucleotide-gated potassium channel-4. It contributes to the pacemaker current in the heart. An increase in HCN2 and HCN4 activity in hypertrophied ventriculomyocytes prolonged the repolarization stage [389].

[87]Reduced miR133a expression is associated with a lower IGF1R concentration and suppression of vSMC growth via the PKB–FoxO3a pathway [389].

[88]LASP1: LIM and SH3-domain-containing protein-1. It is a member of the ubiquitous LIM protein subfamily of the nebulin group. LASP2 is a splice variant of the nebulin gene and is thus also termed nebulette. The association between the chemokine receptors and LASP1 regulates the migration of inflammatory leukocytes [389].

[89]PNP: purine nucleoside phosphorylase. It is involved in energy-deprived conditions in the metabolism of adenosine during ATP degradation and hence regulation of activated T cells [389].

[90]Tagln: transgelin. MiR133a connects to the 3′-UTR of TAGLN2 mRNA, thereby affecting differentiation of smooth myocytes. It intervenes in hypoxia-induced apoptosis via caspase-8 [389].

[91]Colony-stimulating factors CSF1 and CSF2 control the development, functional and phenotypical differentiation, and proliferation of monocytes and macrophages in cooperation with cytokines such as IL4 and IL34, the second CSF1R ligand [390]. The *mononuclear phagocyte system*, which participates in inflammation via the elimination of foreign bodies and the organization of different inflammatory phases, includes lineage-committed bone marrow precursors, circulating monocytes, tissue-resident macrophages, and myeloid dendrocytes [390]. Monocytes reside in the

involved in the development of mononuclear phagocytes only during inflammation, favors the TIMP3− phenotype of macrophages, which are more invasive and sensitive to apoptosis than TIMP3+ macrophages [391]. The density of TIMP3− macrophages is higher in unstable than in stable plaques. In macrophages, CSF2 reduces TIMP3 production via miR181b, thereby favoring plaque instability and aneurysm development [392]. Tissue inhibitors of metallopeptidases protect against the progression of atherosclerosis and AAAs.[92] In addition, miR181b, which is overexpressed in developed atherosclerotic plaques and AAAs, precludes elastin production by vSMCs. Moreover, miR181b is a potent regulator of NFκB signaling in the vascular endothelium, as it targets the KPNA4 (or IPOA3) gene, which encodes karyopherin-α4 (or importin-α3), required for the nuclear translocation of NFκB, thereby downregulating Kpnα4 and NFκB nuclear translocation in the vascular endothelium [391, 392].[93] MiR181b also favors the M2 phenotype in atherosclerotic lesions, as it also targets notch-1 [393], in addition to promoting development of NK cells from hematopoietic progenitor cells [394].

Some miRs (miR29b-3p, miR126-3p, miR145-5p, and miR155-5p) exhibit a transcoronary concentration gradient (TCG) within the coronary circulation between its entry (aortic orifices) and exit (venous coronary sinus; Table 2.22) [382]. They appear in the coronary circulation in the early stages of coronary atherosclerosis. They are related to vulnerable plaques observed by optical coherence tomography with the presence of thin-cap fibroatheroma [382]. On the other hand, miR29b-3p, miR126-3p, miR145-5p, and miR155-5p have negative TCGs in subjects without vulnerable plaques. Uptake of circulating atheroprotective miR126-3p and miR145-5p in the coronary vessels stabilizes atherosclerotic lesions. Platelets can contribute to transcoronary miR concentrations; miR223-3p, which is highly expressed in platelets, but weakly expressed in ECs, also has a negative TCG [382].

Cardiomyocytes cultured under ischemic conditions release exosomes that promote angiogenesis via miR143 and miR222, the most abundant microRNAs in

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bloodstream and the bone marrow and spleen before being released into the blood and any tissue before becoming macrophages. Anticipatory inflammation describes the liberation of Ly6C+ classical monocytes from the bone marrow with a diurnal rhythm under the control of BMAL1. Classical monocytes replenish monocyte-derived macrophages in tissues, differentiate to take an [M1] functional phenotype and into Ly6c− cells in blood and bone marrow, initiate the inflammatory response, and serve as antigen-presenting tissular monocytes following antigen uptake in tissues and recirculation to lymph nodes. Patrolling Ly6C− monocytes constitutes a blood cell subset that scans the endothelial surface in search of damage and pathogens and phagocytose endothelial particles. Macrophages maintain tissue integrity, as they eliminate damaged cells, cellular debris, and degraded matrix components. [M2]macrophage (anti-inflammatory phenotype) can promote tumor growth, whereas [M1]macrophage (pro-inflammatory phenotype) removes pathogens and virally infected and cancerous cells.

[92]TIMP2 plays a greater protective role than TIMP1 in atherosclerosis and aneurysm formation [392]. TIMP2 suppresses MMP14 formation by monocytes and macrophages. CSF2 also raises MMP12 expression in macrophages.

[93]MiR205 targets Timp3 in ECs [392].

Table 2.22 MicroRNA (miR) transcoronary concentration gradients (TCGs) during the main evolution phases of coronary atherosclerosis (Source: [388])

Type	Positive TCG	Negative TCG
Endothelial dysfunction	MiR92/126/133, miR145/155	MiR17a/181a/222
Atherosclerosis	MiR126/145/155, miR208a/221	ND
Acute coronary syndrome	MiR133a/208a, miR499	MiR92a/126/155

Transcoronary concentration gradients of several miRs are associated with atherosclerotic lesion vulnerability. In particular, miR126-3p and miR126-5p are secreted into the coronary circulation by activated ECs covering atherosclerotic plaques via release of apoptotic bodies and/or protein-bound miRs. Negative transcoronary gradients of miR126-3p, miR126-5p, and miR145-5p may result from the uptake and/or degradation during the transcoronary passage in subjects with predominantly stable coronary atherosclerotic plaques [382]

ischemic exosomes [395]. Intramyocardial delivery of ischemic exosomes improves neovascularization following MI.

2.2.1.10 MicroRNAs Involved in Stroke

Two major categories of strokes include *ischemic* (∼87%) and *hemorrhagic strokes*, which are mainly caused by emboli and bleeding respectively. Ischemic stroke can result from carotid artery atherosclerosis, either from hypoperfusion due to high-grade stenosis or emboli upon plaque ulceration, even in relatively small lesions (∼20%) [396].

MicroRNAs can serve as markers to assess atherosclerotic plaque rupture and embolic stroke risk. MicroRNA expression is deregulated in human carotid plaques, causing lesion growth, instability, and rupture (Tables 2.23, 2.24, 2.25, and 2.26).

After a stroke, expression of Let7f, miR15b, miR126, miR142-3p, miR186, miR519e, miR768-5p, and miR1259 is downregulated [396]. Circulating concentrations of miR21 and miR221 are higher in patients with stroke and carotid atherosclerosis than in healthy controls. Plasmatic concentrations of miR106b-5p and miR4306 increase after stroke, whereas those of miR320d and miR320e decline. In peripheral blood mononuclear cells (lymphocytes and monocytes) of patients with stroke, intracellular concentrations of Let7i, miR19a, miR122, miR148a, miR320d, and miR4429 are lower and those of miR363 and miR487b are elevated. Blood concentrations of miR151a-3p and miR140-5p increase, whereas that of miR18b-5p decreases. Concentrations of miR100, miR125a, miR127, miR133a, miR133b, miR145, and miR221 are higher in symptomatic than in asymptomatic plaques examined after endarterectomy. MiR145 concentration is higher in carotid plaques from hypertensive patients; plasmatic miR92a concentration is higher in hypertensive patients. Prognosis is better in patients with a high miR210 level

Table 2.23 MicroRNAs and atherogenesis (**Part 1**; Source: [396]; *asterisk* [*miR**] microRNAs regulated by hemodynamic stress, + [−] miR protecting [amplifying] effect on atherosclerogenesis)

Process	Involved miR species
NOS activity	+: miR153/221/222
Collagen synthesis (fibrous cap)	+: miR21/29b/126/145/192
Collagen degradation by MMPs (fibrous cap thinning)	+: miR24/29
Endotheliocyte activation and inflammation	+: miR/92a*/217/663*/712*
	−: Let7g, miR10a*, miR17-3p/31/124a/125, miR126/126-5pa*/143/145a*/146a/146b, miR155/181b/221/222
Monocyte differentiation	+: miR222/424/503
	−: miR17/20a/106a
Macrophage activation	+: miR125b/155/342-5p
	−: miR9/21/124/125a-5p/146a/146b/147/223
Apoptosis	MiR24/155

Table 2.24 MicroRNAs and atherogenesis (**Part 2**; Source: [396]; *asterisk* [*miR**] microRNAs regulated by hemodynamic stress, + [−] miR's protective [amplifying] effect on atherosclerogenesis)

Process	Involved miR species
Smooth myocyte proliferation and migration	+: miR26a/31/146a/155/208/221/222
	−: Let7d, miR1/10a/29/100/132/133/143/145, miR195/204/424/638/663
Apoptosis	MiR21/126
T-cell differentiation and activation	+: miR17–92/146a/155/182/326
	−: miR125b/181a
Foam cell formation	−: miR9/125a-5p/146a/155
Cholesterol egress	−: miR10b/26/27/33a/33b/106b/144/145/155, miR302a/758
	−: miR223/378
Intraplaque angiogenesis	+: Let7f, miR23/24/27/92a/126/130a/132/150, miR210/218/378
	−: miR15b/16/20a/21/26a/100/200/221/222/223
Wall remodeling	+: miR21/155/221/222
	−: miR26/143/145

in peripheral blood leukocytes, especially if measurement is combined with those of fibrin degradation product and IL-6 [396]. An elevated miR17 concentration predicts early stroke recurrence. A high exosomal miR223 level in stroke patients is related to a poor outcome.

Table 2.25 Targets and effects of main microRNA species involved in atherosclerosis (**Part 1**; Source: [396]; *ABC* ATP-binding cassette transporter, *BCL* B-cell lymphoma protein, *DNMT* DNA methyltransferase, *FABP* fatty acid-binding protein, *LDLR* low-density lipoprotein receptor, *LPL* lipoprotein lipase, *MMP* matrix metallopeptidase, *Sema* semaphorin, *SIRT* sirtuin, *TGF* transforming growth factor, *TIMP* tissue inhibitor of metallopeptidase)

microRNA	Main targets	Effects
Let7f	Timp1	Matrix degradation by MMPs, macrophage infiltration
Let7g	TGFB, Sirt1	Repression of endothelial inflammation
MiR17–92	ABCA1, Ldlr,	Cholesterol efflux
	SEMA6A	Angiogenesis
MiR21	Mmp9, Bcl2,	Macrophage infiltration
	FABP7	SMC proliferation
MiR29a	Lpl	Lipid uptake and inflammatory cytokine secretion
MiR29b	DNMT3B	MMP2/9 synthesis (cell migration)
MiR92a	Integrin	NFκB activation, monocyte adhesion
MiR99a	TGFB	Chemotaxis, proliferation, apoptosis, fibrosis

Table 2.26 Targets and effects of main microRNA species involved in atherosclerosis (**Part 2**; Source: [396]; *AT1R* angiotensin-2 type-1 receptor, *Col* collagen, *CKI* cyclin-dependent kinase inhibitor, *ELk* ETS-like transcription factor, *ETS* E26 transformation-specific sequence transcription factor, *IRAK* interleukin-1 receptor-associated kinase, *IRF* interferon regulatory factor, *KLF* Krüppel-like factor, *OSBPL* oxysterol-binding protein-like protein, *PTGS* prostaglandin endoperoxide synthase [prostaglandin-G/H synthase and cyclooxygenase], *ScaR* scavenger receptor, *SCFR* stem cell factor receptor, *TLR* toll-like receptor, *TOR* target of rapamycin, *TNFSF* TNF superfamily member, *TRAF* TNFR-associated factor, *vcam* vascular cell adhesion molecule, *VEGF* vascular endothelial growth factor)

microRNA	Main targets	Effects
MiR100	Tor	Angiogenesis hindrance
MiR125a-5p	SCARB1/D1/E1,	Lipid uptake, oxLDL internalization,
	OSBPL9	cytokine production
MiR125b	Irf4	Inflammation by macrophages
MiR126	Tnfsf1, Vegf,	Endothelial dysfunction,
	VCAM1	vascular wall remodeling
MiR127	Tnfrsf5, Bcl6	Vascular inflammation, redox stress
MiR133a	Mmp9, Col1	VSMC proliferation, collagen synthesis
MiR143	PTGS2	Atherogenesis
MiR145	Klf4, Elk-1,	SMC differentiation,
		SMC proliferation repression
MiR146a/b	Tlr4, Irak1,	Lipid accumulation and inflammation
	Traf6	
MiR155	Ets1, At1r	Protection against endothelial inflammation
MiR221/222	SCFR, CKI1B/C	SMC proliferation, contractile gene transcription
MiR223	Tissue factor	Atherogenesis, thrombosis

Acute ischemic stroke is associated in a small sample of patients with abnormal blood concentrations of miR125a-5p, miR125b-5p, and miR143-3p, concentrations of which increase significantly, platelets rather than cerebral cells being the major source of these microRNAs [397].

2.2.2 Circular RNAs

Circular RNAs (circRNAs) can serve as miR sponges, which sequester miRs, and nuclear transcriptional regulators. They can be generated from back-spliced exons or intron-derived RNAs.

Platelets and RBCs contain large amounts of ubiquitous cell type-unspecific circular RNAs. Circular RNAs commonly derive from exons of protein-coding genes via backsplicing [360]. On the other hand, some arise from intronic or intergenic regions via alternative splicing. They generally localize to the cytoplasm. RNA-binding proteins, such as quaking homolog and muscleblind-like protein, affect the amount of circRNA in cells, as the interaction of RNA-binding proteins and each RNA end facilitates the formation of a circle. Quaking elicits circRNA genesis.

In the heart, highly expressed circRNAs correspond to key cardiac genes such as desmin (DES), dystrophin, four and a half LIM domain-containing protein-2 (FHL2), myoglobin (MB), cardiac myosin heavy chain-7 (MYH7), cardiac myosin regulatory light chain-2 (MYL2), cardiac ryanodine receptor-2 (RYR2), Na^+–Ca^{2+} exchanger NCX1 (SLC8A1), tropomyosin-1 (TPM1), titin (TTN), and cardiac type-2 troponin-T (TNNT2) [398]. The longest human transcript TTN generates up to 415 different exonic circRNA isoforms.

