Hindawi Publishing Corporation
Disease Markers
Volume 2014, Article ID 218169, 13 pages
http://dx.doi.org/10.1155/2014/218169

Review Article

MicroRNAs-Role in Lung Cancer

Małgorzata Guz,[1] Adolfo Rivero-Müller,[2] Estera Okoń,[1] Agnieszka Stenzel-Bembenek,[1] Krzysztof Polberg,[3] Maria Słomka,[4] and Andrzej Stepulak[1,3]

[1] *Chair and Department of Biochemistry and Molecular Biology, Medical University of Lublin, Ulica Witolda Chodźki 1, 20-093 Lublin, Poland*
[2] *Department of Physiology, Institute of Biomedicine, University of Turku, 20520 Turku, Finland*
[3] *Department of Otolaryngology, MSW Hospital, 20-331 Lublin, Poland*
[4] *Chair and Department of Gastroenterology with Endoscopic Unit, Medical University of Lublin, 20-093 Lublin, Poland*

Correspondence should be addressed to Małgorzata Guz; malg.guz@gmail.com

Received 6 August 2013; Revised 28 January 2014; Accepted 7 February 2014; Published 13 March 2014

Academic Editor: Luisella Bocchio-Chiavetto

Regulation of gene expression is essential for normal physiological functions; thus deregulation of gene expression is common in disease conditions. One level of regulation of gene expression is performed by noncoding RNAs, among which microRNAs (miRNA) are the best studied. Abnormal expression of these molecular players can lead to pathogenic processes such as heart disease, immune system abnormalities, and carcinogenesis, to name but a few. Of a length of 18–25 nucleotides miRNAs are involved in binding partial complementary sequences within the 3′-UTR (3′-untranslated region) of the target mRNAs. Depending on the type of neoplastic transformation, miRNAs can act both as oncogenes (oncomirs) or as tumor suppressors. Because of the great importance of miRNAs, most researches focus on either their role as biomarkers or their potential as therapeutic targets. Herein, we present the review of microRNA biology, function, and tumorigenic potential with emphasis on their role in lung cancer.

1. Introduction

Cancer represents a heterogeneous group of diseases characterized by uncontrolled cell growth promoting tumor formation and metastasis [1]. Tumors are characterized by six essential alterations in cell physiology: self-sufficiency in growth signals, insensitivity to growth-inhibition signals, evasion of apoptosis, limitless replicative potential, sustained angiogenesis, and tissue invasion and metastasis [2, 3]. The latter two are the most deadly hallmarks of malignant tumors [4].

During the past decade, the discovery of the regulatory role of microRNAs (miRNAs) upon gene expression has extensively shed light on cancer biology [1]. In normal cells, miRNAs control genes were involved in normal rates of cellular growth, proliferation, differentiation, and apoptosis. Unsurprisingly, miRNAs who inhibit genes involved in cell cycle progression and drive terminal differentiation are often downregulated in cancer cells, while others regulating genes involved in cell cycle progression and resistance to apoptosis are overexpressed [5]. Concomitantly, miRNA loci frequently map to genomic regions that are commonly amplified or deleted in human cancers [6–10]. Indeed, many tumor cell lines as well as human tumors have been found to have widespread deregulation of miRNA expression [7, 11, 12]. Furthermore, the expression pattern of certain miRNAs correlates with diverse clinicopathological parameters [6, 12, 13] and prognosis [4, 14]. These findings highlighted the potential role of miRNAs as new diagnostic or prognostic biomarkers. There is mounting evidence that specific miRNAs have tumor suppressor or prooncogenic functions making them novel targets for cancer therapy including lung cancer [15].

Lung cancer (LC) is the leading cause of cancer-related deaths all over the world, among both men and women, with an incidence of over 200 000 new cases per year and a very high mortality rate [16]. Indeed, lung cancer is responsible for more deaths than breast, colon, and prostate tumors combined [14]. LC is comprised into two major clinicopathological categories: small-cell (SCLC) and non-small-cell lung carcinoma (NSCLC).

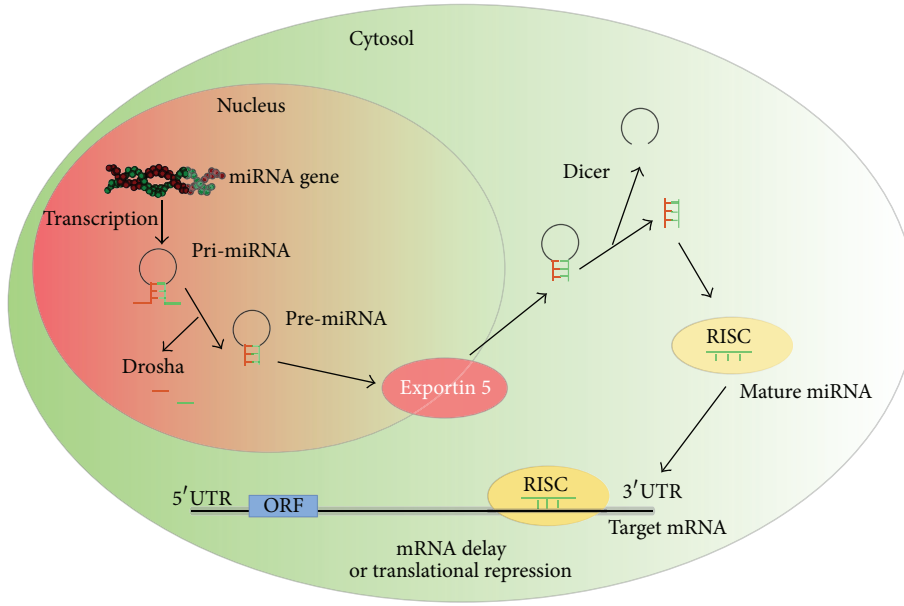

Figure 1: miRNA biogenesis and mechanism of action. miRNAs are transcribed by polymerase II into primary transcripts (pri-miRNAs). Pri-miRNAs are cleaved by the Drosha resulting in the formation of a hairpin precursor (pre-miRNAs). Exportin 5 transports pre-miRNAs to the cytoplasm, where Dicer processes them into miRNAs duplexes. One strand of the duplex (mature miRNA) is incorporated into the RNA-induced silencing complex (RISC) and binds to 3′-UTR of target mRNA resulting in either its degradation or translational repression.

SCLC accounts for around 12% of all cases, it is more aggressive than NSCLC, and it frequently metastasizes [17]. NSCLC tumors consist mainly of three subtypes: adenocarcinoma (40% of lung cancers), squamous cell carcinoma (25% of lung cancers), and large cell carcinoma (10% of lung cancers). NSCLC is less aggressive and more common, accounting for at least 88% of all lung cancer cases.

2. miRNA Biogenesis

Discovered in the early 1990s by Victor Ambros and colleagues, miRNAs comprise an abundant class of endogenous, small noncoding RNAs 18–25 nucleotides in length that repress protein translation through binding to (often only partial) complementary target mRNAs [18]. miRNA genes are evolutionarily conserved and are located within the introns of protein-coding genes, as well as within intergenic areas [19] and transcribed as long transcripts up to 1000 nucleotides long, named primary miRNAs (pri-miRNAs), which are processed by RNase III (Rnasen) and Microprocessor complex subunit DGCR8 (DGCR8) into precursor stem-loop structures 44–180 nt long termed "pre-miRNA" [20–22].

Pre-miRNAs are transported to cytoplasm by exportin 5 (Ran GTP-dependent dsRNA-binding protein) where they are further processed to mature miRNAs by a second RNase III, named Dicer. The resulting 18–25-nucleotide mature miRNA ultimately gets integrated into the RNA-induced silencing complex (RISC). Mature miRNAs exert their regulatory effects by binding to perfect or imperfect complementary sites within the 3′-untranslated region (3′-UTR) of their mRNA targets, thereby posttranscriptionally repressing target-gene expression [23, 24]. Each miRNA may have as many as hundreds of mRNA targets [25, 26]. In general, miRNAs act in the cytoplasm, yet mature miRNAs has also been found in the nucleus [27–31] and nucleolus [32, 33] but the mechanisms of miRNAs subcellular localization and function are still not well understood [31] (Figure 1).

miRNAs were originally found to play a role in the timing of larval development in *Caenorhabditis elegans*, which lead to the identification of *lin-4* and *let-7* miRNAs [18, 34]. Initial understanding of miRNA-mRNA target recognition came from observations of sequence complementarity between *lin-4* and multiple conserved sites within the gene *lin-14* 3′-UTR [35]. Notably, depending on the degree of homology with the target sequence, miRNAs induce translational repression or degradation of mRNAs [36]. At present, more than 2000 miRNAs have been characterized in humans (miRBASE, release 19, February 2013), which target at least 60% of all mRNAs [37]. In this regard, miRNAs control a wide range of biological processes including apoptosis, development, proliferation, and differentiation [15]. Given their integral role in development, it was no surprise that miRNAs were soon found to play a role in tumorigenesis [38]. Their roles in tumor development are so evident that their expression profiles can be used to classify human tumors and identify molecular signatures associated with the corresponding clinical status [9, 39].

3. Proliferation and Cell Cycle Are Affected by miRNAs

Uncontrolled proliferation is a crucial step in cancer progression. Recent evidence demonstrated that aberrant miRNAs expression is a critical factor influencing tumor cell growth

and thus acting as either tumor suppressors or oncogenes [40]. In human lung cancer tissues and corresponding cell lines many miRNAs are usually downregulated when compared with adjacent lung tissue, as demonstrated for *let-7* [41], *miR-15a*, *miR-16* [42], *miR-34a* [43], *miR-34b* [44], *miR-125* [45], *miR-155* [46], *miR-192* [47], and *miR-486* [48], while others are overexpressed such as *miR-21* [49], *miR-194* [50], and *miR-186* [51], when compared to normal human bronchial epithelial cells [49]. As it could be expected, in experimental conditions enforced overexpression of *miR-101* [52] and *miR-186* [51] inhibited NSCLC cell proliferation, whereas forced *miR-200* expression inhibited tumor growth and metastasis [53] Lentiviral-mediated overexpression of *miR-129* blocked proliferation of several tumor cell lines, including lung adenocarcinoma [54].

Likewise overexpression of *miR-192* inhibited cell proliferation in NSCLC cell lines A549, H460, and 95D cells and inhibited tumorigenesis in an *in vivo* model [47], while overexpression of *miR-34b* [55] and *miR-193b* [56] significantly reduced A549 cell survival. Transient introduction of *miR-34a* into NSCLC cell line A549 and SCLC cell line SBC-5 also caused complete suppression of cell proliferation [43]. Recent studies demonstrated that inhibition of individual miRNAs delay lung cancer cell proliferation, as reported for for *miR-150* [57] and *miR-223* [58]. Nevertheless, miRNAs seem to work differently depending of the cellular context; for example, *miR-34a*, which has been previously correlated to prostate cancer inhibition [59], did not influence SCLC cell viability, suggesting that *miR-34a* is unrelated to the malignant behavior of SCLC cells [60].

