REVIEW

MicroRNA-145 targets in cancer and the cardiovascular system: evidence for common signaling pathways

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Abstract

miRNAs are small regulatory RNAs which govern gene expression post-transcriptionally by primarily binding to the 3'-UTR of mRNA target genes. miR-145 is a well-studied miRNA that has been implicated in controlling a range of biological processes. miR-145 is expressed in a variety of tissues and cell types and acts as a tumor-suppressor by regulating target gene signaling pathways involved in different aspects of tumor growth and progression. There is also strong evidence that highlights the important functions of miR-145 in the cardiovascular system. Here, we review the mechanisms of miR-145 in tumorigenesis and cancer progression and compare and contrast with the roles of miR-145 in cardiovascular development and disease. We discuss the important targets of miR-145 in cancer and their possible link to the cardiovascular system.

Key Words

- *miR-145*
- cancer
- cardiovascular
- smooth muscle

Introduction

miRNAs are a group of small (~22 nucleotides in length) ncRNA molecules that functionally fine tune gene expression through post-transcriptional regulation (1). Since their discovery in the nematode *Caenorhabditis elegans* in 1993 (2, 3), there have been significant discoveries steering the field of small RNA biology that have modified the longstanding dogmas of gene regulation. Multiple miRNAs across different species of animals and plants have been discovered and an estimated 2000 mature miRNAs are encoded by the human genome (4).

miR-145 was first identified as a novel tissue-specific miRNA expressed in murine hearts (5). Since this initial report in 2002, miR-145 has garnered much attention, with 1708 PubMed articles with miR-145 in the title or abstract as of October 2020. Nearly 60%, or 1017 of these publications are cancer-related studies, while 210 or only 12% are cardiovascular-associated. The importance of miR-145 in the cardiovascular system became apparent in 2009, when a group of high-profile papers collectively demonstrated its tissue-specific expression during mouse development and its regulatory role in vascular smooth muscle cells (6, 7, 8, 9, 10, 11). miR-143 and miR-145 are co-transcribed as a single bicistronic primary transcript (8, 12). Even though miR-143 and miR-145 are co-transcribed, the absence of homology in their mature sequences, highlights their ability to bind to different targets and therefore have distinct functions (9). The data show that miR-145 targets Krüppel-like factors, *Klf4* and *Klf5*, both of which stimulate proliferation, while increasing myocardin expression to promote a differentiated vascular smooth muscle phenotype (7, 8, 9). Furthermore, miR-145 targets several mediators that govern actin dynamics

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and polymerization in support of a contractile smooth muscle phenotype (9). These and more recent studies in vascular injury models suggest a more complex role, with miR-145 having an anti-proliferative and beneficial role in disease pathology in certain instances (7, 13) while having a distinctly opposite effect in other models (9). In addition to its actions in vascular smooth muscle cells, miR-145 has been reported to have functions in other cardiovascular cell types. These publications have reported on miR-145 activities in cardiac fibroblasts and myofibroblasts and endothelial cells (14, 15). Despite these informative reports, the function of miR-145 specifically in the cardiovascular system remains incomplete. Given the wealth of studies on miR-145 in cancer cells (16), it is intriguing to consider that the functions of miR-145 in tumor environments may shed light on its activities in cardiovascular cells. In this review, we highlight the roles and particular pathways that miR-145 regulates in cancer cells and attempt to link these with the functions of miR-145 in the cardiovascular system. While, it is well known that miRNAs functions are context dependent, understanding miR-145 in cancer could possibly inform our understanding of its critical functions in cells within the cardiovascular system.

Mechanisms of miR-145 in cancer

miRNAs mediate post-transcriptional regulation of target genes by binding to gene-specific seed sequences (17, 18). More than 50% of miRNA genes are located in genomic regions associated with cancer or fragile sites, which are often deleted or repeated in cancer (19, 20), and many miRNAs are commonly dysregulated in cancer (20). miRNAs can function as oncogenes as well as tumor suppressor genes by targeting gene signaling pathways that influence tumor growth, invasion, metastasis and angiogenesis (21). The earliest reports that linked miRNAs and cancer discussed miR-15 and miR-16 as tumor suppressors in B-cell chronic lymphocytic leukemia. miR-15 and miR-16 are expressed from chromosome region 13q14, which is frequently deleted in lymphocytic leukemia (22, 23). Since then many miRNAs have been associated with cancer, one such cluster being the miR-143/145 cluster, which is frequently downregulated in many cancers. Studies focused solely on miR-145 show that in normal cells, it is highly expressed in adult mesodermal tissues, such as uterus, ovaries, testis, heart and prostate (24, 25). The expression of miR-145 is downregulated in a wide range of cancers, including colorectal cancer (CRC), non-small-cell lung cancer (NSCLC), breast cancer (BCa), prostate cancer (PCa), gastric cancer (GC), ovarian cancer (OC), and bladder cancer (BC) (16, 25, 26, 27, 28). miR-145 mainly acts as a tumor suppressor and inhibits cancer stem cells, tumor growth, invasion and metastasis, and tumor angiogenesis (29, 30, 31, 32). Thus, these miRNAs have widely been categorized as tumor-suppressors, although, more recent reports also highlight them as oncogenes (33, 34, 35, 36). Of note, one such report investigated the expression profile of miR-145 in samples of colorectal cancer with and without metastasis to lymph node (34). When compared to normal tissue, miR-145 expression is downregulated in both metastatic and non-metastatic samples. However, miR-145 is dramatically upregulated in cancer samples associated with metastasis when compared to the non-metastatic samples. In addition, overexpression of miR-145 leads to an increase in colorectal cancer cell migration *in vitro,* and an enhanced metastasis to lymph nodes *in vivo* in mice (34). Similarly, miR-145 has opposing roles in different types of esophageal cancer; esophageal squamous cell carcinoma (ESCC), derived from squamous cells in the esophageal lining and esophageal adenocarcinoma (EAC), derived from glandular cells not normally part of the esophageal lining. The expression of miR-145 in esophageal squamous cell carcinoma inhibits cell proliferation and invasion and enhances anoikis (cell death triggered by detachment from extracellular matrix (ECM); an ability that is reduced during metastasis) (35). These results confirm previous studies that highlight miR-145 as a tumor suppressor. However, in esophageal adenocarcinoma cells, miR-145 expression leads to an increase in metastatic potential and protection against anoikis (35). Thus, in certain contexts of cancer, miR-145 functions as a pro-metastatic miRNA rather than a tumor suppressor miRNA. Taken together, the expression and function of miR-145 in cancer depends upon various factors such as the type of cancer, the stage of cancer development and the cell origin of the tumor. These factors become absolutely critical to evaluate when contemplating the use of miR-145 as a therapeutic or as a potential biomarker in cancer.