In vascular ECs, hypoxia upregulates many circRNAs, such as proangiogenic znf292,[94] caff1,[95] cdennd4c,[96] and slightly csrsf4,[97] but downregulates cthsd1 [399].[98] Cznf292 promotes angiogenesis via AP1, E2F1, HIF1, NFκB, SP1, STAT3, and STAT5.

In atherosclerotic plaques, the atheroprotective circANRIL regulates ribosome genesis in vSMCs and macrophages [399].

Atherosclerosis and post-stenting restenosis are characterized by the dedifferentiation of vascular smooth myocytes and intimal hyperplasia. Differentiated vSMCs synthesize SMC-specific contractile proteins and related molecules, such as myosin heavy chain MHC11, actin Actα2, transgelin, and calponin, under control of the transcription factor serum response factor and its cofactor myocardin, which maintain SMC differentiation via the transcription factors and regulators

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[94]ZNF292: zinc finger-containing protein-292.

[95]AFF1: AF4–FMR2 family member-1.

[96]DENND4c: DENN domain-containing protein-4C.

[97]SRSF4: Ser/Arg-rich splicing factor-4.

[98]Thsd: thrombospondin.

Nkx3.1 and Nkx3.2, GATA4 and GATA6, SMADs, and Prx1, and the cysteine-rich LIM-only proteins, CRP1 and CRP2, which are associated with SRF and GATA proteins [400]. PTen interacts with SRF in the nucleus to maintain the differentiated SMC phenotype. In addition, TGFβ, notch, RhoA, and P38MAPK promote SMC differentiation via transcriptional regulators that interact with SRF–myocardin. Furthermore, among epigenetic factors, histone modifications promote SMC contractile gene expression via SRF binding to genes, such as histone modifiers that acetylate (e.g., KAT3b) and methylate histones flanking CArG boxes in the MYH11 and ACTA2 genes. Short and long nonprotein-coding RNAs stimulate or preclude differentiated SMC phenotype. Components of the miR143–miR145 cluster target transcriptional repressors such as KLF4. On the other hand, miR21 and components of the miR221–miR222 cluster favor SMC dedifferentiation via growth repressors (e.g., PTen). Dedifferentiation of SMCs leads to a highly proliferative, migratory, and inflammatory phenotype characterized by the downregulated transcription of SMC-specific genes and upregulated production of inflammatory mediators and matrix components.

Transforming growth factor-β induces the formation and cleavage of transmembrane neuregulin-1.[99] The extracellular EGF-like domain-containing domain ($Nrg1^{ECD}$) binds to nearby receptors such as receptor protein Tyr kinases of the EGFR family, thereby acting as an auto- and paracrine messenger, in particular, during cardiovasculogenesis. Neuregulin-1, which has 11 isoforms, binds to HER3 and HER4 [401]. Released soluble Nrg1 suppresses SMC proliferation via the HER receptor and then activates ERK1 and ERK2 [402].

On the other hand, in response to TGFβ1, the intracellular domain of neuregulin-1 ($Nrg1^{ICD}$) translocates to the nucleus and interacts with the transcription factor Ikaros family zinc finger protein IkZF1 (ZnFn1a1 or simply Ikaros) [402].[100] In the nucleus, the $Nrg1^{ICD}$–IkZF1 complex binds to the first intron of the ACTA2 gene and launches the formation of the circRNA, circActa2, thereby promoting Actα2 production, as it operates as a sponge for miR548f-5p repressing Actα2 synthesis. In human intimal hyperplasia, expression of circActa2 decays and miR548f-5p dissociates from circActa2. On the other hand, PDGFbb decreases NRg1 expression.

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[99]Neuregulins (NRg1–NRg4) belong to the EGF family.

[100]DNA-binding protein ikaros is a transcriptional regulator of hematopoietic cell differentiation, which, in particular, operates in the development of B and T lymphocytes. It targets two chromatin-remodeling complexes, nurd and BAF, in RBCs [108]. It is implicated in normal apoptosis in adult erythroid cells.

The Ikaros family of zinc finger-containing transcription factors include several subtypes encoded by five genes (IKZF1–IKZF5), that is, Ikaros, Aiolos, Helios, Eos, and Pegasus (in Greek mythology, Ικαρος [Latin *Icarus*], is the son of Δαιδαλος [Latin *Daedalus*], the craftsman, artist, and creator of the Labyrinth, Αιολος [Latin *Aeolus*] the god keeper of winds, Ηλιος [Latin *Helius*] one of the Titans and personification of the Sun, his Titan sister Ηως [Latin *Aurora*] the goddess of the Dawn, and Πηγασος [Latin *Pegasus*], the winged horse). Alternative splicing produces additional isoforms from each of the five homologous transcripts. They also lodge in nonhematopoietic organs [403].

2.2.3 ^{Y}*RNAs*

^{Y}RNAs are small RNA species, the size of which ranges from 112 (^{Y1}RNA), 101 (^{Y3}RNA), 98 (^{Y4}RNA), to 84 nucleotides (^{Y5}RNA). They originate from four genes (RNY1 and RNY3–RNY5) and about 900 pseudogenes [360]. Full-length ^{Y}RNAs reside in the nucleus or cytoplasm. They are involved in the initiation of DNA replication and ribosomal RNA quality control. They are components of Ro ribonucleoproteins. Fragments of ^{Y}RNAs generated during cell stress or apoptosis circulate in the bloodstream, $5'$ fragments being much more abundant than $3'$ fragments for most RNY gene products.

2.2.4 *Long Nonprotein-Coding RNAs*

In addition to proteins and microRNAs, long nonprotein-coding RNAs (lncRNAs; content >200 nt), lack significant open reading frames and hence a protein-coding potential. Nevertheless, lncRNAs can act as cofactors that permit the interaction of a transcription factor with a gene promoter, thereby regulating gene transcription. In addition, they can interact with histone modifiers to repress or activate gene expression.

LncRNAs can be used as markers to identify and categorize individuals at risk for developing any type of cardiovascular pathological condition. They can hence support prevention, diagnosis, surveillance, and prognosis of cardiovascular maladies and help to adapt lifestyle and eventually optimize treatments.

Numerous genes encoding lncRNAs have been identified [376]. LncRNAs can be categorized into different groups of transcripts (Vol. 11, Chap. 2. Nonprotein-Coding Transcripts and Regulation of Protein-Coding Gene Transcription).

1. *Sense lncRNAs* overlap exons or introns of messenger RNAs.
2. *Antisense lncRNAs* originate from the opposite strand of protein-coding genes; their transcription start site localizes downstream from that of the coding gene; these RNAs transcribed in the antisense direction often display at least partial overlap with the coding sequence of the corresponding mRNA.
3. *Long intergenic ncRNAs* are encoded between coding genes and do not overlap with exons of other genes; they are transcribed independently.
4. *Circular lncRNAs* form closed loops derived from the splicing of protein-coding RNAs.
5. *Enhancer RNAs* are located to genomic loci that activate gene expression.
6. *Bidirectional RNAs* are transcribed from the same promoter as coding genes, but in the opposite direction.

LncRNA genesis encompasses chromatin remodeling, lncrna gene transcription, and generally, but not always, transcript $5'$-capping, polyadenylation, and alternative splicing [287].

Chromatin that packages DNA controls access to genetic information; it must be remodeled for gene transcription. Messenger RNAs, synthesized in the nucleus and

packaged by RNA-binding proteins that form ribonucleoparticles, are exported to the cytoplasm.

Certain enzymes change states of DNA methylation and histone modifications, whereas ATP-dependent chromatin-remodeling complexes modify nucleosome composition or positioning.

LncRNAs contribute to the regulation of gene expression, as they are involved in epigenetic, transcriptional, or post-transcriptional regulation. They can partner with DNA- and histone-modifying enzymes, such as DNA methyltransferase DNMT1 and histone demethylase KDM1a, polycomb repressive complex and mixed lineage (myeloid–lymphoid) leukemia (MLL) factor [101] activatory complex, in addition to nucleosome remodelers such as the Swi/SNF-like BAF complex [404]. They can also serve as scaffolds.

Nuclear lncRNAs are involved in epigenetic and transcriptional regulation of neighboring (in *cis*) or distant genes (in *trans*). Cis-acting lncRNAs can regulate lncRNA transcription. Trans-acting lncRNAs operate as recruiters or decoys of chromatin modifiers and transcription factors to activate or silence gene transcription. They can interact with RNA-binding proteins, such as members of the polycomb and trithorax groups. LncRNAs that recruit the polycomb repressive complex, a chromatin remodeler, provoke gene silencing. Other lncRNAs sequester RNA-binding proteins and transcription factors. On the other hand, some lncRNAs enhance protein-coding gene transcription. Activating nonprotein-coding RNAs have enhancer-like activity; they activate neighboring genes using a mechanism involving DNA looping between the lncRNA and its target gene [287]. In addition, lncRNAs can bind to RNA polymerase-2, thereby regulating the transcriptional machinery.

Cytoplasmic lncRNAs control mRNA splicing, translation, stability, and degradation, as they bind to mRNAs or proteic components of ribonucleoproteic complexes. Additionally, LncRNAs can act as decoys or sponges for proteins or microRNAs, preventing their activity, and they can serve as templates for small peptide synthesis. Some lncRNAs undergo a posttranscriptional processing. The lncRNA neat1 menε[102] and neat2 (malat1),[103] which mainly localize to the nucleus, are processed into menRNA and mascRNA[104] respectively, the latter lodging

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[101] Also known as trithorax homolog Trx1 and histone Lys methyltransferase KMT2a.

[102] Neat1: nuclear paraspeckle-enriched assembly transcript-1. It is also dubbed virus-inducible noncoding RNA (vinc) and menε. The mouse locus Men1 (multiple endocrine neoplasia-1) in the chromosomal region 19qA produces two ncRNA isoforms, the menβ and smaller menε transcripts.

[103] Neat2 is also called metastasis-associated lung adenocarcinoma transcript malat1. It was first found in metastasizing nonsmall-cell lung carcinomas. It is upregulated by hypoxia and regulates EC proliferation and hence angiogenesis, among other tasks.

In addition, neat2 can serve as both a diagnostic and a prognostic marker in cancers. Inhibition of neat2 can decrease cell motility and increase apoptosis [405]. Neat2 may predict tumor recurrence after liver transplantation.

[104] mascRNA: malat1-associated small cytoplasmic RNA.

exclusively in the cytoplasm [406]. Both neat2 and its product mascRNA serve as immunoregulators.

Unlike microRNAs and ^{Y}RNA fragments, which mainly lodge in the cytosol, lncRNAs are predominantly retained in the nucleus. Whereas microRNAs and ^{Y}RNA fragments are secreted in microvesicles with cytoplasmic components, even in the absence of injury, lncRNAs are released upon cellular damage with nuclear material [360]. In general, because their longer sequence is more vulnerable to cleavage and less protected by RNA-binding proteins, lncRNAs are more susceptible to ribonuclease than small ncRNAs.

LncRNAs cross the plasma membrane, their egress being regulated by ceramide.[105] They can thus reside in body fluids (e.g., blood, urine, and breast milk) [376]. They are observed in exosomes, microvesicles, and apoptotic bodies and are linked to RNA-binding proteins, such as nucleolin, nucleophosmin NPm1, and argonaute protein Ago2, a factor of the RINC, LDLs, and HDLs. A substantial number of circulating ncRNAs are associated with ribonucleoproteins.

Chromatin remodeling determines, at least partly, transcriptome programming during cardiogenesis and its reprogramming during pathogenesis. Improper reorganization of epigenetic marks leads to aberrant gene expression and organ dysfunction. LncRNAs participate in epigenetic regulation; they are designated ^{Epi}lncRNAs.

For example, HOX transcript antisense intergenic RNA (hotair) binds to PRC2 and promotes methylation of histone H3 at Lys27 trimethylation (H3K$_{27}$me^3) at the promoter region of target genes, causing gene silencing, in addition to the demethylase KDM1a that suppresses methylation of histone H3 at Lys4 (H3K4) carried out by the MLL complex [407].

Antisense long nonprotein-coding RNA in the CDKN2B gene locus in the chromosomal 9p21 region suppresses synthesis of the cell cycle regulator CKI2b. It is linked to AAA, myocardial infarction, and stroke [408].

Hypoxia upregulates some lncRNAs. Hypoxia-induced endoplasmic reticulum stress (ERS)-regulating lncRNA (hyperlnc)[106] enables expression of human pericyte markers, such as PDGFRβ, DES, α-smooth muscle actin, and neural and glial antigen NG2 [409]. In addition, hyperlnc supports the connection between pericytes and ECs, thereby reducing endothelial permeability. On the other hand, hyperlnc deficiency favors ERS, ERS-related transcription factors being activated, and reduces the viability and proliferation of pericytes and primes their dedifferentiation. Therefore, hyperlnc modulates pericyte differentiation, proliferation, and recruitment to ECs.

Pericytes stabilize the microvasculature (arterioles, venules, and lymphatics). They can be a source of regenerative cells that participate in the repair after injury and ischemia [408]. A subset of pericytes can serve as a reservoir of multipotential mesenchymal stem cells that may replace lost CMCs or other cardiovascular cell types.

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[105]Neutral sphingomyelinase-2 regulates secretion of exosomes and microRNAs [376].

[106]Also known as ENSG00000262454.

Hyperlnc concentration is markedly reduced in the myocardium of failing hearts, which can be linked to abnormal microvasculature and fibrosis [409]. In addition, hyperlnc expression correlates significantly with pericyte markers in human lungs from patients with idiopathic pulmonary arterial hypertension.

Liver-expressed LXR-induced sequence lncRNA (lexis) regulates cholesterol efflux and synthesis.

The role played by 16401 lncRNAs was assessed in seven types of human cell lines (six transformed cell lines and one human-induced pluripotent stem cells) [410]. The function of most of the tested lncRNAs was specific to a given cell type and often limited to a single cell type. A total of 499 lncRNAs influence cell proliferation, exclusively for a single cell type, among which 372 and 299 have loci distal from a protein-coding gene or mapped enhancer, respectively [410].

Endotheliocytes express relatively high concentrations of the lncRNAs neat2,[107] tug1,[108] meg3,[109] linc00657, and linc00493 [411]. Production of the lncRNAs neat2, tug1, meg3, and linc00657 increases in ECs exposed to hypoxia, but not that of linc00493. Neat2 controls both epigenetic gene regulation and RNA splicing. It promotes proliferation of ECs, but prevents their migration. It supports expression of endothelial cyclins CcnA2, CcnB1, and CcnB2 and hampers that of CKI1a and CKI1b. It interacts with CBx4,[110] assisting recruitment of coactivators to the promoters of cell cycle control genes, which are linked to an increased number of transcriptionally active histone marks.