Given that reduced proliferation is often the result on inhibition of cell division, several studies have been focusing on the role of miRNAs of cell cycle progression. A typical eukaryotic cell cycle is divided into two basic processes: mitosis and interphase. Interphase, the period between mitoses, is the time during which cell growth and DNA replication are started in preparation for cell division. The cycle of eukaryotic cells consists of four phases: G1 phase (gap 1, between mitosis and initiation of DNA replication), S phase while DNA replication begins, and G2 phase (gap 2) during which cell growth is in progress and proteins are synthesized for the next mitosis (M phase) [61]. *In vitro* studies have demonstrated that several deregulated miRNAs are involved in LC cell division. *Let-7* was the first identified miRNAs in *C. elegans* as a regulator of the timing of cell fate determination and is evolutionarily conserved across species. *C. elegans* stem cells carrying a mutant *let-7* fail to exit the cell cycle; they do not differentiate and continue to divide typical characteristics of cancer cells [34]. In humans, reduced expression of *let-7* is often present in certain types of lung adenocarcinoma and bronchioloalveolar carcinoma [62, 63]. Transient introduction of *miR-34a* into A549 and SBC-5 cell lines induced cell cycle arrest at the G1 phase [43]. Moreover, *miR-34a* and *miR-15/16* act synergistically, potentiating the impact on inhibition of G1/S progression [64]. Similarly, ectopic expression of *miR-137* in A549, NCI-H460, and NCI-H520 resulted in G1 cell cycle arrest [65]. *miR-129* induced G1 phase arrest in multiple human lung adenocarcinoma cell lines, suggesting *miR-129* targeting of G1/S phase-specific regulators [54]. *Pre-miR-630* arrested A549 cells in the G0/G1 phase of the cell cycle, resulting in greatly diminished sensitivity of A549 cells to the late S/G2/M cell cycle arrest [66]. In contrast, only *miR-223* was found to induce G2/M arrest in NSCLC cells [58].

The transition from one cell cycle phase to another is regulated by different cellular proteins. A crucial role is played by the cyclin-dependent kinases (CDKs), who become activated via phosphorylation at particular time-points of cell cycle. Five of them are active during the cell cycle: in G1 phase they are CDK4, CDK6, and CDK2 and in S phase it is CDK2, whereas in G and M phases it is CDK1 [67]. CDKs are stable during the cell cycle in opposition to their activating proteins, cyclins. At different phases of the cell cycle different cyclins are required. Essential for entry to G1 phase are CDK-cyclin D complexes formed by three D type cyclins (D1, D2, and D3) with CDK4 and CDK6 [68]. Another G1 phase cyclin is cyclin E that forms a complex with CDK2 and regulates progression from G1 to S phases [69], whereas cyclin A/CDK2 complex is required during S phase [70, 71]. In late G2 and early M phase, complex cyclin A with CDK1 promotes entry into M phase, and afterwards M phase is regulated by cyclin B associated with CDK1 [72, 73]. Since most of the miRNAs act on genes involved in G1/S phases of the cell cycle, their targets include Cdk2/Cdk6 and related cyclins. Indeed, CDK2 was identified as direct targets of *miR-223* in a Lewis lung carcinoma (LLC) cell line [58], and, according to gene expression and bioinformatic analyses of NSCLC cells, CDK6 was identified as a target gene of *miR-129* [54] and miRNA-214 [74]. Ectopic expression of *miR-137* in NSCLC cell lines (A549, NCI-H460, and NCI-H520) downregulated Cdc42 and Cdk6 and induced G1 cell cycle arrest, resulting in decreased cell growth *in vitro* and *in vivo* [65]. Likewise, CDK2 and CDK6 were each directly targeted by *miR-186* and restoring their expression reversed *miR-186*-mediated inhibition of cell cycle progression [51]. Whereas, cyclin D1 (CCND1) and CDK6 seem to be targets of *miR-34a* that results in a G1 cell cycle arrest [75]. CCND1 expression is controlled by *miR-193b* and *miR-206* [76] in the A549 cell line [56], suggesting an involvement of *miR-206* in tumorigenesis [76]. In NSCLC cells cyclins D1, D2, and E1 are directly regulated by physiologic concentrations of *miR-15a*/*miR-16*, showing inverse correlation between *miR-15a*/*miR-16* and CCND1 expressions [64]. Direct evidence for the effect of *miR-34c* on cyclin E regulation was provided by using cells extracted from a mouse model overexpressing cyclin E, who develops multiple pulmonary adenocarcinomas, and human LC cell lines transfected with *miR-34c* resulting in repression of cell proliferation [77].

CDKs activity can be regulated by cell cycle inhibitory proteins (CKI) that bind to CDKs or CDK-cyclin complexes. There are two families of CDKIs: INK4 family (p15, p16, p18, and p19) and Cip/Kip family (p21, p27, and p57) [78]. Members of these families form stable complexes with CDK4/6 before cyclin binding, which prevents the activation and formation of CDK-cyclin D and CDK1-cyclin B complexes [79, 80]. To date, there are no reports describing miRNA-regulation of INK4 family members. However, it was demonstrated that several miRNAs affect cell cycle progression at

different phases of cell cycle acting on members of Kip/Cip family. *Let-7a* elevates p21 (CDKN1A) levels via UHRF2 (ubiquitin-like with PHD and ring finger domains 2) inhibition and suppresses the growth of A549 lung cancer cells [41], whereas *miR-128-2* posttranscriptionally targets E2F5 and leads to the abrogation of its repressive activity on p21 (waf1) transcription in a human NSCLC cell line (H1299) [81]. Likewise, *pre-miR-630* expression arrests A549 cells in the G0/G1, which correlates with the increased levels of p27 (CDKN1B), another cell cycle inhibitor [66]. In contrast, direct inhibition of p27 by at least two micrRNAs, *miR-221*, *miR-222,* and *miRNA-194* results in enhanced cell survival to the NSCLC cells [82].

Another well-known example of a miRNA target gene is the tumor suppressor p53, the most frequently mutated gene in human cancers. p53 protein regulates genes and their products associated with cell cycle progression, apoptosis, DNA repair, or genomic stability [83]. As miRNAs control p53, the opposite is also possible as p53 affects the expression of several miRNAs. The *miR-34* family members are downstream transcription targets of p53; thus *miR-34* is reduced in p53 mutant tumors. Exogenous addition of *miR-34* to tumor cells reduced proliferation and invasiveness *in vitro* and tumor formation *in vivo* [84]. Besides *miR-34a*, other miRNAs (*miR-184*, *miR-181a,* and *miR-148*) are also regulated by p53 protein *in vitro* and *miR-150* expression was correlated with p53 in human NSCLC patients tissues samples [85]. However, the mechanism how p53 affects the expression of these miRNAs is still unknown. Studies on the restoration of p53 pathway may provide effective approaches for anticancer therapy. p53 expression was higher when cancer NSCLC cells were treated with anti-*miR-150* expression vector that indicates that the upregulation of p53 contributes to cancer growth [86]. *miR-150* targets p53 in NSCLC cell lines (SPCA-1, A549, HCC827, 95-D, and BEAS-2B), which in turn regulates the expression of various tumor-suppressor miRNAs participating in cell cycle progression [85]. Earlier reports suggested that some small noncoding RNAs take part in the regulation of p53 expression [87]. Indeed, p53 expression was found to be downregulated by *miR-98*, *miR-453* [88], and *miR-378* [89]. In contrast, wild type p53 mRNA and protein were increased by *miR-125a* overexpression [45] or activated by *miR-29* via p85α (PI3K) and CDC42 [90]. Thereby, the importance of miRNAs to be regulated and regulating p53 in tumorigenesis are highlighted, indirectly involved in the cell cycle progression. Accordingly, targeted deletion of *miR-31* results in the repression of nonsmall cell lung cancer cells growth and *in vivo* tumorigenicity due to higher expression of LATS2 (human large tumor suppressor 2) and PPP2R2A (PP2A regulatory subunit B alpha isoform) [91]. *LATS 2* encodes a putative serine/threonine kinase that exerts tumor-suppressive effects by inhibition of the G1/S cell cycle transition [92]. PPP2R2A is a regulatory subunit of protein phosphatase 2 (PPA2), a highly conserved serine/threonine phosphatase, which exerts its function in tumorigenesis by negative regulation (dephosphorylation) of cell cycle regulators such as cell division control protein 2 homolog (CDC2) and M-phase inducer phosphatase 3 (CDC25C) [93]. Such modulation of the LATS2/PPP2R2A pathway by *miR-31* constitutes a novel growth regulator in lung cancer [94].

4. miRNAs Regulating Apoptosis

Defective programmed-cell death determines a major causative factor in the development and progression of tumor. Central biochemical machinery of apoptosis underlies the activity of the caspases, an aspartate-specific cysteine proteases which cleave to activate/inactivate targets within the cell. The sequence of events culminating in the activation of caspases is categorized into extrinsic and intrinsic pathways [95]. The extrinsic pathway can be mediated by one or several death receptors when bound by the appropriate ligand [96]. The death receptor family also known as the tumor necrosis factor (TNF) receptor family includes TNF, Fas (CD95), and TRAIL 1–5 (TNF-related apoptosis-inducing ligand) receptors. A still growing number of experimental data suggest that miRNAs have an inhibition effect on apoptosis-related molecules, that is, TNF-α and mainly TRAIL. TNF-α is a target for *miR-19a* and a member of *miR-17-92* cluster, and this cluster is often found overexpressed or amplified in many malignant tumors including lung cancers [97], suggesting that *miR-19a* could be a novel target to sensitize cancer cells to apoptosis [97]; however this issue requires further studies.

As mentioned above, *miR-34a* and *miR-34c* are downregulated in NSCLC cell lines, and overexpression of these two noncoding molecules is linked to TRAIL-induced apoptosis, reducing the invasive capacity of NSCLC cells [98]. In addition, ectopic expression of *miR-212* increases TRAIL-induced cell death in lung cancer cells [99]. However, several tumors, including lung cancer, have developed resistance for TRAIL induced apoptosis using miRNA depending mechanisms, such as *miR-494a*, whose downregulation made the A549 cell line more sensitive to TRAIL-induced apoptosis [100]. Two additional miRNAs, *miR-221* and *miR-222,* impair TRAIL-induced apoptosis, thus transfection blocking these miRNAs with *anti-miR-221* and *anti-miR-222* results in TRAIL-sensitivity in NSCLC, a mechanism involving the downregulation of p27, demonstrating that high expression of *miR-221* and *miR-222* maintains the TRAIL-resistant phenotype [101]. In turn, *miR-130a* was able to reduce TRAIL resistance in NSCLC cells through c-Jun downregulation of *miR-221* and *miR-222* expression [82].