2.2.4.1 Endothelial Long Nonprotein-Coding RNAs

Endotheliocytes respond to local and systemic stimuli and can change from quiescence to activation, then becoming a source of prooxidant, proinflammatory, vasoconstrictory, and clotting mediators. LncRNAs operate at the onset and after EC activation. The formation of 27 lncRNAs is markedly changed (two-fold), but among them, that of a single one, the PDZD2-206 transcript (ENST00000509256.1), which encodes PDZ domain-containing protein PDZD2,[111] is downregulated [412]. Many genes with a regulatory function are coexpressed with lncRNAs (e.g., CDK13, EIF3A, HNRNPH2, LHX2, PDSS2, SMEK2, RIF1, SPRY2, STAU2, Wdr20, and WDR42A [Dcaf8]).

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[107]Neat2: nuclear-enriched assembly transcript, or metastasis-associated lung adenocarcinoma transcript malat1. It was first found in metastasizing nonsmall-cell lung carcinomas and is upregulated by hypoxia. It regulates EC proliferation and hence angiogenesis.

In addition, neat2 can serve as both a diagnostic and a prognostic marker in cancers. Inhibition of neat2 can decrease cell motility and increase apoptosis [405]. Neat2 may predict tumor recurrence after liver transplantation.

[108]Tug1: taurine upregulated gene transcript-1.

[109]Meg3: maternally expressed gene transcript-3.

[110]Polycomb chromobox homolog CBx4, a sumo ligase, is also called polycomb protein-2 (Pc2).

[111]Also known as PDZ domain-containing protein PDZK3.

The proangiogenic lncRNA linc00323-003[112] and mir503hg[113] are induced in ECs subjected to hypoxia [413]. However, linc00323-003 elicits tube formation, whereas mir503hg does not markedly affect tube formation.

The lncRNA meg3[114] represses the production of VEGFa and VEGFR1 [413]. Its expression increases in senescent ECs, precluding their angiogenic activity.

The lncRNA neat2[115] is proangiogenic [413]. It upregulates VEGF expression. In particular, it favors tumor angiogenesis, as it promotes FGF2 production in tumor-associated macrophages.

Formation of the lncRNA igf2as[116] increases in myocardial microvascular ECs of T2DM rats, impeding cell proliferation via downregulation of IGF2 and VEGF expression [413].

The lncRNA miat[117] is an angiogenic activator [413]. Hyperglycemia can launch miat formation in microvascular and retinal ECs.

The epigenetically controlled endothelial nuclear lncRNA mantis,[118] the gene of which lodges in the antisense strand of an intron of the gene encoding annexin-A4, is upregulated during angiogenesis; it maintains the endothelial angiogenic phenotype [414]. Its synthesis is prevented by the histone demethylase KDM5b. Its production is downregulated in patients with idiopathic pulmonary arterial hypertension. It favors not only angiogenic sprouting, but also alignment of ECs subjected to hemodynamic stress. It interacts with SMARCa4 (BRG1), the catalytic subunit of the Swi/SNF-related, matrix-associated, actin-dependent regulator of chromatin, thereby promoting transcription of the NR2F2, Smad6, and SOX18 genes.

In ECs, the lncRNA sencr[119] induces cell proliferation and migration in addition to differentiation (i.e., embryonic stem cell commitment into ECs) and hence angiogenesis [415]. It stimulates EC migration, as it upregulates proangiogenic chemokines CCL5 and CX_3CL1. It also governs the differentiation status of vascular smooth myocytes. This EC- and vSMC-enriched lncRNA exists in a full-length (sencr^{V1}) and alternatively spliced variant (sencr^{V2}).

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[112]Also known as ENST00000446910, chromosome 21 open reading frame C21orf130, DSCAM1-3, and pred42.

[113]Mir503hg: miR503 host gene. lincRNA maps onto chromosome X (Xq26). It may serve as a tumor marker.

[114]Meg3: maternally expressed gene transcript-3.

[115]Neat2: nuclear-enriched assembly transcript-2. It is also called metastasis-associated lung adenocarcinoma transcript malat1.

[116]Igf2as: IGF2 antisense RNA.

[117]Miat: myocardial infarction-associated transcript.

[118]It corresponds to n342419. Mantises are carnivorous insects.

[119]Sencr: smooth myocyte- and EC-enriched migration/differentiation-associated lncRNA.

2.2.4.2 Long Nonprotein-Coding RNAs and Mitochondrial Function

Mitochondria serve as controllers of the cellular redox status and as stress sensors via proteins encoded by mitochondrial and nuclear DNA and integrators of stressors.[120]   These hubs also integrate cues from several metabolic pathways (ATP production and fatty acid and amino acid metabolism), modulating nuclear gene expression[121] implicated in cell survival and growth [368].

Mitochondria and the nucleus mutually communicate to adapt their respective activities to the environment. The *anterograde signaling*, that is, the nuclear-to-mitochondrial control, modulates mitochondrial genesis and activity according to cellular needs. The nuclearly encoded mitochondrial proteome is regulated in a coordinated manner by transcription factors (e.g., NRF1–NRF2), among which are some nuclear receptors (e.g., ERRs and PPARs) and their cofactors (PGC1α–PGC1β). These effectors are regulated by sensors of changes in metabolic conditions.[122] Under nuclear stress (e.g., DNA damage), the anterograde signaling lowers mitochondrial metabolism.[123]

On the other hand, mitochondria signal their functional state and can trigger a compensatory cellular response. The *retrograde signaling* launches a metabolic reprogramming, ultimately leading to cell adaptation or death. In addition, when mitochondria are severely damaged, the retrograde signaling engages cellular stress responses without nuclear involvement, such as the intrinsic apoptotic pathway [368].

Mitochondrial function is monitored via their products, which act as signaling effectors that modulate the activity of regulators (e.g., sirtuins, HIF1α, AMPK, TOR, and calmodulin).[124]

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[120]Human mitochondrial DNA encodes 2 rRNAs, 22 tRNAs, and 13 proteins of the respiratory chain.

[121]About 99% of the mitochondrial proteome is encoded by the nuclear genome and synthesized in the cytoplasm as precursors imported into mitochondria, especially for proteins involved in replication, transcription, and translation of the mitochondrial genome and for several components of oxidative phosphorylation. Altered mitochondrial protein import causes accumulation of mistargeted, nuclearly encoded mitochondrial protein precursors in the cytosol, priming *mitochondrial precursor protein accumulation stress* response, which comprises a global reduction in protein translation and an increase in the proteasomal degradation of proteins.

[122]A decrease in ATP production is sensed by AMPK, which increases cellular NAD^+ concentration, thereby activating sirtuin-1, which activates PGC1α and subsequently mitochondrial genesis and energy metabolism [368].

[123]Impaired mitochondrial genesis and function stimulate P53, which represses PGC1α- and PGC1β-driven programs [368]. Activation of PARP1 and PARP2 in response to DNA breaks lowers cellular NAD^+ concentration, thereby precluding the activity of sirtuin-1 and hence mitochondrial function.

[124]Acetyl-CoA and ^{S}adenosylmethionine, which are substrates for acetylation and methylation of histones, respectively, affect the chromatin state and hence gene transcription. Calcium release from mitochondria stimulates transcriptional programs that rely on NFAT and NFκB. The NAD^+/NADH ratio favors mitochondrion genesis and fatty acid oxidation via sirtuin-1 and -3 and represses ribosomal RNA transcription in the nucleolus. The AMP/ATP imbalance activates

Table 2.27 Long nonprotein-coding RNAs (lncRNAs) and mitochondrial function (Source: [368]; *GAS* growth arrest-specific noncoding, single-stranded RNA, *lipcar* long intergenic noncoding RNA predicting cardiac remodeling, *sammson* survival-associated mitochondrial melanoma-specific oncogenic long intergenic noncoding RNA, *sncmtRNA* sense nonprotein-coding mitochondrial RNA)

Type	Location	Targets	Effect
GAS5	Ubiquitous	TOR (?)	Increases mitochondrial protein synthesis and activity
Lincrnap21	Ubiquitous	HIF1α, VHL	Increases HIF1α stability, promotes glycolysis
Lipcar	Plasma	ND	ND
SncmtRNA	Proliferating cells	^{Mt}16S rRNA, chromatin	Cell proliferation
Sammson	Melanoma	C1qBP	Regulates mitochondrial protein synthesis

Question mark (?) denotes denotes uncertainty

Redox stress inactivates TOR, the master cytosolic modulator of protein synthesis. In addition, TOR controls mitochondrial genesis and activity; this hub integrates antero- and retrograde signaling [368].

Nonprotein-coding RNAs act as messengers between the nucleus and mitochondria, hence contributing to the synchronization of cellular processes. The term *mitolncRNAs* (^{Mt}lncRNAs) refers to lncRNAs lodging inside the mitochondrion, which are encoded either by the mitochondrial genome (category 1)[125] or in the nucleus and subsequently enter the mitochondrion (category 2; Table 2.27) [368].[126]

In addition to functioning as messengers, lncRNAs can also indirectly modulate mitochondrial function via regulation of metabolic components, such as AMPK, TOR, HIF1α, and sirtuins. The TOR kinase regulates synthesis of mitochondrial

catabolism. The NO gas produced by mitochondria hampers oxidative phosphorylation and rRNA maturation.

[125]SncmtRNA, is preferentially expressed in highly proliferating normal and cancerous cells. Normal proliferating cells express two antisense transcripts of this lncRNA, both of which are downregulated in tumoral cells. Both sense and antisense transcripts of sncmtRNA can reside in the nucleus and may participate in retrograde signaling [368]. Three other cell-specific lncRNAs are transcribed by the mitochondrial genome and processed by the mitochondrial RNase-P protein MRPP1 encoded in the nucleus. Another lncRNA encoded by the mitochondrial genome is the chimeric (fusion) transcript lipcar formed between the 5′-end of the PGHS2 gene and 3′-end of the CYTB gene, which is detected in plasma from HF patients.

[126]The lincRNA sammson, which predominantly localizes to the cytoplasm of human melanoblasts, is associated with mitochondria. Among sammson interactors, the complement component-1 q-subcomponent-binding protein (C1qBP; or glycoprotein GC1qBP), a trimer that primarily localizes to the mitochondrial matrix and multifunctional chaperone, interacts with hyaluronan (hence its other name hyaluronan-binding protein [HaBP1]) regulates mitoribosome assembly and mitochondrial protein synthesis; sammson hence supports mitochondrial protein synthesis. Another sammson binding partner, 5′–3′-exoribonuclease XRN2, is required for rRNA maturation in the nucleus.

proteins encoded by the nuclear genes such as ^{Mt}TFa, components of ^{ETC}complex-*I* and -*V*, and mitochondrial ribosomal proteins. The gas5 lncRNA promotes growth arrest upon TOR inhibition. The hif1as lncRNA is associated with angiogenesis. The hypoxia-induced lincrnap21 disrupts the connection between VHL and HIF1α, hence impeding HIF1α ubiquitination and proteasomal degradation.

2.2.4.3 Long Nonprotein-Coding RNAs in Adverse Cardiac Remodeling

As CMCs are particularly enriched in mitochondria, mitochondrial lncRNAs are released during myocardial infarction [360].

Three lncRNAs, CoroMarker,[127] bat5,[128] and il21ras1,[129] are CoAD markers (Table 2.28) [420]. In a population of patients suffering from chest pain, Coro-Marker correlates with genes involved in atherosclerosis, such as IL15RA and ADORA1, which encode IL15 receptor-α and adenosine receptor-A1, respectively.

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[127]CoroMarker is the lncRNA AC100865.1, which is stably detected in plasmatic vesicles probably released from monocytes [416]. It is sensitive to coronary artery disease.

[128]BAT: human lymphocyte antigen HLA$_B$-associated transcript (protein-coding mRNA).

Spliceosome ATP-dependent RNA helicase HLA$_B$-associated transcript product BAT1, which is a splicing factor required for association with U2 small nuclear ribonucleoprotein (hence its other alias UAP56), is also called DEAD (Asp–Glu–Ala–Asp) box-containing protein DDx39b.

Large proline-rich HLA$_B$-associated transcript product BAT2, which is also termed proline-rich coiled coil-containing protein PrRC2a, may contribute to pre-mRNA splicing regulation.

HLA$_B$-associated transcript BAT3 is also named large proline-rich protein scythe, an inducer of apoptosis, which is involved in DNA damage-induced apoptosis, and BCL2-associated athanogene (BAG) family molecular chaperone regulator BAG6. It prevents aggregation of misfolded proteins as a component of the BAT3 cytosolic protein quality control complex. It maintains target proteins in a soluble state and participates in their proper delivery to the endoplasmic reticulum; otherwise, it promotes their sorting to the proteasome for degradation after ubiquitination.

The protein BAT5 is also termed αβ-hydrolase domain -containing protein αβ Hd16a, a lipase that preferentially processes long-chain unsaturated monoacylglycerols. It hydrolyzes medium-chain saturated (C14:0) and long-chain unsaturated (C18:1, C18:2, and C20:4) monoacylglycerols and 15-deoxy (12,14)-prostaglandin-J$_2$ 2-glycerol ester and has slight diacylglycerol and TAG activity [417].

The human major histocompatibility complex (MHC), which mediates interactions among leukocytes and between leukocytes and other cell types, is categorized into class-*I* (with three major sets: HLA$_A$–HLA$_C$) and -*II* (HLA$_{DP}$, HLA$_{DQ}$, and HLA$_{DR}$) immunity genes (i.e., antigen-processing genes), which encode plasmalemmal receptors for antigens that mediate the clonal activation of cytotoxic and helper T lymphocytes, respectively [418]. The MHC class-*III* region also contains numerous genes, such as those that encode complement system agents (e.g., factor-B, -C2, and -C4), cytokines (e.g., TNFSF1–TNFSF3), heat shock proteins (HSP70s), and the immunity-related genes encoding BAT1 to BAT9. The BAT1–BAT5 gene cluster localizes to the vicinity of the genes encoding TNFα (TNFSF1) and TNFβ (TNFSF2).

Single nucleotide polymorphisms BAT2 (8671*G), BAT3 (8854*C), and BAT5 (22655*C and 9569*A) are correlated with the development of Kawasaki disease and coronary artery aneurysm; BAT2, BAT3, and BAT5 haplotypes (ATTGTG and ATCATG) are associated with a higher susceptibility of these maladies [419].

[129]il21ras1: IL21R antisense RNA-1.

Table 2.28 Long nonprotein-coding RNA (lncRNA) markers in CVD (Source: [376]; *lipcar* long intergenic ncRNA predicting cardiac remodeling, *myheart* myosin heavy-chain-associated RNA transcript, *sencr* smooth muscle and endothelial cell-enriched migration and differentiation-associated lncRNA, *uca1* urothelial carcinoma-associated lncRNA-1)

LncRNA	Disease
CoroMarker	Coronary atherosclerosis
Lipcar	Myocardial infarction
Myheart	Myocardial infarction
Lncpparδ	Coronary atherosclerosis
Sencr	Heart failure
Uc004cov4	Hypertrophic cardiomyopathy
Uc022bqu1	Hypertrophic cardiomyopathy
Uca1	Myocardial infarction

On the other hand, CoroMarker correlates negatively with genes implicated in apoptosis and redox stress, such as BAX and GSTT1, which encodes glutathione ^{S}transferase GSTθ1. CoroMarker plays an important role in inflammation involved in atherogenesis, as it favors expression of the proinflammatory cytokines IL1β, IL6, and TNFSF1 [420].

LncRNAs include regulators of CMC differentiation, such as braveheart and fendrr,[130] and of cardiac remodeling, such as myheart or chast.[131]

In hypertrophic cardiomyopathy, BRG1,[132] which interacts with multiple transcription regulators, in addition to several HDACs and poly^{ADP}ribose polymerases, is activated. The epigenetic regulator BRG1 induces MYH7 gene transcription and represses that of the MYH6 gene, which encode fetal β- and adult α-myosin

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[130]Braveheart (Bvht) is required for the evolution of the nascent mesoderm to a cardiac fate. It enables activation of the core cardiac transcription factors (e.g., GATA4, GATA6, HAND1, HAND2, MesP1, NKx2-5, and TBx5) [287]. It functions upstream from mesoderm posterior homolog MesP1 (or bHLHc5), a master regulator of multipotent cardiovascular progenitors, as it targets the core cardiac transcription factors [421]. It interacts with the suppressor of zeste SuZ12, a component of the polycomb repressive complex, PRC2, during CMC differentiation, acting as a decoy for PRC2 to exclude PRC2 from the MESP1 promoter in cardiac progenitor cells. MesP1+ progenitors engender all cardiac cell types (CMCs, ECs, and smooth myocytes). In ESCs, Bvht then contributes to the epigenetic regulation of cardiac lineage commitment. Other regulators control mesoderm evolution. The transcription factor FoxF1 allows the adequate partition of the lateral mesoderm into splanchnic and somatic mesoderm lineages. The lncRNA FenDRR (FoxF1 adjacent enhancer-associated noncoding developmental regulatory RNA) is transiently expressed in the lateral mesoderm of mid-gestational mouse embryos and interacts with both repressive PRC2 and activatory MLL complexes, enabling proper differentiation of cells derived from the lateral mesoderm, such as those of the future heart [422].

[131]Expression of the lncRNA myheart is primed during CMC maturation and reduced in hypertension-induced cardiac hypertrophy and HF, an event that correlates with the change in the MYH7/MYH6 ratio during maladaptive cardiac remodeling.

[132]BRG: brahma (SMARCa2)-related gene product.

heavy chain, respectively.[133]   Pathological cardiac stress activates the BRG1–HDAC2–HDAC9–PARP1 chromatin repressor complex to complex on MYH6 and MYH7 promoters for antithetical control, thereby precluding transcription of the lncRNA myheart (mhrt; myosin heavy chain [MYH7]-associated RNA transcript) in the heart, activating aberrant MYH7 gene expression and repressing MYH6 gene transcription, hence re-inducing fetal gene expression that is linked to maladaptive hypertrophy and that can evolve to HF.

The lncRNA cardiac hypertrophy-related factor (chrf) regulates MYH7 expression, as it acts as a sponge for antihypertrophic miR489 [384].   Whereas miR489 reduces adverse hypertrophy initiated by angiotensin-2 via MyD88, chrf downregulates miR489 expression [423].

Cardiac-specific myheart, which abounds in adult CMCs, protects the heart from adverse remodeling. Its concentration is elevated after myocardial infarction [376].

The most abundant isoform. Mhrt779, is a potent inhibitor of adverse cardiac hypertrophy. Alternative splicing of MYH7 antisense transcripts engenders a cluster of Mhrts (size 709–1147 nucleotides) [424].   The mhrt lncRNAs are linked to a transcriptional start site overlapping, but in the opposite direction, the MYH6 promoter, and the coding sequence extends into that of the MYH7 gene; hence, Mhrts correspond to both promoter-associated lncRNAs and antisense lncRNAs with respect to MYH6 and MYH7 respectively [384]. In addition, these alternatively spliced antisense transcripts are implicated not only in cis-regulation at the MYH6–MYH7 locus, but also in the trans-regulation of other cardiac genes.

The Mhrt splice variants abound in the adult myocardium (but in neither endo-nor the epicardium) [424]. Cellular stress activates the BRG1–HDAC–PARP chromatin repressor complex and prevents Mhrt transcription, enabling cardiomyopathy development. In a given cell, the BRG1 complex inhibits both MYH6 and Mhrt and reactivates MYH7 expression. On the other hand, the Mhrt spliced isoforms tether to and antagonize the chromatin remodeler and helicase BRG1, which can connect to mhrt and chromatinized (but not naked) DNA, preventing cardiac adverse hypertrophic gene reprogramming. Mhrt directly interacts with BRG1 helicase domain [424], as a decoy to regulate its chromatin remodeling activity, similar to braveheart, which operates as a decoy for the PRC2 complex [383].

Cardiac apoptosis-related lncRNA, which was identified in CMCs exposed to anoxia, is another sponge for miR539 that regulates PHB2 expression [425]. The prohibitin complex, with its two subunits (Phb1–Phb2), which localizes to the inner mitochondrial membrane, operates in mitochondrial genesis. In addition, Phb2 is an estrogen receptor-binding protein that represses gene transcription and a regulator of mitochondrial fission and fusion dynamics. Its production declines with the increased formation of miR539, causing mitochondrial fission and CMC apoptosis.

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[133]During postnatal cardiac maturation, a switch occurs from β- to α-myosin heavy chain owing to reciprocal up- and downregulation of MYH6 and MYH7 gene expression.

In addition to carl and chrf, hulc[134] lincMD1[135] LncRNA H19, which contributes to cardiofibroblast proliferation and fibrosis via repression of the DuSP5–ERK1/2 axis [428], and the circular RNAs cdr1as[136] also sequester miRs.

The long nonprotein-coding RNA cardiac hypertrophy-associated epigenetic regulator (chaer) acts at the onset of cardiac hypertrophy via a stress-induced transient interaction between chaer and the polycomb repressor complex PRC2, which depends on TOR [430]. Chaer recognizes the epigenetic modulator KTM6a, a PRC2 component, and sequesters PRC2, which forms repressive histone marks, thereby hampering histone H3 Lys27 methylation at the promoter regions of genes involved in cardiac hypertrophy and enabling prohypertrophic gene expression [407]. Interaction between chaer and PRC2 is transiently induced after hormone or stress stimulation via TORC1, and causes epigenetic reprogramming.[137] Inhibition of chaer production in the heart before, but not after, the onset of hypertension attenuates cardiac hypertrophy.

The cardiac hypertrophy-associated transcript chast is upregulated in maladaptive cardiac hypertrophy [432]. Chast impedes the autophagy regulator PlekHm1[138] impeding CMC autophagy.

The mitochondrial long intergenic ncRNA predicting cardiac remodeling lipcarin (i.e., lncRNA lipcar or uc022bqs.1) is downregulated in an early phase after MI but is upregulated during later stages [433]. It has a strong prognostic power for the future development of detrimental cardiac remodeling, HF after MI, and death.

Concentration of urothelial carcinoma-associated transcript-1 (uca1), an indicator of pulmonary cancer, declines shortly after MI and increases 3 days after cardiac injury. Concentration of miR1, which may regulate uca1 expression, follows an opposite time course [376].

Blood concentrations of uc004cov4 and uc022bqu1 are higher in hypertrophic obstructive cardiomyopathy than in hypertrophic non-obstructive cardiomyopathy and healthy subjects [376].

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[134]Hulc: highly upregulated in liver cancer lncRNA. It raises the level of the transcription factor PPARα (nuclear receptor NR1c1), which activates the ACSL1 gene promoter, launching long-chain acylCoA synthase formation [426]. It also suppresses miR9 targeting of PPARA mRNA. It thus supports lipogenesis and the accumulation of intracellular TGs and cholesterol.

[135]lincMD: long intergenic ncRNA muscle differentiation-1. It is a sponge for miR133 and miR135 [427]. It regulates specific myogenic factors required for the onset of late muscle gene transcription, such as mastermind-like protein MamL1 and myocyte enhancer factor MEF2c.

[136]Cdr1as: cerebellar degeneration-related protein-1 (circular) antisense lncRNA. It targets miR7a and miR7b, at least in murine hearts, the expression of which rises in myocardial ischemia–reperfusion injury. These miRs protect myocardium, as they inhibit PARP, an executioner of apoptosis [429]. In addition, miR7a synthesis is upregulated by UPR in MI.

[137]During the cell cycle, cyclin-dependent kinase CDK1 phosphorylates KTM6a (Thr345) and thus enhances the KTM6a–hotair and –xist binding affinity [407].

XX individuals silence one of their X chromosomes using xist, which coats one of the X chromosomes and recruits chromatin-silencing factors such as the polycomb repressor complexes PRC1 and PRC2 via the PCGF3–PCGF5–PRC1 complex (PCGF: polycomb group RING finger protein) [431].

[138]PlekHm1: pleckstrin homology domain-containing protein-M1. The CHAST gene lodges in the opposite PLEKHM1 gene strand.

The long nonprotein-coding RNAs wisper[139] abounds in cardiofibroblasts and regulates their survival, proliferation, and migration in addition to extracellular matrix protein deposition and hence cardiac fibrosis after injury [434]. Repression of wisper attenuates fibrosis after MI. Wisper cooperates with T-cell intracellular antigen TIA1-related protein (TIAR), an RNA-binding protein involved in the regulation of alternative pre-mRNA splicing, to control the expression of a profibrotic form of procollagen lysine 2-oxoglutarate 5-dioxygenase PLOD2,[140] which is implicated in collagen crosslinking and stabilization of the matrix.

2.2.5   Ribosomal RNA

Ribosomal RNA participates in protein synthesis. It is also detected in the blood circulation. It is released upon tissue damage and cell death.

Extracellular ribosomal RNA may play several pathophysiological roles in atherosclerotic plaques, as it can increase vascular permeability, initiate blood coagulation and hence thrombus formation, recruit leukocytes and support a proinflammatory phenotype of monocytes and macrophages, and favor CMC death [376].

2.3   Clinical Types of Markers

Markers of diseases can be classified into three main groups:

1. *Screening markers* for individuals who have not yet developed any apparent disease (i.e., for early diagnosis and evolution prevention), as these markers modulate diet and activity in addition to potentially beneficial drug effects
2. *Diagnostic markers* in subjects in whom advanced-stage disease is already suspected
3. *Prognostic markers* in patients who have the disease

2.3.1   Screening

Potential screening markers comprise molecules associated with [435]:

1. *Inflammation*, such as CRP (or pentraxin-1; Sects. 2.3.2.3 and 5.4.5.22), IL-6 (Sect. 5.4.5.25), and secreted and lipoprotein-associated phospholipase-A2.

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[139]wisper; wisp2 superenhancer-associated RNA.

[140]Also known as lysyl hydroxylase LH2.

2. *Hemostasis*, such as fibrinogen and plasminogen-activator inhibitor-1 (or serpin-E1)
3. *Neurohormonal control*, such as renin and BNP
4. *Insulin resistance*, such as insulin and hemoglobin-A1c.[141]
5. *Endothelial dysfunction*, such as homocysteine and albuminuria (urinary albumin).
6. *Myocardial damage*, such as cardiac FABP ([He]FABP), which diffuses rapidly into the circulation after CMC injury, and growth differentiation factor GDF15, which also rises sharply after myocardial ischemia

Screening tests incorporate two features: *sensitivity* and *specificity*. Sensitivity and specificity refer to the proportion of diseased persons with a positive test and to the proportion of healthy individuals with a negative test, respectively.

Markers fulfill several criteria:

- Marker expression in the vascular tissue correlates with blood concentration.
- Marker blood level is related to structural and functional changes.
- Its method of identification is easy, reproducible, and has a low cost.
- The marker yields good sensitivity and specificity in detecting the pathological condition.

2.3.1.1   Hemostasis Factors

Many components of hemostasis, such as fibrinogen, [D]dimer, coagulation factor-*VIII*, plasmin–antiplasmin complexes, and von Willebrand factor, in addition to adamts13, correlate with an increased risk of coronary atherosclerosis complications, stroke, and mortality [436].

Von Willebrand factor (vWF) carries coagulation factor-*VIII* and promotes platelet adhesion and aggregation at sites of vascular injury. Its activity depends on the metallopeptidase adamts13, which cleaves large and highly procoagulant vWF multimers into smaller and less procoagulant forms. Deficiency in vWF quantity or activity causes bleeding in von Willebrand disease. Severe deficiency in adamts13 engenders the clotting thrombocytopenic purpura. Elevated plasmatic vWF and reduced adamts13 concentrations provoke a procoagulant state, and hence the combination of the two is associated with an increased risk of atherosclerosis

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[141]Glycation of hemoglobin, an heterotetrameric ($[\alpha\beta]_2$) metalloprotein, results from a non-enzymatic reaction between glucose and the β chain. When the blood glucose concentration is elevated, glucose attaches to hemoglobin in RBCs. Glycated hemoglobin (HbA1c) is measured to identify the average plasma glucose concentration over prolonged periods. High HbA1c amounts indicate defective control of the blood glucose concentration and is associated with CVD. Even with a glycohemoglobin concentration below 6.9% (52 mmol/mol), the CVD risk of T1DM patients is on average twice that of the general population [51].

complications [437]. In addition, both vWF and adamts13 are dysregulated in inflammation.

2.3.1.2 Periodontitis

Periodontitis is a chronic inflammatory disease caused by bacterial infections that destroy the tissue between the tooth surface and gingiva and provoke the loss of connective tissue attachment and teeth in addition to erosion of alveolar bone, which defines mild and moderate (radiographic bone loss 21–44%) or severe periodontitis (<66% remaining bone).

Periodontitis is more commonly observed in patients with atherosclerosis and its acute complications (MI and stroke) along with abdominal aortic aneurysm [438]. The risk for coronary atherosclerosis increases in these individuals.