The intrinsic apoptosis pathway is characterized by the rapid release of cytochrome c (CYCS) from the mitochondrial intermembrane space into the cytosol. The releasing mechanism of CYCS requires permeabilization of the outer mitochondrial membrane, with a loss of membrane potential and the presence of members of pro- and antiapoptotic Bcl-2 proteins [102, 103]. In the cytosol CYCS binds an adapter protein Apaf-1 (apoptotic protease activating factor 1) that in the presence of dATP/ATP forms Apaf-1 multimer recruiting procaspase [104, 105]. Procaspase-9 becomes activated and in turn it activates caspase-3, an effector caspase involved in the dismantling of the cell structures during apoptosis. Effector caspases, caspase-3, caspase-6, and caspase-7, when activated cleave cytoskeletal and nuclear proteins [106].

There are miRNAs known to regulate proapoptotic (BAK, Bax, Bcl-rambo, Bcl-xs, BOK/Mtd, and BH-3 proteins) as well as antiapoptotic (Bcl-2: Bad, BID, Bik/Nbk, BIM, BLK, Bmf, Hrk/DP5) or antiapoptotic (Bcl-2, Bcl-xL, Bcl-w, Mcl-1, Bcl-10, and Bcl-2 related protein A1). In experimental conditions, ectopic expression of *miR-503* causes reduced expression of antiapoptotic Bcl-2 protein in NSCLC A549 cells [107]. Enforced *miR-181b* [108], *miR200b/c-429* cluster [109], and *miR-497* [110] expression downregulated Bcl-2, suggesting that Bcl-2 was the target gene of these miRNAs in NSCLC cell lines. Different miRNAs have also been shown to affect expression and function of other antiapoptotic proteins of the Bcl-2 family. For example, *Let-7c* significantly reduced luciferase activity of Bcl-xL 3′UTR-based reporter, simultaneously reducing Bcl-xL protein levels [111], whereas *miR-125b* expression induced spontaneous apoptosis in various cell lines derived from lung cancer and sensitized cancer cells to different apoptotic stimuli, probably by suppressing the antiapoptotic molecules Mcl-1 and/or Bcl-w, suggesting that *miR-125b* downregulation facilitates tumor development [112]. Likewise, several miRNAs modulate expression of proapoptotic members of Bcl-2 family. Luciferase reporter assays demonstrated that in lung cancer cells *miR-221/222* comodulated PUMA (p53 upregulator of apoptosis) expression, by directly targeting the binding site within its 3′-UTR [113]. Overexpression of *miR-494* [100] and *miR-17* [114] downregulates BIM protein (Bcl-2-like 11). Similarly, *in vitro* and *in vivo* studies showed the inhibited expression of BIM by *miR-30b*, *miR-30c*, *miR221*, *miR-222*, *miR-103*, and *miR-203* in NSCLC cells [115]. Silencing of *miR-155* elevated expression of the Apaf-1 proteins, whereas Apaf mRNA levels were unchanged [46]. Mitochondrial/postmitochondrial steps of the intrinsic pathway of apoptosis, including Bax oligomerization, mitochondrial transmembrane potential dissipation, and the proteolytic maturation of caspase-9 and caspase-3, were modulated by *pre-miR-181a* and *pre-miR-630* in A549 lung cancer cell line [66].

Several studies have investigated the role of miRNAs in the induction of apoptosis through caspase activation via extrinsic and intrinsic pathways, finding that a number of miRNAs may rescue lung cancer cells from caspase-8 activation in death receptor-mediated apoptosis. These miRNAs included members such as *miR-17*, *miR-135,* and *miR-520* [116]. On the other hand, it was confirmed that *miR-186* promotes apoptosis by targeting caspase-10 in A549 cells [117].

A number of miRNAs were shown to interfere in apoptosis by affecting expression and activation of effector caspases. This is the case for *miR-1* in A549 lung cancer cells which enhances activation of caspase-3 and caspase-7 [118]. The same was demonstrated for *miR-15a-3p*, a novel member of the proapoptotic miRNA cluster *miR-15a/16* [119]. Caspase-3, together with caspase-7, is also activated by inhibition of *miR-133a/b* and *miR-361-3p* in 95D lung cancer cell line [120]. Apart from caspase-3, the proteolytic activation of caspase-9 is modulated by *pre-miR-181a* and *pre-miR-630* in A549 cell line [66]. Cells viability are also reduced by overexpression of *miR-192* by caspase-7 activation in *in vivo* and *in vitro* studies [47], demonstrating that the modulation of caspases activity is a promising therapeutic target.

5. miRNAs Regulate Lung Cancer Angiogenesis

As tumors expand, the distance between the centre of the tumor and blood vessels becomes too large so the centre turns hypoxic. The sustained cell growth in the tumor requires that oxygen and metabolites can be delivered to the tumor cells and thus the formation of new blood vessels. Hypoxia triggers a series of molecular events that result in the activation of angiogenesis (see below) so these two mechanisms are closely related [121].

Studies focused on the role of specific miRNAs in the regulation of angiogenesis are increasingly being performed. The growth of the new blood vessels is necessary for solid tumors to keep growing and spreading in a process called angiogenic switch. In lung tumor tissues where angiogenesis is taking place with higher microvessel density, there is a decreased expression of *let-7b* and *miR-126*, as compared to normal lung tissues, suggesting that low expression of these miRNAs could have antiangiogenic role in lung cancer [122], whereas *miR-16* overexpression reduced the ability of endothelial cells to form blood vessels *in vivo* [123].

Many miRNAs display organ-specific expression patterns suggesting cell-type-specific functions [124–126]. Increasing evidence indicates that miRNAs are important regulators of tumor angiogenesis as well as hypoxic responses. Hypoxia, a major hallmark of tumorigenesis, modulates activity of hypoxia-inducible factor 1 (HIF-1) [127]. HIF-1-α was identified as a direct target for *miR-17-92* by mass spectrometry analysis [128]. Likewise, *miR-519c* was shown as a hypoxia-independent regulator of HIF-1-α acting through the direct binding to the HIF-1-α 3′UTR. Overexpression of *miR-519c* resulted in a significant decrease of HIF-1-α and the reduction of tumor angiogenesis [129]. Conversely, *miR-210* expression is regulated by hypoxia-mediated HIF-1-α via an HRE (hypoxia-responsive element) in its promoter [130], which in turn stabilizes HIF-1-α through positive regulatory loop [131]. HIF-1-α induces the expression of different angiogenic growth factors, including vascular endothelial growth factor (VEGF) and platelet-derived growth factor (PDGF), acting on its respective receptors and promoting formation of new capillaries of tumor [132–134]. It seems that VEGF expression in lung cancer could be regulated by several miRNAs. The *Vegf* gene produces several alternative splicing forms, $VEGF_{121}$, $VEGF_{145}$, $VEGF_{165}$, $VEGF_{183}$, $VEGF_{189}$, and $VEGF_{206}$, where each of them is involved in different properties and functions, such as association with cell surface or extracellular matrix, and has effects on the diameter of the vessel during tube formation [135]. VEGF receptors (VEGF-R1, 2, and 3) are expressed at high levels particularly during embryogenesis. In adults, VEGF-R1 and VEGFR2 are mainly expressed in the blood vascular system in adults, whereas VEGF-R3 is restricted to the lymphatic endothelium [136]. VEGF-A is directly downregulated by miR-126 in NSCLC cells, while miR-26a expression results in its upregulation through a yet unknown mechanism [137, 138]. Additionally, the VEGFR2 is directly regulated by *miR-200c* in A549 cells [139].

The angiogenesis process in lung cancer is also regulated by many cytokines all of which are regulated by miRNAs;

these cytokines include the fibroblast growth factors (FGF) as well as interleukin-8 (IL-8). FGFs are composed of 22 members that bind and activate the four FGF receptor family members (FGFR1-4); these ligands include the basic FGF (bFGF and FGF2),which plays important role in NSCLC tumor proliferation and angiogenesis [137, 140–143]. In lung tumor tissue of patients with detectable nodal metastasis, FGF seems to be specifically regulated by *miR-155*, while other angiogenic growth factors (VEGF-A, PDGF-B, and HIF-1-alpha) are not [144]. Likewise, angiogenesis-related IL-8 levels [145, 146] often elevated in tumor samples of patients with NSCLC [147] can be regulated by *miR-200* which also targets growth regulated alpha protein (CXCL1) secreted by the tumor endothelial and cancer cells. Indeed, the therapeutic potential of *miR-200* was put to the test by delivery of *miR-200* into the tumor endothelium which resulted in marked reduction in metastasis and angiogenesis, as well as vascular normalization [148]. In contrast, overexpression of *miR-378* enhances the expression of IL-8 and consequently increases stimulation of endothelial cells in NSCLC patients [89].

Other angiogenesis-related proteins are also regulated by miRNAs, for example, ectopic expression of *miR-381* reduced at mRNA and protein level a putative stem cell gene ID1 (inhibitor of differentiation 1), involved in invasion and angiogenesis, and thus significantly decreased lung cancer cells migration and invasion [149], whereas ectopic expression of *miR-26a* enhances lung cancer metastasis potential via modulation of metastasis-related genes, among them matrix metallopeptidase 2 *(MMP-2)* [150].

6. microRNAs as Next-Generation Biomarkers for Lung Cancer

The diagnosis of lung cancer at its early stage is essential for improving the effect of treatment and survival rate of patients. Thus, there is a great need for easy detectable biomarkers determining existence of lung tumors or monitoring disease progression in cancer-bearing patients [151]. Thanks to the advances in sequencing technologies (i.e., next-generation sequencing, NGS) and bioinformatics tools it is possible to screen large data sets of miRNA expression patterns from normal and malignant human cells in order to find novel and reliable biomarkers.

In fact, it has been reported that human serum/plasma contains large amounts of stable miRNAs and their expression patterns in body fluids have a great potential as biomarkers of many diseases [152, 153]. More importantly, the circulating miRNAs levels correlate to cancer progression, therapeutic response, and patient survival. MicroRNAs expression profiles might be diagnostic and prognostic markers for many types of cancers, including lung cancer [154].

One of the pioneering reports using quantitative reverse transcription PCR (qRT-PCR) assays found higher expression levels of four miRNAs (*miR-486, miR-30d, miR-1*, and *miR-499*) in serum of patients with NSCLC. The results demonstrated the similarity between the circulating exosomal miRNA and the tumor-derived miRNA patterns, as well as the clear difference in total exosome and miRNA levels between lung cancer patients and healthy controls [152]. Later on, Rabinowits and collaborators reported the presence of 12 miRNAs in serum of lung adenocarcinoma-bearing patients, *miR-17-3p, miR-21, miR-106a, miR-146, miR-155, miR-191, miR-192, miR-203, miR-205, miR-210, miR-212*, and *miR-214*, in circulating exosomes suggesting that circulating exosomal miRNA might be useful in a screening test for patients with lung adenocarcinoma [155].