2.3.2 Diagnosis

CRP, brain natriuretic peptide, urinary albumin, renin, and homocysteine are predictors of cardiovascular events with possible high death risk, but they moderately yield supplementary prediction in addition to known risk factors. In fact, markers often provide information that correlates with known data.

2.3.2.1 Troponin, Myosin-Binding Protein-C, and CyR61

In emergency departments, triage of patients with acute chest pain relies on the measurements in the blood circulation of cardiac troponins cTnnI or cTnnT, as only a small proportion of patients with acute myocardial infarction (AMI) have ST segment elevation on ECG trace. After the onset of myocardial ischemia, CMC death can occur within 15 min and necrosis within 4–6 h [439].

The fundamental contractile unit of the CMC, *sarcomere*, comprises thick (myosin) and thin filaments (actin; Vol. 5, Chap. 5. Cardiomyocytes). The thin filament is a helix of two filamentous actins, the groove of which contains TPM, to which troponin is attached. The troponin heterotrimer controls the Ca^{2+}-dependent interaction of actin and myosin:

1. Inhibitory cTnnI prevents actin–myosin cross-bridge formation in the absence of Ca^{2+} ions.
2. Tropomyosin-bound cTnnT links TPM on actin to the troponin complex.
3. Calcium-binding cTnnC regulates activation of actin.

Troponins are synthesized in fast- (ftTnns encoded by the TNNC2, TNNI2, and TNNT3 genes) and slow-twitch skeletal (stTnns encoded by the TNNC1, TNNI1, and TNNT1 genes) and cardiac myocytes (cTnns encoded by the TNNC1, TNNI3,

and TNNT2 genes]). Hence, troponins TnnI and TnnT are selectively produced in the heart, but not TnnC, which is shared with the slow-twitch skeletal muscle. Both cTnnI and cTnnT are distinguished by regions of different amino acid sequences.

The TNNT2 mRNA generates multiple alternatively spliced transcripts encoding different proteins (cTnnT1–cTnnT4), cTnnT3 being the dominant isoform in the normal adult heart [439].

During embryo- and fetogenesis, stTnnI is expressed in the heart instead of cTnnI. Troponin fetal isoforms, the expression of which is downregulated postnatally, can be re-expressed postnatally under certain pathological circumstances such as chronic HF. The cTnnT2 subtype is expressed to a greater extent than cTnnT3 in failing hearts.

Cardiac troponins commonly serve as markers for acute myocardial necrosis. Both cTnnI and cTnnT are released in free forms and cleared by cardiac lymphatics. They are also liberated as noncovalent binary (cTnnI–cTnnT and predominantly cTnnI–cTnnC dimers) and ternary complexes (cTnnT–cTnnI–cTnnC trimer) [439]. Moreover, all troponin isoforms undergo redox modifications and circulate as oxidized and reduced forms. Troponin is also released from the sarcomere both as intact proteins and degradation products after proteolysis by calpain-1, caspase, or MMP2 [439]. These enzymes also reside in blood and the circulating cTnn complexes can also be degraded within blood.

However, cTnn concentrations are commonly elevated in acute, reversible or irreversible, myocardial injury and are thus unrelated to necrosis linked to ACS, in addition to chronic diseases (e.g., chronic HF [myocardial strain], diabetes mellitus, pulmonary arterial hypertension, stable coronary artery disease, and chronic kidney disease, especially end-stage renal disease), along with extreme exercise [439]. Cardiac troponins thus serve as organ-specific, disease-nonspecific markers.

In addition, cTnnI and cTnnT are released slowly. Cardiac myosin-binding protein-C (MyBPc3),[142] another marker of cardiac injury, is more abundant than cardiac troponins and is released more rapidly after MI. Its blood concentration rises and falls more rapidly than those of cTnnT and cTnnI [440].

Known diagnostic markers include cardiac troponin-I and -T for MI and B-type natriuretic peptide for HF.

In mouse HF and human cardiomyopathies, accumulation of branched-chain α-keto acids (BCKAs) and associated defective catabolism of branched-chain amino acids (BCAAs) are markers of metabolic reprogramming [441]. The transcription factor KLF15 regulates BCAA catabolism in the heart. Impaired BCAA catabolism causes redox stress in response to hypertension. Elevated BCKA concentrations provoke superoxide production in mitochondria.

Cardiac-specific troponins are characterized by better specificity and sensitivity than that of the cardiac marker CK-MB (which combines two isozymes ^{SkM}CK [muscle] and ^{Br}CK [brain]). Elevated troponin level is a CoAD diagnosis criterion

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[142]Members of the MYBPC family include three isoforms specific to slow and fast skeletal and cardiac muscles.

that can require early revascularization. However, blood troponins are not necessarily augmented before 12 h after the onset of symptoms.[143]

Concentration of MyBPC3 is significantly higher in patients with acute myocardial necrosis than those without AMI [440]. The discriminatory power of MyBPC3 (>10 ng/l) for AMI is similar to that of high-sensitivity troponins hscTnnT and hscTnnI, but higher than that of hscTnnT in patients with chest pain for less than 3 h (i.e., in early attenders to emergency departments).

In coronary thrombi of ACS patients, the amount of cysteine-rich angiogenic inducer CYR61 transcripts[144] is elevated with respect to that in lymphocytes and monocytes [442]. In a murine ischemia–reperfusion model, myocardial Cyr61 expression markedly rises. Concentration of CyR61 also significantly increases in STEMI with respect to non-STEMI, unstable angina, and stable CoAD, whether coronary thrombi are present or not. Therefore, soluble CyR61 can serve as a marker of ACS due to a thromboembolic event or acute myocardial injury. It can surpass the diagnostic potential of troponin [442].

2.3.2.2 Fatty Acid-Binding Proteins

Fatty acid-binding proteins (FABPs) are small cytosolic widespread proteins that are members of the ILBP set[145] (Sect. 5.4.5.20). They reversibly bind to intracellular hydrophobic ligands, such as fatty acids and their acylCoA derivatives, with high affinity and broad specificity for transfer to various cellular compartments, (e.g., endoplasmic reticulum, nucleus, lipid droplets, peroxisomes, and mitochondria) for storage, oxidation, membrane synthesis, signaling, and activation of nuclear receptors, which are transcription factors, such as peroxisome proliferator-activated receptors (PPARs [NR1c1–NR1c3]) [445].

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[143]Other markers, such as $_\mathrm{NT}$proBNP, glycogen phosphorylase-BB, CRP, myeloperoxidase, and MMP-9, do not yield additional information with respect to cardiac troponin-T.

[144]Cysteine-rich angiogenic inducer CyR61 is also named insulin-like growth factor (IGF)-binding protein IGFBP10 and CTGF, CyR61, NOv family member CCN1. In addition to this heparin-binding matrix protein, the CCN family includes connective tissue growth factor (CTGF or CCN2), nephroblastoma overexpressed protein (NOv1 or CCN3), and Wnt-induced secreted protein WISP1 to WISP3 (or CCN4 to CCN6). It promotes cell proliferation, adhesion, and migration, and hence angiogenesis. It is a ligand for activated platelets binding to integrin-$\alpha_{2B}\beta_3$ [442]. This immediate early gene product is synthesized in response to mechanical stress, hypoxia, and exposure to TNFSF1, TGFβ, and PDGF [442]. In cutaneous fibroblasts, it upregulates synthesis of factors involved in angio- and lymphogenesis, inflammation, and matrix remodeling (e.g., VEGFa, VEGFc, MMP1, MMP3, TIMP1, uPA, PAI1, and integrins Itgα_3 and Itgα_5 via ERK1 and ERK2 [443]). In smooth myocytes, it can increase the production of VEGF, α_VItg, smooth muscle α-actin (Actα), along with myosin heavy chain MyH11 [444]. Its gene transcription regulation depends on heparin binding. On the other hand, it downregulates type-*I* collagen α1 and α2 subunits encoded by the COL1A1 and COL1A2 genes.

[145]iLBP: intracellular lipid-binding protein.

Table 2.29 Fatty acid-binding proteins (Source: [445])

Gene	Protein names and aliases	Location
Fabp1	^{Li}FABP (liver)	Liver, intestine, pancreas, stomach, kidney, lung
Fabp2	^{In}FABP (intestine)	Intestine, liver
Fabp3	^{He}FABP (heart), MDGI	Cardiac and skeletal muscle, brain, kidney, lung, stomach, adrenal gland, brown adipose tissue, mammary gland, placenta, ovary, testis
Fabp4	^{AdC}FABP (adipocyte), ALBP, aP2	Adipocyte, macrophage, dendrocyte, skeletal myocyte
Fabp5	^{Ep}FABP (epiderm), ^{KC}FABP, paFABP	Keratinocyte, adipocyte, macrophage, dendrocyte Brain, lung, kidney, liver, heart, skeletal muscle, intestine, stomach, tongue, spleen, retina, lens, testis, mammary gland, placenta
Fabp6	^{Il}FABP (ileum), gastrophin, ^{In}BABP	Ileum, adrenal gland, stomach, ovary
Fabp7	^{Br}FABP (brain), BLBP, MRG	Central nervous system, retina, gliocyte, mammary gland
Fabp8	^{My}FABP (myelin) PMP2	Peripheral nervous system, Schwann cells
Fabp9	^{Te}FABP (testis) TLBP, Perf, Perf15	Testis, salivary gland, mammary gland

These isoforms (FABP1–FABP9) were named according to the organ in which they were first identified or predominate. In fact, they have a widespread distribution in the organism (*ALBP* adipocyte lipid-binding protein, *aP* adipocyte protein, *BABP* bile acid-binding protein, *BLBP* brain lipid-binding protein, *MDGI* mammary gland-derived growth inhibitor, *MRG* MDRI-related gene product, *paFABP* psoriasis-associated-FABP, *Perf* perforatorial protein, *PMP* peripheral myelin protein, *TLBP* testis lipid-binding protein)

Synthesis of FABPs is mostly regulated at the transcriptional level. The human genome encodes at least nine FABPs (Table 2.29). These isoforms differ with regard to ligand affinity and specificity.

Circulating FABPs are markers of injury in the liver (FABP1), intestine (FABP2), myocardium (FABP3), kidney (FABP4), and brain (FABP7) [167].

Cardiac FABP3, which can be detected 1 h after acute coronary occlusion, is a more reliable early index of cardiac damage than MB [446]. Moreover, it has a sensitivity superior to that of cardiac TNNT, but lacks specificity in detecting acute MI [447].

Plasmatic FABP4 concentration is correlated with atherosclerosis, insulin resistance, type-2 diabetes, obesity, hypertension, left ventricular hypertrophy, intimal hyperplasia, and endothelial and cardiac systolic and diastolic dysfunction [167]. FABP4 is synthesized in adipocytes, macrophages, and ECs of capillaries and veins, but not arteries. However, ectopic production in and secretion of FABP4 from coronary arterial ECs contribute to neointima formation and restenosis after stenting [448].

Plasmatic FABP5 concentration is also associated with metabolic syndrome, although the correlation was not as strong as that of FABP4 [167]. The metabolic syndrome results from interference between metabolic and inflammatory pathways, that is, *metaflammation* (Vol. 12, Chap. 3. Metabolic Syndrome).

2.3.2.3   C-Reactive Protein (Pentraxin-1)

The circulating pentameric molecule CRP[146] (median concentration 0.8 mg/l in healthy young adults; half-life ∼19 h [450]), a prototypical acute phase reactant,[147] is the first identified pattern recognition receptor. It binds to phosphocholine on the surface of dead or dying cells and some types of bacteria, thereby activating the complement system via the C1q complex.[148]

It is synthesized mainly in the liver at a low rate in normal conditions. CRP isoforms have distinct functions. Its production increases, and it is secreted following tissue injury and inflammation, especially under the control of IL-6 released by immunocytes [451]. Concentration of CRP hence rises in response to inflammation (>500 mg/l [450]).

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[146]It was discovered in 1930 by W.S. Tillet and T. Francis as a substance in the serum of patients with acute inflammation linked to lobar pneumonias [449]. This substance reacted with the somatic fraction-C derived from pneumococci, a carbohydrate common to the pneumococcus species, which is distinct from both type-specific capsular polysaccharide and nontype-specific somatic nucleoprotein. It is not limited to the serum of individuals with pneumococcal infection but is also observed in sera of patients with streptococcus and staphylococcus infections [449].

[147]The acute-phase response results from tissular damage, inflammation, infection, and cancers. Other acute-phase proteins include peptidase inhibitors in addition to coagulation, complement, and transport proteins, but only CRP and serum amyloid-A have a strong sensitivity and response speed [450].

[148]Extrinsic ligands include many glycans, phospholipids, and other constituents of microorganisms, such as capsular and somatic components of bacteria, fungi, and parasites, along with plant products [450]. Once it is connected to its ligands, CRP is recognized by C1q and activates the classical complement pathway, engaging the main adhesion molecule of the complement system C3 and the terminal membrane attack C5–C9 complex. Bound CRP yields secondary binding sites for complement factor-H, thereby regulating alternative pathway amplification.

C-reactive protein (CRP) pertains to the pentraxin family with pentraxin-2[149] and pentraxin-3.[150] Short monomeric, insoluble, tissue-associated, proinflammatory, pentraxin-1 (Ptx1) dissociates from pentameric CRP.

Circulating pentameric CRP binds to activated platelets and microparticles enriched in lysophosphatidylcholine, which predominantly derive from activated cell membranes and carry microRNAs. On microparticles, the pentamer is converted into CRP monomers that reside on the microparticle surface [452].

In vitro, monomeric CRP provokes synthesis of chemokines in neutrophils and integrins in monocytes, enhances neutrophil adhesion to ECs, and delays neutrophil apoptosis. Monomeric Ptx1 enhances platelet adhesion to ECs and supports formation of neutrophil–platelet aggregates.

Pentraxin-1 can be detected in atherosclerotic plaques and infarcted myocardium, where it colocalizes with inflammatory leukocytes.

Plasmatic concentrations of CRP, nitrotyrosine, a molecular marker for NO-derived oxidants, and the CXCL8 chemokine (or IL-8) are predictors of acute cardiovascular events in patients with stable or unstable angina [453]. Plasmatic CRP concentration is a stronger predictor of cardiovascular events than that of LDL^{CS} [451]. In women, 77% of cardiovascular events occur with LDL^{CS} concentrations below 160 mg/dl (4.1 mmol/l) and 46% below 130 mg/dl (3.4 mmol/l) [454]. Plasmatic CRP concentration rises in patients at moderate (1–3 μ g/ml [or mg/l]) and high risk (>3 μ g/ml) for future cardiovascular events [454].

Plasma concentration of pentraxin-3, which is highly expressed in the heart and mainly by dendrocytes, macrophages, and ECs in response to inflammatory stimuli, reaches a peak approximately 7 h after MI. It also lodges in atherosclerotic lesions and vasculitis in humans. It is induced by oxidized LDLs in smooth myocytes. This acute phase protein, but neither Ptx1 nor other cardiac markers ($_{NT}$proBNP, TNNT, and CK), predicts 3-month mortality [455].

2.3.2.4 Other Inflammatory Indices

The *neutrophil-to-lymphocyte ratio* and *monocyte-to-high-density lipoprotein ratio* are used as indicators of inflammation and redox stress. These markers are related to adverse cardiovascular outcomes, especially after ACS treated by primary percutaneous coronary intervention (e.g., post-stenting restenosis and altered cardiac rhythm such as atrial fibrillation). They are also associated with a bad prognosis in cancers, which is also linked to systemic inflammation (throughout the body).

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[149]Also known as α1-glycoprotein and serum amyloid P-component (SAP). It is encoded by the APCS gene.

[150]Long pentraxin Ptx3 is also called tumor-necrosis factor-α-induced protein TNFAIP5 and TNF-inducible gene product TSG14.

In HF, neurohumoral processes are aimed at preserving contractile performance to compensate for a defective myocardium. However, sympathetic nervous system hyperactivity is associated with a poor prognosis in HF patients.

Concentration of β ARs and GRK2 in circulating lymphocytes correlates with those in the myocardium, particularly in right atrial appendages of HF subjects [456]. Moreover, after left ventricular assist device implantation, partial restoration of myocardial function and, in CMCs, of β AR signaling and attenuated GRK2 activity is associated with diminished GRK2 activity in lymphocytes. Therefore, lymphocytic GRK2 concentration may complement the blood concentration of $_{NT}$proBNP to assess HF severity. Furthermore, lymphocyte GRK2 expression correlates significantly with age and left ventricular ejection fraction [457]. The prognostic power of ^{LC}GRK2 concentration is similar to that of $_{NT}$proBNP in HF patient surveillance, as ^{LC}GRK2 concentration independently predicts mortality.

Inhibitors of the adrenergic and angiotensin signaling are used in HF patients. β-Adrenoceptor antagonists (β-blockers) that prevent CMC apoptosis and normalize inotropy are used in congestive heart failure and hypertension [458]. Among them, carvedilol is a β-arrestin-based agonist of β-adrenoceptors that elicits GPCR phosphorylation by GRK, β-arrestin recruitment, β AR internalization, ERK activation, and EGF receptor transactivation. Pepducins are lipidated peptides derived from the GPCR intracellular loop that can stimulate or inhibit downstream signaling from their cognate receptors. ^{β2AR}Pepducin operates in a similar manner to carvedilol, stabilizing the β2AR conformation used as a substrate for GRK-mediated phosphorylation and β-arrestin-1 binding, but induces CMC contraction, whereas carvedilol does not [458]. Lymphocytic GRK2 concentration is not linked to therapy efficiency and does not predict mortality [456].

Elevated plasmatic concentration of γ-glutamyl transferase (GGT)[151] correlates with hypertension, diabetes, and vascular diseases, in addition to liver damage. GGT is an indicator for an early diagnosis and prognosis in HF patients [459].

Cytokines released by inflammatory leukocytes cause vSMC apoptosis or transdifferentiation to an osteochondrogenic lineage, mineralizing atherosclerotic plaques. Arterial wall calcification (Vol. 10, Chap. 3. Adverse Wall Remodeling), which is linked to altered stress field and phosphate metabolism in addition to hyperglycemia, hyperlipidemia, inflammation, and redox stress, is a frequent and severe complication of atherosclerosis [460]. Calcium released in apoptotic bodies forms a nidus of microcalcification that can destabilize the lesion, but can also later fuse to form macrocalcification, which may protect against plaque rupture.

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[151]γ-Glutamyl transferase, or glutathione hydrolase-1 proenzyme, is a membrane-bound extracellular enzyme that cleaves γ-glutamyl peptide bonds in glutathione and other peptides and transfers the γ-glutamyl moiety to acceptors. In particular, it cleaves the γ-glutamyl bond of extracellular glutathione, releasing free glutamate and the dipeptide cysteinyl–glycine, which is hydrolyzed to cysteine and glycine by dipeptidases, in addition to glutathione conjugates and other γ-glutamyl compounds [108]. It can also catalyze transpeptidation, transferring the γ-glutamyl moiety to an acceptor amino acid to form a new γ-glutamyl compound. It has an optimal activity at 37 °C and pH 8.2 [459].

The cytokine TNFSF1 released primarily by macrophages not only primes apoptosis and hence the accumulation of apoptotic bodies but also activates osteogenic reprogramming in vSMCs via BMP2, Msx2, and Wnt [461].

Signaling by GTPases of the Rac category influences the synthesis of growth factors and cytokines. In macrophages, Rac2 promotes VEGFa formation and hence arteriogenesis in response to ischemia [461]. In addition, Rac2 precludes Rac1 activation. On the other hand, activated Rac1 supports NFκB signaling and ROS production via NOx and hence the action of NLRP3 inflammasome, which promotes IL1β maturation. Pattern recognition receptors and cholesterol crystals, which destabilize lysosomes, trigger the expression of IL1β via the Rac1–NFκB pathway.

Interleukin-1β released by SMC-derived plaque macrophages also favors vascular calcification and can thus serve as a marker of advanced atherosclerotic plaque calcification. On the other hand, Rac2 hinders Rac1-mediated IL1β production in bone marrow-derived macrophages [461]. In the late stages of atherosclerosis, the Rac2 concentration diminishes.

2.3.2.5 Extracellular Vesicles

Information is exchanged between cells using (1) short-distance signaling via intercellular contact, (2) mid-range intercellular transfer of paracrine messengers (autacoids) and EVs (size 50 nm–2 μ m), and (3) remote transmission of hormones, growth factors, and cytokines. Intercellular communication is fostered by juxta-, para-, and endocrine messengers (i.e., autacoids and endocrine regulators), which are secreted either as isolated messengers or contained in EVs.

Once released, EVs travel to neighboring or relatively remote recipient cells, in which they can be incorporated via receptors or fusion of vesicle and target cell plasma membranes and subsequently enter the cytoplasm and even the nucleus.

Extracellular vesicles of multiple cellular origin mediate intercellular communication in physiological and pathological conditions. Circulating EVs yield information on the disease state.

Most cell types, in particular ECs, vascular smooth myocytes, CMCs, monocytes, macrophages, and platelets, release extracellular vesicles of different sizes, composition, and content, and hence subcellular origin. Extracellular vesicle composition and content are markers of cellular activation and injury.

Extracellular vesicles originate from diverse subcellular compartments. They contain cytoplasmic and plasmalemmal components of source cells. Membrane-encapsulated extracellular particles are formed in different pathways, carry diverse cargos to target cells, and have distinct functions. They are detected in all bodily fluids (e.g., cerebrospinal fluid, saliva, and urine), especially in the blood circulation.

A given cell type secretes various types of EVs characterized by their size, density, intracellular origin, and tetraspanin composition. In particular, cells release many types of nanovesicles with similar size, density, and surface markers.

Extracellular vesicles are secreted under basal conditions and upon stress exposure, particularly in inflammatory sites [462]. The origin, composition, and concentration of EVs can also be used as diagnostic and prognostic markers. Their composition, in particular the microRNA content, indeed signals cell activation and injury.

Extracellular vesicles are covered by a lipid bilayer, which contains transmembrane proteins. They serve as containers of numerous soluble proteins (e.g., growth factors, cytokines, chemokines, receptors, and cell adhesion molecules) in addition to genetic information (DNA, mRNA, premature and mature microRNAs, long nonprotein-coding RNAs, and other nucleic acid species) and free ribonucleoprotein complexes.

The vesicular content is transmitted to acceptors (e.g., ECs, monocytes, and platelets). The surface receptors of EVs allow their capture by neighboring recipient cells that can then incorporate proteic, lipidic, and RNA messengers. Extracellular vesicles that circulate in the extracellular space (including the blood) protect RNA from degradation by nucleases.

Plasmatic concentrations of EVs result from the balance between production and rapid clearance by Kupffer cells in the liver[152] and macrophages in the spleen and lung that renders the long-range transmission of information unlikely [462].

Red blood capsule-derived EVs undergo major uptake by the liver ($\sim$45%), followed by bone ($\sim$23%), skin ($\sim$10%), muscle ($\sim$6%), spleen ($\sim$3%), kidney ($\sim$3%), and lung ($\sim$2%) [464].

The EV formation rate depends on the activation status of releasing cells. On the other hand, EVs are cleared using different mechanisms. Endothelial microvesicles are taken up by the endothelium owing to an annexin-5-phosphatidylserine receptor [464]. Platelet-derived microvesicles are taken up by ECs via EDIL3[153] in addition to macrophages via lactadherin[154] Microvesicle clearance also implicate

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[152]Kupffer cells are also named stellate macrophages and Kupffer–Browicz cells ($\sim$15% of the total liver cell population [463]). They line walls within the lumen of hepatic sinusoids formed by sinusoidal ECs (fenestrated discontinuous endothelium), whereas hepatic stellate cells coat sinusoid walls within the perisinusoidal space (or space of Disse) between hepatocytes and sinusoids. Kupffer cells are exposed to bacteria from the gut microbiota in addition to microbial debris and bacterial toxins. These scavenger cells remove material from the portal circulation (dead RBCs, microorganisms, toxins, degenerated cells, immune complexes, and toxics). They are involved in the hepatic response to stressors (e.g., infection, and ischemia). They release chemoattractants for CD8+ cytotoxic and regulatory T lymphocytes. During the progression from non-alcoholic steatosis to steatohepatitis and fibrosis, they produce chitotriosidase, which contributes to hepatic stellate cell activation [463]. They also synthesize thromboxane-A2 using PGhS and thus can cause portal hypertension via endothelin-1.

[153]EDIL3: EGF-like repeat and discoidin-I-like domain-containing protein-3. It is also integrin-binding developmentally regulated EC locus-1 protein DEL1. This angiogenic protein is an extracellular matrix protein expressed by ECs during embryological vascular development. It promotes adhesion of ECs via $\alpha_V\beta_3$-integrin [465]. It also supports EC migration and in vitro angiogenesis, is re-expressed in ischemic adult organs, and stimulates angiogenesis.

[154]Lactadherin, also called milk fat globule EGF-like motif-containing factor MFGE8 and secreted EGF repeat and discoidin domain-containing protein SED1, promotes VEGF-stimulated

Table 2.30 Characteristics of different types of extracellular vesicles, the size of vesicle subtype varying according to investigators (Source: [462])

	Exosomes	Microvesicles	Apoptotic bodies
Caliber	30–150 nm	150–1000 nm	1–5 μm
Content	Proteins Cytosolic content: proteins, lipids, RNA, miRs	Cell surface molecules RNA, miRs	Organelles Cytosolic content: proteins, lipids, nucleic acids (DNA, RNA, miRs)
Membrane	Impermeable	Impermeable	Permeable
Markers	Annexin-5 cell-specific markers	LAMP1, Tspan30, VPS23, lactadherin	Annexin-5,

Apoptotic bodies are formed during the late stage of apoptosis. Microvesicles (or microparticles) are smaller and created by membrane blebbing. They can be released from most cell types (e.g., circulating leukocytes, erythrocytes, and platelets, in addition to vascular cells and CMCs). Their number increases under stress. Exosomes are formed by the fusion of multivesicular bodies with the plasma membrane. Extracellular vesicles transfer their cargos to recipient cells by membrane fusion, endocytosis, or receptor-mediated binding. Microparticle-derived arachidonic acid, oxidized phospholipids, and chemokines may contribute to increased leukocyte adhesion on the vascular endothelium. Apoptotic bodies can prime IL-1α release by ECs. On the other hand, leukocyte-derived microparticles stimulate the secretion of anti-inflammatory cytokines

their uptake from the circulation by Kupffer cells and splenocytes. Exosome turnover relies on uptake via tetraspanins, integrins, and lactadherin [464].

Extracellular vesicles can be categorized into large, mid-size, and small particles. Small EVs can be further subdivided into subcategories according to proteic markers (e.g., Tspan28–Tspan30).

Many subtypes of EVs are described in the literature, such as ectosomes, exosomes, microvesicles, microparticles, membrane particles, and apoptotic vesicles (Vol. 10, Chap. 1. Architecture and Structure of the Vasculature). Extracellular vesicles mainly comprise (Table 2.30):

1. Exosomes (size 30–150 nm) originating from the endosomal circuit that are released upon fusion of multivesicular bodies with the plasma membrane
2. Microvesicles (size 150 nm–1 μm) that are directly shed from the plasma membrane
3. Apoptotic bodies (size 1–5 μm) that are released as blebs of apoptotic cells

Exosomes are permanently exported from cells, but microvesicles are released in response to cell activation or injury [464].

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neovascularization [108, 194]. It tethers to $\alpha_V\beta_3$- and $\alpha_V\beta_5$-integrins. It links apoptotic cells to phagocytes and promotes mucosal healing. It is cleaved into lactadherin short form and medin. Lactadherin on EVs mediates the cellular uptake by binding phosphatidylserine and cell surface integrin subunits α_V and β_3 on recipient cells [464].

Extracellular vesicles formed and released from the plasma membrane are often called microparticles, shed microvesicles, or *ectosomes*. Their sizes range from a few tens of nanometers to a few micrometers.

Intracellular vesicles generated in the endosomal and multivesicular compartment are secreted when they fuse with the plasma membrane. These endosome-derived vesicles released in the extracellular medium are named *exosomes*. Exosomes are defined by their size (<150 nm) and content (i.e., endosomal proteins).

Extracellular vesicles, at least those from monocyte-derived dendrocytes, are categorized into large, medium-sized, and small vesicles [466].

- Large EVs contain HSP90b1 (endoplasmin) and possibly other endoplasmic reticulum-associated proteins.
- Large and medium-sized EVs possess actinin-4, mitofilin, and possibly other mitochondrial proteins, but not small EVs.
- Small EVs are characterized by the presence of flotillins, annexin-5, -6, and -11, syntenin-1, endosomal sorting complexes required for transport (ESCRT1) complex subunits TSG101, ChMP4a, ChMP4b, and VPS4b, ADAM10, ACE, and EHD4. Among them, syntenin-1 and TSG101 are specific to exosomes.

Patients with risk factors, such as hypertension, metabolic syndrome, diabetes, and/or hypercholesterolemia, have elevated concentrations of circulating microvesicles derived from ECs and platelets [464].