The profiles of circulating miRNAs were also compared in patients with lung squamous cell carcinoma before and after tumor removal. The levels of five miRNAs *miR-205, miR-19a, miR-19b, miR-30b*, and *miR-20a* were downregulated in plasma of patients 7–10 days after tumor removal. Another recent study confirmed that *miR-205* level consistently decreased in the serum of lung cancer patients after tumor resection [156]. Large-scale analysis on NSCLC indicated that, among 46 miRNAs differentially expressed in PMBC (peripheral blood mononuclear cells), 42 miRNAs were downregulated after lung cancer resection. Significant changes were related to *let-7c, miR-34a, miR-202, miR-769-5p,* and *miR-642* expression levels [157]. Conversely, Leidinger et al. only found slightly differences in expression pattern of *miR-34a* before and after NSCLC surgery and *let-7c* upregulation after surgery, whereas *miR-202* and *miR-769p* were not expressed neither in pre- nor in in postsurgery samples in limied group of patient's plasma. However, follow-up of the fate of the plasma miRNome of lung cancer patients, starting prior to surgery and ending 18 months after surgery, shows specific fluctuating miRNA patterns with a significant correlation between miRNAs expression level and time distance from surgery [158]. Therefore, it seems that miRNAs are released or leaked by tumor cells and circulate in stable form in the bloodstream, although the mechanism of miRNAs release is not well understood [159, 160]. In the experimental conditions, *miR-451* and *miR-205* are secreted to the medium by cultivated A2182 lung cancer cell line. Likewise, *miR-30b* was found to be excreted by A549 lung adenocarcinoma cells, proving that cancer cells can release miRNAs to the environment [161].

miRNAs are also present in sputum, an aspect that allowed Yu and collaborators to distinguish between patients with lung adenocarcinoma and healthy controls. Analyzing several miRNAs they showed that four of them, *miR 486, miR-21, miR-200b*, and *miR-375,* in combination produced the best prediction in distinguishing lung adenocarcinoma patients from normal subjects with 80.6% sensitivity and 91.7% specificity [162]. Furthermore, they identified another three miRNAs in sputum, *miR-205, miR-210*, and *miR-708,* that characterize squamous cell lung carcinoma patients [163], demonstrating that miRNAs could be specific for histologic type of lung cancer. Performing similar type of study Lee and collaborators investigated the expression of a panel of 7 miRNAs (*miR-21, miR-29b, miR-34a/b/c, miR-155*, and *let-7a*) in 31 SCLC tumors, 14 SCLC cell lines, and 26 NSCLC cell lines and observed significantly lower *miR-21, miR-29b,* and *miR-34a* expression in SCLC cell lines [60]. However, miRNA levels could not distinguish between patients with benign lung disease and lung cancer patients, although the levels of *miR-10b, miR-141,* and *miR-155* were

significantly higher in lung cancer patients than those in patients with benign disease [164].

Nevertheless, tissue samples examination using qPCR or microarray analysis showed significant differences in the amounts of particular miRNAs between lung cancer tissues (LCT) benign disease and tumor adjacent or normal lung tissues (NLT). Twenty-seven miRNAs were observed to be deregulated greater than twofold in LCT compared with NLT by microarray analysis [165]. In detail, the expression levels of *miR-21* [165] and *miR-155* [46, 119] were significantly higher in LCT than in adjacent normal tissue. *MiR-205* was also overexpressed in NSCLC [166, 167], which is supported by new data confirming higher levels of *miR-205* in tissues and serum from NSCLC and SCLC patients [168].

On the other hand, most of the analyzed miRNAs expression in clinical samples showed lower levels in lung cancer tissues compared to adjacent or noncancerous lung tissues. *MiR-15a/miR-16* were frequently deleted or downregulated in squamous cell carcinomas and adenocarcinomas of the lung [64]. Likewise, the expression of *miR-34b* was lower in NSCLC tissues compared to that in pericarcinous tissues of lung cancer [44]. The same was reported for *miR-150* [169], *miR-186* [51], *miR-192* [47], *miR-193b* [56], *miR-486* [48], and *miR-3940-5p* [169] suggesting that loss of these miRNAs may be important in lung cancer development. However, contradicting reports suggest the association of miRNAs expression and tumor size. The expression of *miR-150* in T2 stage tissue samples was higher than in T1 stage [85], whereas other authors presented data showing that *miR-150* was downregulated in a subgroup of patients with tumor diameter more than or equal to 3 cm as well as in clinical stages III and IV [169].

One report showed that *miR-210* was overexpressed at late stages of NSCLC [170], as well as *miR-574p,* previously considered a serum-based biomarker for early stage NSCLC [171].

Late stages of lung cancer are often associated with the presence of nodal and distance metastases. In lung cancer patients high serum *miR-10b* values associated with lymph node metastasis [164]. Likewise, *miR-26a* expression level was higher in lymph node metastasis tumor tissues than in primary tumor tissues [150], suggesting direct involvement of *miR-26a* in the metastatic potential of lung cancer cells. In contrast, the lower *miR-34b* expression in cancer tissue was correlated with higher lymph node metastasis [44], and *miR-126* was a significant negative prognostic factor in lymph node-positive subgroup of patients [137]. Interestingly, several miRNAs were found to be significantly differentially expressed between primary lung tumors and the metastatic tumors to the lung from other localizations. Two of them, *miR-126* and *miR-182,* represent potential biomarkers for distinguishing between primary and metastatic lung tumors, reflecting lung tissue specificity [172].

In the clinical specimens of NSCLC *miR-194* expression was also associated with metastasis. Consequently, overexpression of *miR-194* in lung cancer cell lines resulted in suppressing metastasis of lung cancer cells, while inhibiting its expression through "miRNA sponge" promotes cancer cells to metastasize [50]. Similarly, forced *miR-200* expression inhibits tumor growth and metastasis, whereas in lung cancer patients *miR-200* levels were suppressed in metastasis-prone tumor cells and predicted poor prognosis [53].

High *miR-16* expression levels were associated with shorter disease-free survival (DFS) or overall survival (OS) of lung cancer patients [173]. Likewise, Markou and coworkers found that overexpression of mature *miR-21* was an independent negative prognostic factor for overall survival in NSCLC patients [174], and overexpressed *miR-155* in lung cancer cells correlated with poor patients prognosis [175]. In contrast, overexpression of *miR-519c* was observed in cancer patients with better prognosis [129], whereas low levels of *miR-34a* expression in cancer tissue correlated with high probability of relapse [176], while low levels of *miR-186* in NSCLC cells correlated with shorter patients OS [51]. Similarly, low expression of *let-7b* and *miR-126* correlated with worse progression-free survival and overall survival [122], and coexpression of *miR-126* and VEGF-A had a significant prognostic impact with 5-year survival of lung cancer patients [137].

But not all independent reports support the notion that miRNAs can be used as prognosis markers. Expression of *miR-21, miR-29b, miR-34a/b/c, miR-155,* and *let-7a* was determined from 639 IALT (the International Adjuvant Lung Cancer Trial) patients with NSCLC, finding no significant association between any of the tested miRNAs and survival, with the exception of *miR-21* for which a deleterious prognostic effect of lowered expression was suggested [177]. Likewise, SCLC-bearing patients-*miR-21, miR-29b, miR-34a/b/c, miR-155*, and *let-7a* were unrelated to clinical characteristics and were neither prognostic in terms of overall survival or progression-free survival nor predictive of treatment response in SCLC tumors and cell lines or NSCLC cell lines [60]. The observed discrepancies could result from different methods of miRNAs isolation, storage conditions, type of biological material (serum, plasma, and cancer tissues), or detection methods.

Recent data indicate that not only different miRNAs patterns but also miRNA-related polymorphisms may be associated with NSCLC patients clinical outcomes [178], opening new field for miRNAs investigations in relation to lung cancer progression.

7. Concluding Remarks

Many studies have demonstrated that miRNAs have unique expression profiles in different types of tissues. Among different lung cancer biomarkers miRNAs are the most promising because of remarkable stability, cancer-type specificity, and the presence in body fluids. The relationship between different miRNAs profiles in body fluids and tumor progression, presence of metastasis, and patients clinical prognosis rise the possibility for using miRNAs as biomarkers. However, the identification for specific miRNAs unequivocally associated with clinicopathological features of lung cancer is still work in progress. The growing number of bioinformatic and biochemical analyses opens up new possibilities for the discovery of such biomarkers, especially that the number of existing miRNAs is still growing, bringing new promising targets in the battle against cancer. The role of miRNAs as biomarkers

in the detection of tumorigenesis is of vital importance, since lung cancer has a poor prognosis. So extensive and sensitive studies in this area are necessary to be successful in prospective clinical applications.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

References

[1] C. Lopez-Camarillo, L. A. Marchat, E. Arechaga-Ocampo et al., "MetastamiRs: non-coding microRNAs driving cancer invasion and metastasis," *International Journal of Molecular Sciences*, vol. 13, no. 2, pp. 1347–1379, 2012.

[2] D. Hanahan and R. A. Weinberg, "The hallmarks of cancer," *Cell*, vol. 100, no. 1, pp. 57–70, 2000.

[3] Y. Lazebnik, "What are the hallmarks of cancer?" *Nature Reviews Cancer*, vol. 10, no. 4, pp. 232–233, 2010.

[4] A. J. Schetter, S. Y. Leung, J. J. Sohn et al., "MicroRNA expression profiles associated with prognosis and therapeutic outcome in colon adenocarcinoma," *The Journal of the American Medical Association*, vol. 299, no. 4, pp. 425–436, 2008.

[5] C. M. Croce, "Causes and consequences of microRNA dysregulation in cancer," *Nature Reviews Genetics*, vol. 10, no. 10, pp. 704–714, 2009.

[6] J. Brennecke, D. R. Hipfner, A. Stark, R. B. Russell, and S. M. Cohen, "Bantam encodes a developmentally regulated microRNA that controls cell proliferation and regulates the proapoptotic gene hid in Drosophila," *Cell*, vol. 113, no. 1, pp. 25–36, 2003.

[7] A. Gaur, D. A. Jewell, Y. Liang et al., "Characterization of microRNA expression levels and their biological correlates in human cancer cell lines," *Cancer Research*, vol. 67, no. 6, pp. 2456–2468, 2007.

[8] J. Jiang, E. J. Lee, Y. Gusev, and T. D. Schmittgen, "Real-time expression profiling of microRNA precursors in human cancer cell lines," *Nucleic Acids Research*, vol. 33, no. 17, pp. 5394–5403, 2005.

[9] J. Lu, G. Getz, E. A. Miska et al., "MicroRNA expression profiles classify human cancers," *Nature*, vol. 435, no. 7043, pp. 834–838, 2005.

[10] Y. Murakami, T. Yasuda, K. Saigo et al., "Comprehensive analysis of microRNA expression patterns in hepatocellular carcinoma and non-tumorous tissues," *Oncogene*, vol. 25, no. 17, pp. 2537–2545, 2006.