In addition, microvesicular content changes. Diabetic patients have a reduced amount of endothelial miR26 and miR126-3p in circulating endothelial microvesicles [464]. In EVs from smokers, the quantity of platelet-derived miR223 declines, whereas amounts of miR29b and type-2 U6 small nuclear RNA (RNU6-2) increase with respect to controls. Familial hypercholesterolemia is associated with higher numbers of platelet-derived microvesicles in addition to tissue factor-rich microvesicles, which have a procoagulant activity. Patients with low miR26a and miR126 levels are at a high risk for CoAD.

In patients at a high CoAD risk, levels of circulating CD144+ endothelial microvesicles and/or CD31+ annexin-5+ microvesicles in addition to low concentrations of miR126-3p and miR199-5p predict future cardiovascular events [464]. In patients with pulmonary hypertension, the circulating level of SelE+ endothelial microvesicles is related to a bad mid-term prognosis. Microvesicular cystatin-C (Cst3), an inhibitor of cysteine peptidases, serpin-F2, and CD14, a coreceptor for TLR2–TLR6 heterodimer and bacterial lipopolysaccharide, are related to an elevated risk for future vascular events.

Small Extracellular Vesicles

Small EVs cross 220-nm pore filters. They incorporate subpopulations of exosomes and other types of extracellular vesicles.

Flotillin-1, 70-kDa heat shock proteins (inducible HSPa1 and constitutive HSPa8), and MHC class-*I* and *II* proteins are present in all types of EVs, albeit to various extents [466].

Four subcategories of small extracellular vesicles (SECVs) can be defined according to the types of contained tetraspanins among the three species used as markers (Tspan28–Tspan30) [466].

1. SECVs enriched in Tspan28 to Tspan30 and endosomal markers are genuine exosomes.
2. SECVs devoid of Tspan28 and Tspan30, but enriched in Tspan29 are linked to the plasma membrane and have an early endocytic signature. Tspan29+ small EVs have transmembrane proteins, integrin-α_{2B}, and MMP14.
3. SECVs devoid of Tspan28 to Tspan30 are not associated with the endosomal route.
4. SECVs enriched in matrix constituents (collagen, fibronectin, S100a18, and serpin-F1), complement (C3 and C4a), and serum-derived factors (prothrombin [coagulation factor F*II*] and albumin) are also not linked to the endosomal route.

Pulmonary arterial hypertension (PAHT) is characterized by hyperplasia and hypertrophy of vascular smooth myocytes and proliferation of ECs in small pulmonary arteries, increasing the mean pulmonary arterial pressure and hence right ventricle afterload. The severity of PAHT is associated with elevated amounts of circulating microvesicles released from stressed and apoptotic cells [467].

Circulating small vesicles from PAHT mouse models are heterogeneous in size (30–430 nm) and carry proteins characteristic of exosomes and microvesicles. Administration of lung- and plasma-derived vesicles from these mice to healthy mice increases right ventricular mass and pulmonary vascular wall thickness [468]. Lung-derived small vesicles (LDSVs), but not plasma-derived small vesicles (PDSVs), contain high amounts of endothelial mRNAs. In addition, small vesicles from monocrotaline-treated mice possess altered levels of microRNAs, such as miR145, miR328, and miR451, which are involved in pulmonary arterial hypertensive remodeling. Furthermore, the concentration of phosphodiesterase-5 is augmented in both LDSVs and PDSVs.

Extracellular vesicles from monocrotaline-treated mice induce differentiation of bone marrow cells into endothelial progenitor cells. When these cells are transplanted into healthy mice, they home to the lung, where they raise the muscularization of pulmonary arterial walls, thereby contributing to adverse pulmonary vascular remodeling; mobilization of bone marrow-derived progenitor cells and their recruitment to the pulmonary vasculature are also observed in experimental hypoxia-induced PAHT [467]. Extracellular vesicles from monocrotaline-treated mice upregulate the activity of growth factor implicated in PAHT genesis from bone marrow-derived progenitor cells.

Exosomes

Exosomes are produced on the endosomal route from multivesicular bodies spontaneously or in response to external mechanical and chemical cues (e.g., shear stress, hypoxia, cytokines, and chemotherapeutic agents), whereas larger vesicles are expelled from the plasma membrane.

Exosomes contain membrane-anchored receptors, adhesion molecules, signaling mediators, and nucleic acids. They transfer their cargos to recipient cells, thereby affecting cell fate.

Exosomes influence immune response and are involved in pathogenesis and in tumor growth. Cancerous cells exploit exosomes to favor tumor growth and control premetastatic niche formation [469]. Exosomes can intervene in cancer growth, angiogenesis, metastasis, and immune surveillance.

Syntenin, a cytosolic adaptor that binds to the intracellular domain of syndecans, is implicated in the genesis of a subset of exosomes. Syntenin also binds to programmed cell death-6-interacting protein (PdCD6IP), which is involved in endocytosis, multivesicular body genesis, membrane repair, cytokinesis, apoptosis, and maintenance of tight junction integrity. PdCD6IP connects the syntenin–syndecan complex to the ESCRT machinery, thereby operating in endosomal membrane budding and scission and generating intraluminal vesicles that are released as exosomes when multivesicular endosomes fuse with the plasma membrane [469]. Regulators of these processes include, on the cytosolic side, the small GTPase ARF6 and its effector phospholipase-D2 and, on the luminal side, the endoglycosidase heparanase.

The cytoplasmic protein Tyr kinase Src, which controls cell growth, proliferation, adhesion, and motility, and signal transduction induced by growth factors and integrins, stimulates the secretion of exosomes loaded with syntenin and syndecans [469]. This regulator of exosomal communication phosphorylates syndecans and syntenin and stimulates syndecan internalization, endosomal genesis, and secretion of exosomes having promigratory activity on ECs. It operates in syndecan endocytosis and syntenin–syndecan endosomal budding upstream from the ArfGEF cytohesin-2, ARF6, and PLD2. Syntenin endosomal budding depends on interaction with phosphatidic acid, the PLD2 product [469].

Exosomes can cause pulmonary hypertension, but not microvesicles [470]. Hypoxic lung-derived exosomes from patients with idiopathic pulmonary artery hypertension contain increased concentrations of miR19b, miR20a, miR20b, and miR145. On the other hand, mesenchymal stem cell-derived exosomes have elevated concentrations of anti-inflammatory, antiproliferative, and antimigratory microRNAs (miR34a, miR122, miR124, and miR127),[155] thereby preventing

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[155]For example, miR34a elicits senescence of endothelial progenitor cells, fibroblasts, and vascular smooth myocytes and precludes angiogenesis. MiR124 attenuates proliferation and migration of hypertensive fibroblasts in addition to their chemokine (CCL2) production.

vascular remodeling and the development of hypoxic pulmonary hypertension. However, nonmesenchymal bone marrow stem and progenitor cells have no effect.

Numerous microRNAs of mesenchymal stem cell-derived exosomes (e.g., miR101a, miR122, miR193, miR224, and miR302b) provoke apoptosis or suppress proliferation or migration of cancerous cells [470].

Adipose tissue macrophages (ATMφs) release exosomes containing microRNAs that modulate insulin sensitivity. MicroRNA-155 is differentially produced in ATMs in lean and obese rodents. Diet-induced obesity alters the amounts of various microRNAs in ATMφ-generated exosomes. In particular, the amount of miR155 rises. MicroRNA-155 diminishes glucose uptake and impedes insulin signaling [471].

In addition, macrophage-derived leukotriene-B_4, which operates via its cognate receptor LTb_4R,[156] participates in metabolic dysfunction; it can reduce insulin signaling in hepatocytes and myocytes [472]. It supports macrophage chemotaxis, elicits inflammatory phenotype in macrophages, promotes hepatic lipogenesis, decreases insulin-stimulated glucose uptake in myocytes, increases gluconeogenesis, and alters insulin-mediated suppression of glucose output in hepatocytes, as it reduces insulin-primed Gi- and JNK-dependent PKB phosphorylation and augments IRS1 and IRS2 phosphorylation [472].

Galectin-3 (GaL3), a β-galactoside-binding lectin on monocytes and macrophages, the concentration of which rises progressively during differentiation of monocytes into macrophages, is a macrophage-secreted molecule that can promote inflammation and inhibit insulin receptor. Galectin-3 serves as a chemoattractant for monocytes and macrophages. Intracellular GaL3 participates in cell migration and apoptosis. Extracellular GaL3 mediates cellular adhesion and cytokine production. Homocysteine, which is produced by methionine processing, stimulates activity of the GaL3 inducer, NFκB. Galectin-3 contributes to macrophage activation, especially in atherosclerotic plaques [473]. Hyperhomocysteinemia is observed in patients with unstable CoAD. In monocytes, it participates in the production of superoxide and cooperates with ROS in exosome formation. Galectin-3 is thus involved in inflammation, redox stress, and cardiac fibrosis.

ATMφ-derived TSG101+,[157] syntenin-1+,[158] TSpan29+, and TSpan30+ exosomes are taken up by adipocytes, hepatocytes, and skeletal myocytes, the major

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[156] The LTb_4R receptor (BLT_1) lodges on dendrocytes, granulocytes, macrophages, effector CD8+ T cells, and T_{H1}, T_{H2}, and T_{H17} cells.

[157] TSG101: tumor susceptibility gene product-101. It is a component of the ESCRT*I* complex, also called vesicular protein sorting VPS23. This subunit contributes to regulating the vesicular transfer of material, the sorting of endocytic ubiquitinated cargos into multivesicular bodies. It is involved in the exosomal release of syndecan, syntenin-1, and tetraspanin-30 (a.k.a. granulophysin and lysosomal-associated membrane protein LAMP3).

[158] Syntenin-1 (syndecan-binding protein SdcBP1). It is an adaptor implicated in the trafficking of transmembrane proteins, neuro- and immunomodulation, and exosome genesis in cooperation with syndecans Sdc1 and Sdc4 and programmed cell death-6 protein-interacting protein PdCD6IP, and tumorigenesis.

insulin target cells [471]. In particular, neighboring adipocytes take up exosomes containing myeloid cell-specific microRNAs. MicroRNA-155 contributes to insulin resistance in obesity. MicroRNA-155 released by proinflammatory macrophages targets the ligand-activated transcription factor, nuclear receptor NR1c3 (PPARγ) [471]. However, other exosomal microRNAs contribute to the modulation of insulin sensitivity. ATMφ-derived exosomal miRs from obese mice impair cellular insulin sensitivity. On the other hand, ATMφ-derived exosomal miRs from lean mice attenuate obesity-induced insulin resistance. Lean mice receiving ATMφ-derived exosomes from obese mice develop glucose intolerance and insulin resistance. Conversely, in obese mice, injection of ATMφ-engendered exosomes from lean mice normalizes insulin signaling.

Exosomes mediate intercellular communication among tumoral cells and between them and their surrounding stroma. Sunitinib-resistant renal carcinoma cells[159] transfer the long nonprotein-coding RNA activated in renal cell carcinoma with sunitinib resistance (lncarsr) to sensitive cells via exosomes. Lncarsr competitively connects to tumor-suppressive microRNAs miR34 and miR449 and raises amounts of receptor protein Tyr kinases Axl and hepatocyte growth factor receptor in recipient cells [475]. Lncarsr packaging into exosomes involved the ribonucleoprotein hnRNPa2b1. Inhibitors of both Axl and HGFR resensitize lncarsr+ cells to sunitinib.

Microvesicles

Plasmatic concentration of endothelial microvesicles is a marker of endothelial dysfunction. Plasmatic concentration of platelet and leukocyte microparticles rises in HF and cardiac valvulopathies [462].

Phosphatidylserine+ microvesicles of different cellular origin bind to the platelet scavenger receptor ScaRb3 and increase ADP-dependent platelet activation, thereby facilitating thrombosis. On the other hand, phosphatidylserine of endothelial microvesicles can interact with endothelial phosphatidylserine receptor in an annexin-

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[159]Sunitinib is used in chemotherapy. This drug is a multitargeting receptor protein Tyr kinase inhibitor, especially of VEGFR and hence an angiogenesis inhibitor. A feedforward loop exists between long nonprotein-coding RNA activated in renal cell carcinoma with sunitinib resistance (lncarsr) and yes-associated protein (YAP) [474]. Lncarsr interacts with YAP to hamper its phosphorylation by the large tumor suppressor LaTS1, thereby facilitating YAP nuclear translocation and favoring expansion of renal tumor-initiating cells. Conversely, YAP promotes the transcription of lncarsr.

1-dependent manner to prevent apoptosis [462]. Lactadherin[160] allows the clearance of circulating PSer+ microvesicles by macrophages in the spleen and liver.

Microvesicles contain MMP2, MMP3, MMP7, and MMP13, which can assist crossing of the extracellular matrix to reach target neighboring cells (mainly) or blood circulation [462].[161] Microvesicles derived from atherosclerotic plaques possess the adamalysin ADAM17. Microvesicles can also engender ROS production.

Exposure of ECs to microvesicles shed from senescent ECs or circulating endothelial microvesicles from ACS patients provokes premature aging, elevated β-galactosidase activity, redox stress, abnormal phosphorylation of MAPK and PKB, and upregulation of P53, CKI1a, and CKI2a [476]. Endotheliocyte senescence is associated with a progressive shedding of procoagulant microvesicles, augmented tissue factor activity, attenuated NOS3 activity and hence NO-mediated inhibition of platelet aggregation. These microvesicles are endowed with an angiotensin convertase activity and AT_1 receptors. Inhibitors of AT_1, MAPK, or PKB prevent microvesicle-primed endothelial senescence. Premature endothelial senescence and thrombogenicity result from angiotensin-2-induced redox-sensitive activation of the MAPK module and PKB.

2.3.2.6   Genomic Loci and Variants

New markers can be found from reproducible variants in genes, RNAs, and metabolites associated with the investigated disease. Current technology allows the examination of hundreds to thousands of single-nucleotide polymorphisms to search for significant associations with any disease (Chap. 7).

For example, a locus on chromosome 9p21 that does not contain genes linked to established CoAD risk factors, but near genes encoding cyclin-dependent kinase inhibitors CKI2a and CKI2b implicated in cellular senescence and apoptosis, is associated with the early onset of myocardial infarction [435].