[11] M. V. Iorio, M. Ferracin, C.-G. Liu et al., "MicroRNA gene expression deregulation in human breast cancer," *Cancer Research*, vol. 65, no. 16, pp. 7065–7070, 2005.

[12] M. V. Iorio, R. Visone, G. Di Leva et al., "MicroRNA signatures in human ovarian cancer," *Cancer Research*, vol. 67, no. 18, pp. 8699–8707, 2007.

[13] G. A. Calin, M. Ferracin, A. Cimmino et al., "A microRNA signature associated with prognosis and progression in chronic lymphocytic leukemia," *The New England Journal of Medicine*, vol. 353, no. 17, pp. 1793–1801, 2005.

[14] A. Jemal, R. Siegel, E. Ward, T. Murray, J. Xu, and M. J. Thun, "Cancer statistics, 2007," *CA: A Cancer Journal for Clinicians*, vol. 57, no. 1, pp. 43–66, 2007.

[15] G. A. Calin and C. M. Croce, "MicroRNA signatures in human cancers," *Nature Reviews Cancer*, vol. 6, no. 11, pp. 857–866, 2006.

[16] J. Takamizawa, H. Konishi, K. Yanagisawa et al., "Reduced expression of the let-7 microRNAs in human lung cancers in association with shortened postoperative survival," *Cancer Research*, vol. 64, no. 11, pp. 3753–3756, 2004.

[17] L. Esposito, D. Conti, R. Ailavajhala, N. Khalil, and A. Giordano, "Lung cancer: are we up to the challenge?" *Current Genomics*, vol. 11, no. 7, pp. 513–518, 2010.

[18] R. C. Lee, R. L. Feinbaum, and V. Ambros, "The *C. elegans* heterochronic gene *lin-4* encodes small RNAs with antisense complementarity to *lin-14*," *Cell*, vol. 75, no. 5, pp. 843–854, 1993.

[19] A. Rodriguez, S. Griffiths-Jones, J. L. Ashurst, and A. Bradley, "Identification of mammalian microRNA host genes and transcription units," *Genome Research*, vol. 14, no. 10, pp. 1902–1910, 2004.

[20] A. M. Denli, B. B. J. Tops, R. H. A. Plasterk, R. F. Ketting, and G. J. Hannon, "Processing of primary microRNAs by the Microprocessor complex," *Nature*, vol. 432, no. 7014, pp. 231–235, 2004.

[21] J. Han, Y. Lee, K.-H. Yeom, Y.-K. Kim, H. Jin, and V. N. Kim, "The Drosha-DGCR8 complex in primary microRNA processing," *Genes and Development*, vol. 18, no. 24, pp. 3016–3027, 2004.

[22] Y. Lee, M. Kim, J. Han et al., "MicroRNA genes are transcribed by RNA polymerase II," *The EMBO Journal*, vol. 23, no. 20, pp. 4051–4060, 2004.

[23] A. Esquela-Kerscher and F. J. Slack, "Oncomirs—microRNAs with a role in cancer," *Nature Reviews Cancer*, vol. 6, no. 4, pp. 259–269, 2006.

[24] J. Winter, S. Jung, S. Keller, R. I. Gregory, and S. Diederichs, "Many roads to maturity: microRNA biogenesis pathways and their regulation," *Nature Cell Biology*, vol. 11, no. 3, pp. 228–234, 2009.

[25] C. D. Jeffries, H. M. Fried, and D. O. Perkins, "Nuclear and cytoplasmic localization of neural stem cell microRNAs," *RNA*, vol. 17, no. 4, pp. 675–686, 2011.

[26] M. Selbach, B. Schwanhäusser, N. Thierfelder, Z. Fang, R. Khanin, and N. Rajewsky, "Widespread changes in protein synthesis induced by microRNAs," *Nature*, vol. 455, no. 7209, pp. 58–63, 2008.

[27] Z. Földes-Papp, K. König, H. Studier et al., "Trafficking of mature miRNA-122 into the nucleus of live liver cells," *Current Pharmaceutical Biotechnology*, vol. 10, no. 6, pp. 569–578, 2009.

[28] H.-W. Hwang, E. A. Wentzel, and J. T. Mendell, "A hexanucleotide element directs microRNA nuclear import," *Science*, vol. 315, no. 5808, pp. 97–100, 2007.

[29] D. H. Kim, P. Sætrom, O. Snøve Jr., and J. J. Rossi, "MicroRNA-directed transcriptional gene silencing in mammalian cells," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 105, no. 42, pp. 16230–16235, 2008.

[30] E. Marcon, T. Babak, G. Chua, T. Hughes, and P. B. Moens, "MiRNA and piRNA localization in the male mammalian meiotic nucleus," *Chromosome Research*, vol. 16, no. 2, pp. 243–260, 2008.

[31] T. Ohrt, J. Mütze, W. Staroske et al., "Fluorescence correlation spectroscopy and fluorescence cross-correlation spectroscopy reveal the cytoplasmic origination of loaded nuclear RISC *in vivo* in human cells," *Nucleic Acids Research*, vol. 36, no. 20, pp. 6439–6449, 2008.

[32] J.-Y. Liao, L.-M. Ma, Y.-H. Guo et al., "Deep sequencing of human nuclear and cytoplasmic small RNAS reveals an unexpectedly complex subcellular distribution of mirnas and tRNA 3′ trailers," *PLoS ONE*, vol. 5, no. 5, Article ID e10563, 2010.

[33] J. C. R. Politz, E. M. Hogan, and T. Pederson, "MicroRNAs with a nucleolar location," *RNA*, vol. 15, no. 9, pp. 1705–1715, 2009.

[34] B. J. Reinhart, F. J. Slack, M. Basson et al., "The 21-nucleotide let-7 RNA regulates developmental timing in *Caenorhabditis elegans*," *Nature*, vol. 403, no. 6772, pp. 901–906, 2000.

[35] B. Wightman, I. Ha, and G. Ruvkun, "Posttranscriptional regulation of the heterochronic gene *lin-14* by *lin-4* mediates temporal pattern formation in *C. elegans*," *Cell*, vol. 75, no. 5, pp. 855–862, 1993.

[36] O. Slaby, M. Svoboda, J. Michalek, and R. Vyzula, "MicroRNAs in colorectal cancer: translation of molecular biology into clinical application," *Molecular Cancer*, vol. 8, article 102, 2009.

[37] R. C. Friedman, K. K.-H. Farh, C. B. Burge, and D. P. Bartel, "Most mammalian mRNAs are conserved targets of microRNAs," *Genome Research*, vol. 19, no. 1, pp. 92–105, 2009.

[38] T. A. Farazi, J. I. Spitzer, P. Morozov, and T. Tuschl, "MiRNAs in human cancer," *The Journal of Pathology*, vol. 223, no. 2, pp. 102–115, 2011.

[39] S. Volinia, G. A. Calin, C.-G. Liu et al., "A microRNA expression signature of human solid tumors defines cancer gene targets," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 103, no. 7, pp. 2257–2261, 2006.

[40] B. Zhang, X. Pan, G. P. Cobb, and T. A. Anderson, "MicroRNAs as oncogenes and tumor suppressors," *Developmental Biology*, vol. 302, no. 1, pp. 1–12, 2007.

[41] X. He, C. Duan, J. Chen et al., "Let-7a elevates $p21^{WAF1}$ levels by targeting of NIRF and suppresses the growth of A549 lung cancer cells," *FEBS Letters*, vol. 583, no. 21, pp. 3501–3507, 2009.

[42] L. Ma, J. Liu, J. Shen et al., "Expression of miR-122 mediated by adenoviral vector induces apoptosis and cell cycle arrest of cancer cells," *Cancer Biology and Therapy*, vol. 9, no. 7, pp. 554–561, 2010.

[43] X. Wang, K. Dong, P. Gao et al., "MicroRNA-34a sensitizes lung cancer cell lines to DDP treatment independent of p53 status," *Cancer Biotherapy & Radiopharmaceuticals*, vol. 28, no. 1, pp. 45–50, 2013.

[44] L. G. Wang, Y. Ni, B. H. Su, X. R. Mu, H. C. Shen, and J. J. Du, "MicroRNA-34b functions as a tumor suppressor and acts as a nodal point in the feedback loop with Met," *International Journal of Oncology*, vol. 42, no. 3, pp. 957–962, 2013.

[45] L. Jiang, Q. Huang, J. Chang, E. Wang, and X. Qiu, "MicroRNA HSA-miR-125a-5p induces apoptosis by activating p53 in lung cancer cells," *Experimental Lung Research*, vol. 37, no. 7, pp. 387–398, 2011.

[46] Y. S. Zang, Y. F. Zhong, Z. Fang, B. Li, and J. An, "MiR-155 inhibits the sensitivity of lung cancer cells to cisplatin via negative regulation of Apaf-1 expression," *Cancer Gene Therapy*, vol. 19, pp. 773–778, 2012.

[47] S. Feng, S. Cong, X. Zhang et al., "MicroRNA-192 targeting retinoblastoma 1 inhibits cell proliferation and induces cell apoptosis in lung cancer cells," *Nucleic Acids Research*, vol. 39, no. 15, pp. 6669–6678, 2011.

[48] Y. Peng, Y. Dai, C. Hitchcock, X. Yang, E. S. Kassis et al., "Insulin growth factor signaling is regulated by microRNA-486, an underexpressed microRNA in lung cancer," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 110, no. 37, pp. 15043–15048, 2013.

[49] Q. Sun, M. Hang, X. Guo, W. Shao, and G. Zeng, "Expression and significance of miRNA-21 and BTG2 in lung cancer," *Tumor Biology*, vol. 34, no. 6, pp. 4017–4026, 2013.

[50] X. Wu, T. Liu, O. Fang, L. J. Leach, X. Hu, and Z. Luo, "MiR-194 suppresses metastasis of non-small cell lung cancer through regulating expression of BMP1 and $p27^{kip1}$," *Oncogene*, 2013.

[51] J. Cai, J. Wu, H. Zhang, L. Fang, Y. Huang et al., "MiR-186 downregulation correlates with poor survival in lung adenocarcinoma, where it interferes with cell-cycle regulation," *Cancer Research*, vol. 73, pp. 756–766, 2013.

[52] J.-G. Zhang, J.-F. Guo, D.-L. Liu, Q. Liu, and J.-J. Wang, "MicroRNA-101 exerts tumor-suppressive functions in non-small cell lung cancer through directly targeting enhancer of zeste homolog 2," *Journal of Thoracic Oncology*, vol. 6, no. 4, pp. 671–678, 2011.

[53] J. D. Roybal, Y. Zang, Y.-H. Ahn et al., "MiR-200 inhibits lung adenocarcinoma cell invasion and metastasis by targeting Flt1/VEGFR1," *Molecular Cancer Research*, vol. 9, no. 1, pp. 25–35, 2011.