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[160]Lactadherin is also called sperm-associated antigen (SpAG10), breast epithelial antigen (BA46), secreted EGF repeat and discoidin domain-containing protein (SED1), and milk fat globule-EGF factor (MFGE8). The lactadherin precursor is encoded by the Mfge8 gene [107]. It is produced by mammary epitheliocytes; this cell surface protein is secreted as part of the milk fat globule membrane. This 364-amino acid glycoprotein is cleaved into short lactadherin and medin, the latter deriving from the coagulation factor-like domain of lactadherin. Its structure possesses sequences similar to those of EGF-like domain and blood-clotting factors-V and -$VIII$ (C1 and C2 domains) [194]. In human arteries, it is observed in adventitial microvessels, medial smooth myocytes, and some ECs. It contributes to the phagocytic removal of apoptotic cells and the maintenance of the intestinal epithelial barrier. It supports mucosa healing and angiogenesis [108]. It connects to $\alpha_V\beta_3$- and $\alpha_V\beta_5$-integrins in addition to phosphatidylserine-enriched cell surfaces, hence promoting cell adhesion.

[161]Endothelial EVs released from the wetted and basal surface enter the blood circulation and target medial smooth myocytes, respectively.

2.3.3 Prognosis

Prognosis[162] predicting the likely outcome of a disease, upon treatment or not. In addition, appropriate use of markers can optimize treatment [477]. Prognostic indicators and estimators can be used to assess the disease evolution.

2.3.3.1 Renin

Reninemia (blood [plasmatic] concentration of renin) is inversely related to oral sodium intake. Reninemia can serve as a prognostic index for the cardiovascular event risk, independently of the blood pressure value [478].

2.3.3.2 IL1RL1 (ST2 Marker of Cardiac Stress)

Membrane-bound receptor $_\text{m}$IL1RL1[163] also exists as a soluble truncated form ($_\text{t}^\text{S}$IL1RL1 or ST2), which is secreted into the bloodstream. The messenger RNA of IL33 is markedly upregulated in cultured CMCs exposed to hypertension. Binding of IL33 to IL1RL1 ensures cardioprotection. However, IL33 connects competitively to $_\text{t}^\text{S}$IL1RL1 and is then unavailable for its IL1RL1 receptor, hence functioning as a decoy receptor for the IL33 cytokine. The soluble truncated IL1RL1 can be used to estimate the severity of adverse cardiac remodeling; elevated blood concentration in $_\text{t}^\text{S}$IL1RL1 can then predict outcomes in HF patients [479, 480].

2.3.3.3 S100

The secreted calcium-binding protein S100a9[164] is a discriminator between stable and unstable CoAD. Other proteins can be targeted using nuclear magnetic resonance spectroscopy and tandem mass spectrometry coupled with chromatography, but generally their amounts are low, as they are within nanomolar (e.g., troponin), picomolar (e.g., insulin), or femtomolar (e.g., tumor-necrosis factor) ranges [435].

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[162]προγνωσις: foreknowledge.

[163]The alias ST2 is an example of ambiguous abbreviation, as ST2 can represent suppression of tumorigenicity-2, syndecan-binding protein-2 (or syntenin-2) encoded by the SDCBP2 gene, and cytosolic dehydroepiandrosterone (DHEA)-preferring sulfotransferase-2A1 (a.k.a. bile salt sulfotransferase, dehydroepiandrosterone sulfotransferase [DHEAST] and hydroxysteroid sulfotransferase [HST]) encoded by the SULT2A1 gene, in addition to interleukin-1 receptor-like 1 (IL1RL1). This member of the IL-1 receptor family is also called IL33R, as its ligand is the cytokine IL-33.

[164]Also known as calgranulin-B, myeloid-related protein-14, and migration inhibitory factor-related protein-14 (MRP14).

2.3.3.4 Quinones

Quinones are metabolic intermediates of several endogenous and dietary molecules that can form stable adducts with DNA and proteins, which can be toxic products. The most common quinones are derivatives of hormones and neurotransmitters, such as catecholamines (adrenaline, noradrenaline, and dopamine). Others are produced in the human organism from tea catechins and other vegetable-derived flavonoids (with anti-inflammatory, -oxidant, and -carcinogenic effects) in addition to environmental pollutants such as pesticides. Molecular sources share a catechol (1,2)-dihydroxy benzene group that is generally metabolized to a quinone moiety. Moreover, quinones can also be generated during redox stress, as they can be produced from catechol-containing molecules by reaction with free radicals and other reactive species.

Quinones are involved in the onset of several pathological conditions. Systematic analysis of the reactivity of quinone derivatives with amino acids and DNA bases based on computational chemistry techniques is aimed at addressing the formation and stability of quinone adducts to predict the most reactive quinones and their adducts with biological and pathophysiological relevance. Computational results can then be used to guide experiments to validate the identification of reliable markers in the early detection and prognosis evaluation of pathological conditions.

2.3.3.5 Rock

Two isoforms of rock kinases (rock1–rock2) are effectors of small GTPases of the RHOA subfamily that have different functions in the vascular circuit. Hence, rock is an effector of the small RhoA, RhoB, and RhoC GTPases [481].

The RhoA–rock pathway is implicated in cell contraction (and hence vasospasm and systemic and pulmonary hypertension), motility, proliferation, and apoptosis.

Its major substrates include $PP1_{r12a}$ (Thr697, Ser854, and Thr855, attenuating MLCP activity), $PP1_{r14a}$ (at Thr38), MLC2, LIMKs (LIMK1–LIMK2, which phosphorylate [inhibit] cofilin, hence impeding actin depolymerization), the plasma membrane–actin filament crosslinkers ezrin, radixin, and moesin (phosphorylated in response to vasoconstrictors signaling via PKC and rock), and adducin. These substrates are also phosphorylated by MLCK, PKA, PKC, and PKG [482].

The kinase rock participates in organizing the actin cytoskeleton and controlling its dynamics and hence stress fiber formation, using the contraction-mediating rock–$PP1_{r12a}$–MLC and actin-stabilizing rock–LIMK–cofilin pathways. It is thus involved in cell contraction, adhesion, migration, proliferation, and apoptosis, in addition to remodeling of the extracellular matrix. Its activity is modulated by interaction with lipid mediators (e.g., arachidonic acid and sphingosylphosphorylcholine), mechanical stress, autophosphorylation, and proteolytic cleavage of its inhibitory C-terminus.

Because rock stabilizes PTen, the RhoA–rock–PTen pathway antagonizes the PI3K–PKB pathway. Rock also phosphorylates IRS1 and IRS2, thereby improving or impairing insulin signaling according to the context.

Its ubiquitous isoforms, rock1 and rock2, are functionally redundant. They both homodimerize. The autoinhibitory domain obviates constitutive activation; activation by Rho^{GTP} thus relies on derepression. Rock activity does not depend on autophosphorylation. Full catalytic rock2 activity relies on several phosphorylation sites (PLK1 targets Thr967, Ser1099, Ser1133, and Ser1374).

Cytosolic ubiquitous rock1 is linked to the plasma membrane, centrosome, cellular adhesion sites, and vesicles [481]. Cytosolic and nuclear rock2, which abounds in the brain, heart, muscle, lung, and placenta, localizes to the centrosome and colocalizes with actin and vimentin filaments, in addition to the intercalated and Z discs of striated myocytes. In the myocardium and skeletal muscles, a unique rock2 splice variant exists (^{SkM}rock2) [481]. Both rock1 and rock2 are cleaved and activated by peptidases.

Only rock2 binds directly to and phosphorylates $PP1_{r12a}$ [482]. In addition, rock2 tethers to PIP_3 and $PI(4,5)P_2$, but not rock1 [481]. Rock1, but not rock2, phosphorylates RhoE, a rock-specific substrate [481].

In the vasculature, The RhoA–rock pathway is implicated in vasoconstriction, using the rock–$PP1_{r12a}$–MLC2 pathway, and inflammatory cell recruitment, in addition to endothelial dysfunction and vascular and cardiac remodeling [482]. It is involved in vascular effects of redox stress (ROS production by NOxs) and various vasoconstrictors, in particular the renin–angiotensin–aldosterone axis, and hence hypertension.

The rock is also involved in transcriptional regulation, especially in SRF activity [482]. Myocardin and myocardin-related transcription factors MRTFa and MRTFb are SRF coactivators, the nuclear translocation of which depends on actin dynamics. It can thus regulate MRTFa-mediated myofibroblast activation in cardiac fibrosis.

In addition, rock mediates CMC hypertrophy caused by angiotensin-2, endothelin-1, and α-adrenoceptor [482]. In fact, rock1 and rock2 operates in cardiac fibrosis and hypertrophy, respectively [481].

The rock also favors inflammation, as it can induce expression of proinflammatory cytokines and adhesion molecules in endothelial and smooth muscle cells. It stimulates several molecules involved in inflammation, fibrosis, and thrombosis. Activity of the rock in circulating leukocytes serves as a marker for the assessment of disease severity and therapeutic response [483].

Myosin light chain (MLC) is phosphorylated by Ca^{2+}–Cam-activated MLC kinase (MLCK) and is dephosphorylated by MLC phosphatase (MLCP),[165] hence causing vSMC contraction and relaxation, respectively. In vascular smooth myocytes, the RhoA–rock axis leads to MLC phosphorylation directly and via $PP1_{r12a}$

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[165] Also known as myosin phosphatase target subunit MyPT1 and MyPT2 or $PP1_{r12a/b}$.

inhibition. The substrates of rock include not only MLC and MLCP, but also the members of the ERM (ezrin–radixin–moesin) family, adducin, PTen phosphatase, and LIM kinases [483].

The vascular effect of the rock is augmented by mechanical stress, hypoxia, growth factors (e.g., PDGF), and some vasoactive agents (e.g., angiotensin-2, thrombin, thromboxanes, extracellular nucleotides, and urotensin) [483]. The rock upregulates NADPH oxidase expression.

The rock participates in interactions between vascular endothelial and vSMCs. Endotheliocytes release vasodilators, such as NO and prostacyclin, and endothelium-derived hyperpolarizing factors such as hydrogen peroxide. Hydrogen peroxide transmitted through myoendothelial junctions rapidly cause vasodilation via PGhS (cyclooxygenase), cAMP, and the cGMP–PKG1α–K_{Ca} pathway (vSMC membrane hyperpolarization) [483].

In ECs, the RhoA–rock axis prevents nitric oxide synthesis. It also supports actin–myosin contractility, hence disrupting the endothelial barrier and raising endothelial permeability.

In addition, activated rock enhances transcriptional regulation of SRF, provoking stress fiber formation and vSMC contraction. It also mediates vSMC contraction elicited by vasoconstrictory GPCR ligands sent by ECs.

Cyclophilin-A mediates redox stress. Reactive oxygen species and the RhoA–rock axis promote cyclophilin-A secretion via VAMP2+[166] vesicle formation and actin–myosin-2 cytoskeletal dynamics [483].

In ECs, released cyclophilin-A decreases NOS3 activity. In addition, it stimulates expression of E-selectin and vcam1 on vECs. It is also a chemoattractant for inflammatory leukocytes and activates MMPs.

In vascular smooth myocytes, extracellular cyclophilin-A activates ERK1, ERK2, PKB, and JaK, contributing to ROS production. Reactive oxygen species foster vSMC proliferation in an auto- and paracrine manner.

The rock is active not only in vascular cells, but also in leukocytes. It inhibits reverse cholesterol transport in macrophages, thereby promoting foam cell formation [484].

Extracellular cyclophilin-A also activates platelets via basigin and the PI3K–PKB axis, enhancing their adhesion and thrombus formation.

Platelets are not only involved in hemostasis but also in inflammation and immunity. Platelet activation results from signaling initiated and then amplified by plasmalemmal receptors and involves re-organization of the actin cytoskeleton for the secretion of granule content, adhesion, spreading, aggregation, and blood clot retraction, and hence monomeric GTPases of the RHO superfamily (RhoA, Rac1, and CDC42). RhoA is implicated in platelet contractility and thrombus stability, Rac1 in lamellipodium formation, and CDC42 in filopodium formation and granule secretion [484].

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[166]VAMP: vesicle-associated membrane protein.

Platelet activators such as thrombin connect to their cognate GPCRs and launch RhoA–rock2 signaling. In addition, RhoA and Rock2 regulate platelet formation from megakaryocytes, albeit to a considerably lesser extent than Rac1 and CDC42 [484].

Rock2 operates in platelet activation and thrombosis. It promotes $\alpha_{2B}\beta_3$-integrin binding to fibrinogen and expression of P-selectin, which elicits hetero- (platelet–leukocyte) and homotypic aggregates [485]. Rock2 does not influence expression of plasmalemmal receptors, but their activation and function, as binding of fibrinogen to $\alpha_{2B}\beta_3$Itg relies on its structural changes to bind to fibrinogen. Rock2 is necessary for initial activation of $\alpha_{2B}\beta_3$Itg, but not for the maintenance of of activation. α-Granules contain P-selectin. Upon platelet activation, P-selectin lodges on the plasma membrane. Rock2 is necessary for the initial release of granules at low thrombin concentration, but not for release maintenance at a higher thrombin concentration.

Rock2 influences blood clot formation, but not its growth. In platelets, rock2 may also regulate rock1 concentration. Rock2 deficiency does indeed mildly reduce rock1 concentration [485].

Coronary microvascular resistance is controlled by numerous myogenic, metabolic, neurohumoral, and endothelium-derived factors to adjust myocardial nutrient oxygen delivery to its metabolism. Microvascular dysfunction is generally characterized by the decreased production of vasodilators (NO, hydrogen peroxide, prostacyclin, and endothelium-derived hyperpolarizing factors) and increased formation of vasoconstrictors (endothelin, thromboxane-A2, and superoxide). Adverse remodeling and hypoperfusion perpetuate cardiac dysfunction. Activation of the RhoA–rock axis and redox stress in different cardiac cell types alters cardiac function either directly via hypertrophy and fibrosis or indirectly via hypoperfusion, that is, whether abnormal activation of the RhoA–rock axis and redox stress occur in cardiac myocytes and fibroblasts or in arteriolar endothelial and smooth muscle cells [486].

Hypertensive cardiac hypertrophy is associated with reduced coronary flow reserve. In hypertension, rock1 and rock2 cause NO deficiency, augment ET activity, and increase superoxide production, thereby provoking redox stress and contributing to coronary arteriolar dysfunction, that is, the inability to increase blood flow in response to metabolic stimulation. Myocardial and microvascular redox stress, elevated vasoconstriction, perivascular fibrosis, and CMC hypertrophy are attenuated by rock inhibition [487]. Expression of both rock isoforms is significantly upregulated in the coronary arterioles of hypertensive mice. In mice, deletion of rock1 reduces fibrogenesis, but not ventricular hypertrophy, although it improves cardiac contractility. On the other hand, rock2 deletion reduces cardiac cell apoptosis and ventricular fibrosis and hypertrophy. In addition, rock inhibition improves aortic endothelial and cardiac function in rat models of streptozotocin-induced diabetes [486].