[54] J. Wu, J. Qian, C. Li et al., "MiR-129 regulates cell proliferation by downregulating Cdk6 expression," *Cell Cycle*, vol. 9, no. 9, pp. 1809–1818, 2010.

[55] J. Balca-Silva, S. S. Neves, A. C. Goncalves et al., "Effect of miR-34b overexpression on the radiosensitivity of non-small cell lung cancer cell lines," *Anticancer Research*, vol. 32, no. 5, pp. 1603–1609, 2012.

[56] H. Hu, S. Li, J. Liu, and B. Ni, "MicroRNA-193b modulates proliferation, migration, and invasion of non-small cell lung cancer cells," *Acta Biochimica et Biophysica Sinica*, vol. 44, no. 5, pp. 424–430, 2012.

[57] N. Zhang, X. Wei, and L. Xu, "MiR-150 promotes the proliferation of lung cancer cells by targeting P53," *FEBS Letters*, vol. 587, no. 15, pp. 2346–2351, 2013.

[58] W. Nian, X. Ao, Y. Wu et al., "MiR-223 functions as a potent tumor suppressor of the Lewis lung carcinoma cell line by targeting insulin-like growth factor-1 receptor and cyclin-dependent kinase 2," *Oncology Letters*, vol. 6, no. 2, pp. 359–366, 2013.

[59] C. Liu, K. Kelnar, B. Liu et al., "The microRNA miR-34a inhibits prostate cancer stem cells and metastasis by directly repressing CD44," *Nature Medicine*, vol. 17, no. 2, pp. 211–215, 2011.

[60] J.-H. Lee, J. Voortman, A.-M. C. Dingemans et al., "MicroRNA expression and clinical outcome of small cell lung cancer," *PLoS ONE*, vol. 6, no. 6, Article ID e21300, 2011.

[61] G.M. Cooper and National Center for Biotechnology Information (U.S.), *The Cell: A Molecular Approach*, ASM Press, Washington, DC, USA; Sinauer Associates, Sunderland, Mass, USA, 2nd edition, 2000.

[62] K. Inamura, Y. Togashi, K. Nomura et al., "Let-7 microRNA expression is reduced in bronchioloalveolar carcinoma, a non-invasive carcinoma, and is not correlated with prognosis," *Lung Cancer*, vol. 58, no. 3, pp. 392–396, 2007.

[63] N. Yanaihara, N. Caplen, E. Bowman et al., "Unique microRNA molecular profiles in lung cancer diagnosis and prognosis," *Cancer Cell*, vol. 9, no. 3, pp. 189–198, 2006.

[64] N. Bandi, S. Zbinden, M. Gugger et al., "MiR-15a and miR-16 are implicated in cell cycle regulation in a Rb-dependent manner and are frequently deleted or down-regulated in non-small cell lung cancer," *Cancer Research*, vol. 69, no. 13, pp. 5553–5559, 2009.

[65] X. Zhu, Y. Li, H. Shen, H. Li, L. Long et al., "MiR-137 inhibits the proliferation of lung cancer cells by targeting Cdc42 and Cdk6," *FEBS Letters*, vol. 587, no. 1, pp. 73–81, 2013.

[66] L. Galluzzi, E. Morselli, I. Vitale et al., "MiR-181a and miR-630 regulate cisplatin-induced cancer cell death," *Cancer Research*, vol. 70, no. 5, pp. 1793–1803, 2010.

[67] K. Vermeulen, D. R. van Bockstaele, and Z. N. Berneman, "The cell cycle: a review of regulation, deregulation and therapeutic targets in cancer," *Cell Proliferation*, vol. 36, no. 3, pp. 131–149, 2003.

[68] C. J. Sherr, "G1 phase progression: cycling on cue," *Cell*, vol. 79, no. 4, pp. 551–555, 1994.

[69] M. Ohtsubo, A. M. Theodoras, J. Schumacher, J. M. Roberts, and M. Pagano, "Human cyclin E, a nuclear protein essential for the G1-to-S phase transition," *Molecular and Cellular Biology*, vol. 15, no. 5, pp. 2612–2624, 1995.

[70] F. Girard, U. Strausfeld, A. Fernandez, and N. J. C. Lamb, "Cyclin A is required for the onset of DNA replication in mammalian fibroblasts," *Cell*, vol. 67, no. 6, pp. 1169–1179, 1991.

[71] D. H. Walker and J. L. Maller, "Role for cyclin A in the dependence of mitosis on completion of DNA replication," *Nature*, vol. 354, no. 6351, pp. 314–317, 1991.

[72] M. Arellano and S. Moreno, "Regulation of CDK/cyclin complexes during the cell cycle," *International Journal of Biochemistry and Cell Biology*, vol. 29, no. 4, pp. 559–573, 1997.

[73] R. W. King, P. K. Jackson, and M. W. Kirschner, "Mitosis in transition," *Cell*, vol. 79, no. 4, pp. 563–571, 1994.

[74] H. Salim, A. Arvanitis, L. de Petris et al., "MiRNA-214 is related to invasiveness of human non-small cell lung cancer and directly regulates alpha protein kinase 2 expression," *Genes Chromosomes and Cancer*, vol. 52, no. 10, pp. 895–911, 2013.

[75] F. Sun, H. Fu, Q. Liu et al., "Downregulation of CCND1 and CDK6 by miR-34a induces cell cycle arrest," *FEBS Letters*, vol. 582, no. 10, pp. 1564–1568, 2008.

[76] A. Alteri, F. de Vito, G. Messina et al., "Cyclin D1 is a major target of miR-206 in cell differentiation and transformation," *Cell Cycle*, vol. 12, no. 24, pp. 3781–3790, 2013.

[77] X. Liu, L. F. Sempere, F. Galimberti et al., "Uncovering growth-suppressive microRNAs in lung cancer," *Clinical Cancer Research*, vol. 15, no. 4, pp. 1177–1183, 2009.

[78] C. J. Sherr and J. M. Roberts, "Inhibitors of mammalian G1 cyclin-dependent kinases," *Genes and Development*, vol. 9, no. 10, pp. 1149–1163, 1995.

[79] A. Carnero and G. J. Hannon, "The INK4 family of CDK inhibitors," in *Cyclin Dependent Kinase (CDK) Inhibitors*, vol. 227 of *Current Topics in Microbiology and Immunology*, pp. 43–55, Springer, Berlin, Germany, 1998.

[80] L. Hengst and S. I. Reed, "Inhibitors of the Cip/Kip family," in *Cyclin Dependent Kinase (CDK) Inhibitors*, vol. 227 of *Current Topics in Microbiology and Immunology*, pp. 25–41, Springer, Berlin, Germany, 1998.

[81] S. Donzelli, G. Fontemaggi, F. Fazi et al., "MicroRNA-128-2 targets the transcriptional repressor E2F5 enhancing mutant p53 gain of function," *Cell Death and Differentiation*, vol. 19, pp. 1038–1048, 2012.

[82] M. Acunzo, R. Visone, G. Romano et al., "MiR-130a targets MET and induces TRAIL-sensitivity in NSCLC by downregulating miR-221 and 222," *Oncogene*, vol. 31, no. 5, pp. 634–642, 2012.

[83] S. L. Harris and A. J. Levine, "The p53 pathway: positive and negative feedback loops," *Oncogene*, vol. 24, no. 17, pp. 2899–2908, 2005.

[84] A. L. Kasinski and F. J. Slack, "MiRNA-34 prevents cancer initiation and progression in a therapeutically resistant K-ras and p53-induced mouse model of lung adenocarcinoma," *Cancer Research*, vol. 72, no. 21, pp. 5576–5587, 2012.

[85] D. T. Wang, Z. L. Ma, Y. L. Li et al., "MiR-150, p53 protein and relevant miRNAs consist of a regulatory network in NSCLC tumorigenesis," *Oncology Reports*, vol. 30, no. 1, pp. 492–498, 2013.

[86] Y.-J. Li, Y.-X. Zhang, P.-Y. Wang et al., "Regression of A549 lung cancer tumors by anti-miR-150 vector," *Oncology Reports*, vol. 27, no. 1, pp. 129–134, 2012.

[87] L. He, X. He, L. P. Lim et al., "A microRNA component of the p53 tumour suppressor network," *Nature*, vol. 447, no. 7148, pp. 1130–1134, 2007.

[88] S. Zhang, C. Zhang, Y. Li, P. Wang, Z. Yue, and S. Xie, "MiR-98 regulates cisplatin-induced A549 cell death by inhibiting TP53 pathway," *Biomedicine and Pharmacotherapy*, vol. 65, no. 6, pp. 436–442, 2011.

[89] K. Skrzypek, M. Tertil, S. Golda et al., "Interplay between heme oxygenase-1 and miR-378 affects non-small cell lung carcinoma growth, vascularization, and metastasis," *Antioxidants & Redox Signaling*, vol. 19, no. 7, pp. 644–660, 2013.

[90] S.-Y. Park, J. H. Lee, M. Ha, J.-W. Nam, and V. N. Kim, "MiR-29 miRNAs activate p53 by targeting p85α and CDC42," *Nature Structural and Molecular Biology*, vol. 16, no. 1, pp. 23–29, 2009.

[91] X. Liu, L. F. Sempere, H. Ouyang et al., "MicroRNA-31 functions as an oncogenic microRNA in mouse and human lung cancer cells by repressing specific tumor suppressors," *Journal of Clinical Investigation*, vol. 120, no. 4, pp. 1298–1309, 2010.

[92] Y. Li, J. Pei, H. Xia, H. Ke, H. Wang, and W. Tao, "Lats2, a putative tumor suppressor, inhibits G1/S transition," *Oncogene*, vol. 22, no. 28, pp. 4398–4405, 2003.

[93] P. R. Clarke, I. Hoffmann, G. Draetta, and E. Karsenti, "Dephosphorylation of cdc25-C by a type-2A protein phosphatase: specific regulation during the cell cycle in Xenopus egg extracts," *Molecular Biology of the Cell*, vol. 4, no. 4, pp. 397–411, 1993.

[94] P.-Y. Lin, S.-L. Yu, and P.-C. Yang, "MicroRNA in lung cancer," *British Journal of Cancer*, vol. 103, no. 8, pp. 1144–1148, 2010.

[95] D. R. Green and G. I. Evan, "A matter of life and death," *Cancer Cell*, vol. 1, no. 1, pp. 19–30, 2002.

[96] R. M. Locksley, N. Killeen, and M. J. Lenardo, "The TNF and TNF receptor superfamilies: integrating mammalian biology," *Cell*, vol. 104, no. 4, pp. 487–501, 2001.

[97] M. Liu, Z. Wang, S. Yang et al., "TNF-α is a novel target of miR-19a," *International Journal of Oncology*, vol. 38, no. 4, pp. 1013–1022, 2011.

[98] M. Garofalo, Y. J. Jeon, G. J. Nuovo et al., "MiR-34a/c-dependent PDGFR-α/β downregulation inhibits tumorigenesis and enhances TRAIL-induced apoptosis in lung cancer," *PLoS ONE*, vol. 8, no. 6, Article ID e67581, 2013.

[99] M. Incoronato, M. Garofalo, L. Urso et al., "MiR-212 increases tumor necrosis factor-related apoptosis-inducing ligand sensitivity in non-small cell lung cancer by targeting the antiapoptotic protein PED," *Cancer Research*, vol. 70, no. 9, pp. 3638–3646, 2010.

[100] G. Romano, M. Acunzo, M. Garofalo et al., "MiR-494 is regulated by ERK1/2 and modulates TRAIL-induced apoptosis in non-small-cell lung cancer through BIM down-regulation," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 109, no. 41, pp. 16570–16575, 2012.

[101] M. Garofalo, C. Quintavalle, G. Di Leva et al., "MicroRNA signatures of TRAIL resistance in human non-small cell lung cancer," *Oncogene*, vol. 27, no. 27, pp. 3845–3855, 2008.

[102] J.-C. Martinou and R. J. Youle, "Mitochondria in apoptosis: bcl-2 family members and mitochondrial dynamics," *Developmental Cell*, vol. 21, no. 1, pp. 92–101, 2011.

[103] N. J. Waterhouse, J. C. Goldstein, O. von Ahsen, M. Schuler, D. D. Newmeyer, and D. R. Green, "Cytochrome c maintains mitochondrial transmembrane potential and ATP generation after outer mitochondrial membrane permeabilization during the apoptotic process," *Journal of Cell Biology*, vol. 153, no. 2, pp. 319–328, 2001.

[104] H. Zou, W. J. Henzel, X. Liu, A. Lutschg, and X. Wang, "Apaf-1, a human protein homologous to *C. elegans* CED-4, participates in cytochrome c-dependent activation of caspase-3," *Cell*, vol. 90, no. 3, pp. 405–413, 1997.

[105] P. Li, D. Nijhawan, I. Budihardjo et al., "Cytochrome c and dATP-dependent formation of Apaf-1/caspase-9 complex initiates an apoptotic protease cascade," *Cell*, vol. 91, no. 4, pp. 479–489, 1997.

[106] T. Nakagawa, H. Zhu, N. Morishima et al., "Caspase-12 mediates endoplasmic-reticulum-specific apoptosis and cytotoxicity by amyloid-β," *Nature*, vol. 403, no. 6765, pp. 98–103, 2000.

[107] T. Qiu, L. Zhou, T. Wang et al., "MiR-503 regulates the resistance of non-small cell lung cancer cells to cisplatin by targeting Bcl-2," *International Journal of Molecular Medicine*, vol. 32, no. 3, pp. 593–598, 2013.

[108] W. Zhu, X. Shan, T. Wang, Y. Shu, and P. Liu, "MiR-181b modulates multidrug resistance by targeting BCL2 in human cancer cell lines," *International Journal of Cancer*, vol. 127, no. 11, pp. 2520–2529, 2010.

[109] W. Zhu, H. Xu, D. Zhu et al., "MiR-200bc/429 cluster modulates multidrug resistance of human cancer cell lines by targeting BCL2 and XIAP," *Cancer Chemotherapy and Pharmacology*, vol. 69, no. 3, pp. 723–731, 2012.

[110] W. Zhu, D. Zhu, S. Lu et al., "MiR-497 modulates multidrug resistance of human cancer cell lines by targeting BCL2," *Medical Oncology*, vol. 19, no. 1, pp. 384–391, 2012.

[111] S. Y. Cui, J. Y. Huang, Y. T. Chen et al., "Let-7c governs the acquisition of chemo- or radioresistance and epithelial-to-mesenchymal transition phenotypes in docetaxel-resistant lung adenocarcinoma," *Molecular Cancer Research*, vol. 11, pp. 699–713, 2013.

[112] J. Gong, J. P. Zhang, B. Li et al., "MicroRNA-125b promotes apoptosis by regulating the expression of Mcl-1, Bcl-w and IL-6R," *Oncogene*, vol. 32, no. 25, pp. 3071–3079, 2013.

[113] C. Zhang, J. Zhang, A. Zhang et al., "PUMA is a novel target of miR-221/222 in human epithelial cancers," *International Journal of Oncology*, vol. 37, no. 6, pp. 1621–1626, 2010.

[114] B. Dai, J. Meng, M. Peyton et al., "STAT3 mediates resistance to MEK inhibitor through microRNA miR-17," *Cancer Research*, vol. 71, no. 10, pp. 3658–3668, 2011.

[115] M. Garofalo, G. Romano, G. Di Leva et al., "EGFR and MET receptor tyrosine kinase-altered microRNA expression induces tumorigenesis and gefitinib resistance in lung cancers," *Nature Medicine*, vol. 18, no. 1, pp. 74–82, 2012.

[116] S. Catuogno, L. Cerchia, G. Romano, P. Pognonec, G. Condorelli, and V. de Franciscis, "MiR-34c may protect lung cancer cells from paclitaxel-induced apoptosis," *Oncogene*, vol. 32, no. 3, pp. 341–351, 2013.

[117] J. Zhang, Y. Du, C. Wu et al., "Curcumin promotes apoptosis in human lung adenocarcinoma cells through miR-186* signaling pathway," *Oncology Reports*, vol. 24, no. 5, pp. 1217–1223, 2010.

[118] M. W. Nasser, J. Datta, G. Nuovo et al., "Down-regulation of micro-RNA-1 (miR-1) in lung cancer: suppression of tumorigenic property of lung cancer cells and their sensitization to doxorubicin-induced apoptosis by miR-1," *The Journal of Biological Chemistry*, vol. 283, no. 48, pp. 33394–33405, 2008.

[119] A. Druz, Y. C. Chen, R. Guha, M. Betenbaugh, S. E. Martin, and J. Shiloach, "Large-scale screening identifies a novel microRNA, miR-15a-3p, which induces apoptosis in human cancer cell lines," *RNA Biology*, vol. 10, no. 2, pp. 287–300, 2013.

[120] L. Du, R. Borkowski, Z. Zhao et al., "A high-throughput screen identifies miRNA inhibitors regulating lung cancer cell survival and response to paclitaxel," *RNA Biology*, vol. 10, no. 11, pp. 1700–1713, 2013.

[121] D. Liao and R. S. Johnson, "Hypoxia: a key regulator of angiogenesis in cancer," *Cancer and Metastasis Reviews*, vol. 26, no. 2, pp. 281–290, 2007.

[122] E. Jusufovic, M. Rijavec, D. Keser et al., "Let-7b and miR-126 are down-regulated in tumor tissue and correlate with microvessel density and survival outcomes in non-small-cell lung cancer," *PLoS ONE*, vol. 7, no. 9, Article ID e45577, 2012.

[123] A. Chamorro-Jorganes, E. Araldi, L. O. F. Penalva, D. Sandhu, C. Fernández-Hernando, and Y. Suárez, "MicroRNA-16 and MicroRNA-424 regulate cell-autonomous angiogenic functions in endothelial cells via targeting vascular endothelial growth factor receptor-2 and fibroblast growth factor receptor-1," *Arteriosclerosis, Thrombosis, and Vascular Biology*, vol. 31, no. 11, pp. 2595–2606, 2011.

[124] C.-Z. Chen, L. Li, H. F. Lodish, and D. P. Bartel, "MicroRNAs modulate hematopoietic lineage differentiation," *Science*, vol. 303, no. 5654, pp. 83–86, 2004.

[125] M. N. Poy, L. Eliasson, J. Krutzfeldt et al., "A pancreatic islet-specific microRNA regulates insulin secretion," *Nature*, vol. 432, no. 7014, pp. 226–230, 2004.

[126] E. van Rooij, L. B. Sutherland, X. Qi, J. A. Richardson, J. Hill, and E. N. Olson, "Control of stress-dependent cardiac growth and gene expression by a microRNA," *Science*, vol. 316, no. 5824, pp. 575–579, 2007.

[127] R. Pocock, "Invited review: decoding the microRNA response to hypoxia," *Pflügers Archiv*, vol. 461, no. 3, pp. 307–315, 2011.

[128] A. Taguchi, K. Yanagisawa, M. Tanaka et al., "Identification of hypoxia-inducible factor-1α as a novel target for miR-17-92 microRNA cluster," *Cancer Research*, vol. 68, no. 14, pp. 5540–5545, 2008.

[129] S.-T. Cha, P.-S. Chen, G. Johansson et al., "MicroRNA-519c suppresses hypoxia-inducible factor-1α expression and tumor angiogenesis," *Cancer Research*, vol. 70, no. 7, pp. 2675–2685, 2010.

[130] X. Huang, L. Ding, K. L. Bennewith et al., "Hypoxia-inducible mir-210 regulates normoxic gene expression involved in tumor initiation," *Molecular Cell*, vol. 35, no. 6, pp. 856–867, 2009.

[131] S. Grosso, J. Doyen, S. K. Parks et al., "MiR-210 promotes a hypoxic phenotype and increases radioresistance in human lung cancer cell lines," *Cell Death and Disease*, vol. 4, article e544, 2013.

[132] L. Geng, E. Donnelly, G. McMahon et al., "Inhibition of vascular endothelial growth factor receptor signaling leads to reversal of tumor resistance to radiotherapy," *Cancer Research*, vol. 61, no. 6, pp. 2413–2419, 2001.

[133] H. Harada, S. Kizaka-Kondoh, G. Li et al., "Significance of HIF-1-active cells in angiogenesis and radioresistance," *Oncogene*, vol. 26, no. 54, pp. 7508–7516, 2007.

[134] B. J. Moeller, Y. Cao, C. Y. Li, and M. W. Dewhirst, "Radiation activates HIF-1 to regulate vascular radiosensitivity in tumors: Role of reoxygenation, free radicals, and stress granules," *Cancer Cell*, vol. 5, no. 5, pp. 429–441, 2004.

[135] K. A. Houck, D. W. Leung, A. M. Rowland, J. Winer, and N. Ferrara, "Dual regulation of vascular endothelial growth factor bioavailability by genetic and proteolytic mechanisms," *The Journal of Biological Chemistry*, vol. 267, no. 36, pp. 26031–26037, 1992.

[136] T. Veikkola, M. Karkkainen, L. Claesson-Welsh, and K. Alitalo, "Regulation of angiogenesis via vascular endothelial growth factor receptors," *Cancer Research*, vol. 60, no. 2, pp. 203–212, 2000.

[137] T. Donnem, K. Lonvik, K. Eklo et al., "Independent and tissue-specific prognostic impact of miR-126 in non-small cell lung cancer: coexpression with vascular endothelial growth factor-A predicts poor survival," *Cancer*, vol. 117, no. 14, pp. 3193–3200, 2011.

[138] X. Zhu, H. Li, L. Long et al., "MiR-126 enhances the sensitivity of non-small cell lung cancer cells to anticancer agents by targeting vascular endothelial growth factor A," *Acta Biochimica et Biophysica Sinica*, vol. 44, no. 6, pp. 519–526, 2012.

[139] L. Shi, S. Zhang, H. Wu et al., "MiR-200c increases the radiosensitivity of non-small-cell lung cancer cell line A549 by targeting VEGF-VEGFR2 pathway," *PLoS ONE*, vol. 8, no. 10, Article ID e78344, 2013.

[140] R. M. Bremnes, C. Camps, and R. Sirera, "Angiogenesis in non-small cell lung cancer: the prognostic impact of neoangiogenesis and the cytokines VEGF and bFGF in tumours and blood," *Lung Cancer*, vol. 51, no. 2, pp. 143–158, 2006.

[141] M. Korc and R. E. Friesel, "The role of fibroblast growth factors in tumor growth," *Current Cancer Drug Targets*, vol. 9, no. 5, pp. 639–651, 2009.

[142] M. Presta, P. Dell'Era, S. Mitola, E. Moroni, R. Ronca, and M. Rusnati, "Fibroblast growth factor/fibroblast growth factor receptor system in angiogenesis," *Cytokine and Growth Factor Reviews*, vol. 16, no. 2, pp. 159–178, 2005.

[143] G. Seghezzi, S. Patel, C. J. Ren et al., "Fibroblast growth factor-2 (FGF-2) induces vascular endothelial growth factor (VEGF) expression in the endothelial cells of forming capillaries: an autocrine mechanism contributing to angiogenesis," *Journal of Cell Biology*, vol. 141, no. 7, pp. 1659–1673, 1998.

[144] T. Donnem, C. G. Fenton, K. Lonvik et al., "MicroRNA signatures in tumor tissue related to angiogenesis in non-small cell lung cancer," *PLoS ONE*, vol. 7, no. 1, Article ID e29671, 2012.

[145] G. Murugaiyan and B. Saha, "Protumor vs antitumor functions of IL-17," *The Journal of Immunology*, vol. 183, no. 7, pp. 4169–4175, 2009.

[146] A. Yuan, J. J. W. Chen, P.-L. Yao, and P.-C. Yang, "The role of interleukin-8 in cancer cells and microenvironment interaction," *Frontiers in Bioscience*, vol. 10, pp. 853–865, 2005.

[147] M. Orditura, F. de Vita, G. Catalano et al., "Elevated serum levels of interleukin-8 in advanced non-small cell lung cancer patients: relationship with prognosis," *Journal of Interferon and Cytokine Research*, vol. 22, no. 11, pp. 1129–1135, 2002.

[148] C. V. Pecot, R. Rupaimoole, D. Yang et al., "Tumour angiogenesis regulation by the miR-200 family," *Nature Communications*, vol. 4, article 2427, 2013.

[149] S. I. Rothschild, M. P. Tschan, R. Jaggi, M. F. Fey, M. Gugger, and O. Gautschi, "MicroRNA-381 represses ID1 and is deregulated in lung adenocarcinoma," *Journal of Thoracic Oncology*, vol. 7, no. 7, pp. 1069–1077, 2012.

[150] B. Liu, X. Wu, B. Liu et al., "MiR-26a enhances metastasis potential of lung cancer cells via AKT pathway by targeting PTEN," *Biochimica et Biophysica Acta* , vol. 1822, no. 11, pp. 1692–1704, 2012.

[151] N. Kosaka, H. Iguchi, and T. Ochiya, "Circulating microRNA in body fluid: a new potential biomarker for cancer diagnosis and prognosis," *Cancer Science*, vol. 101, no. 10, pp. 2087–2092, 2010.

[152] H. Hu, B. Wang, M. Borde et al., "Foxp1 is an essential transcriptional regulator of B cell development," *Nature Immunology*, vol. 7, no. 8, pp. 819–826, 2006.

[153] P. S. Mitchell, R. K. Parkin, E. M. Kroh et al., "Circulating microRNAs as stable blood-based markers for cancer detection," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 105, no. 30, pp. 10513–10518, 2008.

[154] M. S. Kumar, J. Lu, K. L. Mercer, T. R. Golub, and T. Jacks, "Impaired microRNA processing enhances cellular transformation and tumorigenesis," *Nature Genetics*, vol. 39, no. 5, pp. 673–677, 2007.

[155] G. Rabinowits, C. Gerçel-Taylor, J. M. Day, D. D. Taylor, and G. H. Kloecker, "Exosomal microRNA: a diagnostic marker for lung cancer," *Clinical Lung Cancer*, vol. 10, no. 1, pp. 42–46, 2009.

[156] H. B. Le, W. Y. Zhu, D. D. Chen et al., "Evaluation of dynamic change of serum miR-21 and miR-24 in pre- and post-operative lung carcinoma patients," *Medical Oncology*, vol. 29, no. 5, pp. 3190–3197, 2012.

[157] A. V. Kossenkov, A. Vachani, C. Chang et al., "Resection of non-small cell lung cancers reverses tumor-induced gene expression changes in the peripheral immune system," *Clinical Cancer Research*, vol. 17, no. 18, pp. 5867–5877, 2011.

[158] P. Leidinger, A. Keller, C. Backes, H. Huwer, and E. Meese, "MicroRNA expression changes after lung cancer resection: a follow-up study," *RNA Biology*, vol. 9, no. 6, pp. 900–910, 2012.

[159] J. Skog, T. Würdinger, S. van Rijn et al., "Glioblastoma microvesicles transport RNA and proteins that promote tumour growth and provide diagnostic biomarkers," *Nature Cell Biology*, vol. 10, no. 12, pp. 1470–1476, 2008.

[160] G. Zhuang, X. Wu, Z. Jiang et al., "Tumour-secreted miR-9 promotes endothelial cell migration and angiogenesis by activating the JAK-STAT pathway," *The EMBO Journal*, vol. 31, no. 17, pp. 3513–3523, 2012.

[161] V. N. Aushev, I. B. Zborovskaya, K. K. Laktionov et al., "Comparisons of microRNA patterns in plasma before and after tumor removal reveal New biomarkers of lung squamous cell carcinoma," *PLoS ONE*, vol. 8, no. 10, Article ID e78649, 2013.

[162] L. Yu, N. W. Todd, L. Xing et al., "Early detection of lung adenocarcinoma in sputum by a panel of microRNA markers," *International Journal of Cancer*, vol. 127, no. 12, pp. 2870–2878, 2010.

[163] L. Xing, N. W. Todd, L. Yu, H. Fang, and F. Jiang, "Early detection of squamous cell lung cancer in sputum by a panel of microRNA markers," *Modern Pathology*, vol. 23, no. 8, pp. 1157–1164, 2010.

[164] C. Roth, S. Kasimir-Bauer, K. Pantel, and H. Schwarzenbach, "Screening for circulating nucleic acids and caspase activity in the peripheral blood as potential diagnostic tools in lung cancer," *Molecular Oncology*, vol. 5, no. 3, pp. 281–291, 2011.

[165] W. Gao, Y. Yu, H. Cao et al., "Deregulated expression of miR-21, miR-143 and miR-181a in non small cell lung cancer is related to clinicopathologic characteristics or patient prognosis," *Biomedicine and Pharmacotherapy*, vol. 64, no. 6, pp. 399–408, 2010.

[166] J. A. Bishop, H. Benjamin, H. Cholakh, A. Chajut, D. P. Clark, and W. H. Westra, "Accurate classification of non-small cell lung carcinoma using a novel microRNA-based approach," *Clinical Cancer Research*, vol. 16, no. 2, pp. 610–619, 2010.

[167] Y. Lu, R. Govindan, L. Wang et al., "MicroRNA profiling and prediction of recurrence/relapse-free survival in stage I lung cancer," *Carcinogenesis*, vol. 33, no. 5, pp. 1046–1054, 2012.

[168] M. Jiang, P. Zhang, G. Hu et al., "Relative expressions of miR-205-5p, miR-205-3p, and miR-21 in tissues and serum of non-small cell lung cancer patients," *Molecular and Cellular Biochemistry*, vol. 383, no. 1-2, pp. 67–75, 2013.

[169] Y. Sun, B. Su, P. Zhang et al., "Expression of miR-150 and miR-3940-5p is reduced in non-small cell lung carcinoma and correlates with clinicopathological features," *Oncology Reports*, vol. 29, no. 2, pp. 704–712, 2013.

[170] M.-P. Puisségur, N. M. Mazure, T. Bertero et al., "MiR-210 is overexpressed in late stages of lung cancer and mediates mitochondrial alterations associated with modulation of HIF-1 activity," *Cell Death and Differentiation*, vol. 18, no. 3, pp. 465–478, 2011.

[171] K. M. Foss, C. Sima, D. Ugolini, M. Neri, K. E. Allen, and G. J. Weiss, "MiR-1254 and miR-574-5p: serum-based microRNA biomarkers for early-stage non-small cell lung cancer," *Journal of Thoracic Oncology*, vol. 6, no. 3, pp. 482–488, 2011.

[172] I. Barshack, G. Lithwick-Yanai, A. Afek et al., "MicroRNA expression differentiates between primary lung tumors and metastases to the lung," *Pathology Research and Practice*, vol. 206, no. 8, pp. 578–584, 2010.

[173] A. Navarro, T. Diaz, E. Gallardo et al., "Prognostic implications of miR-16 expression levels in resected non-small-cell lung cancer," *Journal of Surgical Oncology*, vol. 103, no. 5, pp. 411–415, 2011.

[174] A. Markou, E. G. Tsaroucha, L. Kaklamanis, M. Fotinou, V. Georgoulias, and E. S. Lianidou, "Prognostic value of mature MicroRNA-21 and MicroRNA-205 overexpression in non-small cell lung cancer by quantitative real-time RT-PCR," *Clinical Chemistry*, vol. 54, no. 10, pp. 1696–1704, 2008.

[175] I. A. Babar, J. Czochor, A. Steinmetz, J. B. Weidhaas, P. M. Glazer, and F. J. Slack, "Inhibition of hypoxia-induced miR-155 radiosensitizes hypoxic lung cancer cells," *Cancer Biology and Therapy*, vol. 12, no. 10, pp. 908–914, 2011.

[176] J. F. Wiggins, L. Ruffino, K. Kelnar et al., "Development of a lung cancer therapeutic based on the tumor suppressor microRNA-34," *Cancer Research*, vol. 70, no. 14, pp. 5923–5930, 2010.

[177] J. Voortman, A. Goto, J. Mendiboure et al., "MicroRNA expression and clinical outcomes in patients treated with adjuvant chemotherapy after complete resection of non-small cell lung carcinoma," *Cancer Research*, vol. 70, no. 21, pp. 8288–8298, 2010.

[178] X. Pu, J. A. Roth, M. A. Hildebrandt et al., "MicroRNA-related genetic variants associated with clinical outcomes in early-stage non-small cell lung cancer patients," *Cancer Research*, vol. 73, no. 6, pp. 1867–1875, 2013.