miR-145 and stem cells

miR-145 represses pluripotency of human embryonic stem cells (hESCs) by directly targeting core pluripotency factors (37). Core transcription factors (TFs) such as Octamer-binding transcription factor 4 (OCT4/POU5F1), SRY-box 2 (SOX2), NANOG control a wide range of downstream genes required for the self-renewal and

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pluripotency properties of embryonic stem cells. Other factors such as KLF4, c-MYC (MYC) and E-RAS are required for the maintenance of pluripotency and self-renewal of embryonic stem cells and are often upregulated in cancer. The core factors decrease during differentiation of embryonic stem cells. Contrastingly, miR-145 levels are low in human embryonic stem cells but increase during their differentiation. Xu *et al*. demonstrated that miR-145 represses pluripotency and disrupts the self-renewal state of embryonic stem cells by targeting the 3'UTRs of *OCT4*, *SOX2* and *KLF4* (37). Additionally, OCT4 can bind to the promoter and represses miR-145, indicating a double-negative feedback loop involving pluripotency and miR-145 (37). Further investigation of the function of miR-145 during human embryonic stem cell differentiation, revealed that expression of miR-145 leads to an increase in differentiation markers of mesoderm lineage (α-smooth muscle actin (*ACTA-2*)) and ectoderm lineage (β-III tubulin (*TUBB3*)). This ectopic expression of miR-145 acts through the repression of *OCT4* and *SOX2* to induce differentiation (37). Another study confirmed that miR-145 directly targets *OCT4*, *SOX2* and *NANOG* and that ectopic expression of miR-145 during differentiation of embryonic stem cells leads to the degradation of these core factors (38). Thus, miR-145 plays a critical role in controlling stem cell self-renewal and driving differentiation by suppressing pluripotency factors summarized in Table 1.

Similar to its role in controlling pluripotency in embryonic stem cells (37), miR-145 regulates the cell fate of cancer stem cells by suppressing the same pluripotency factors that function in embryonic stem cells. Tumors harbor a small population of cells called cancer stem cells (CSCs)/cancer stem-like cells (CSLCs), which highly express OCT4, NANOG, SOX2 and KLF4 (39, 40). The stem-like characteristic of cancer stem cells has been speculated to drive tumor progression, metastasis, relapse, and drug resistance (39, 41). Evidence from different research studies reveal a common underlying mechanism of miR-145 function in the regulation of cancer stem cells in different cancer types. Overexpression of miR-145 suppresses *OCT4*, *SOX2*, *NANOG* and *KLF4* in cancer stem cells in colorectal cancer, laryngeal squamous cell carcinoma (LSCC) and cervical cancer (CC) (32, 42, 43). Further, overexpression of miR-145 inhibits stemness property of cancer stem cells, induces differentiation, and reduces tumor growth and progression in these cancers. Additionally, miR-145 plays an important role in inhibiting tumorigenicity of bone metastatic prostate cancer (PCa) cell line and its metastasis to the bone *in vivo*, by suppressing cancer stem cell markers, *OCT4*, *KLF4*, and *c-MYC* (44). Taken together, these findings demonstrate that miR-145 plays an important role in regulating cancer stem cell characteristics to inhibit tumor growth, progression, and metastasis.

Table 1 Shared miR-145 targets in human embryonic stem cells and cancer stem cells.

Target genes	3'UTR target site location	Types of cancer	References
OCT4 (*POU5F1*)	138–157; 1276–1297	LSCC, cervical, NSCLC	(37, 38, 42, 43, 48)
SOX2	1–20; 1391–1411	Colorectal, LSCC, cervical	(32, 37, 38, 42, 43)
KLF4	256–285	LSCC	(37, 42)
NANOG	764–790	Cervical	(38, 43)
c-MYC (*MYC*)	Unknown	Prostate, breast, colon, NSCLC	(44, 46, 47)

LSCC, laryngeal squamous cell carcinoma; NSCLC, non-small cell lung cancer.

miR-145 and tumor growth and angiogenesis

miR-145 reduces tumor growth by regulating the expression of genes that are critical in cell proliferation and apoptosis in various cancers. c-MYC is a widely studied oncogene and is often dysregulated in many tumors. c-MYC regulates numerous genes, which play pivotal roles in cell proliferation, apoptosis and differentiation (45). Moreover, *c-MYC* is a direct target of miRNA-145. Ectopic expression of miR-145 suppresses *c-MYC* and delays cell cycle progression and inhibits tumor cell proliferation and tumor growth *in vitro* and *in vivo* in breast, colon cancer and non-small lung cancer (46, 47). In addition, in non-small lung cancer, miR-145 inhibits tumor cell proliferation by targeting *OCT4* and impairs the progression of lung cancer development (48). Thus, miR-145 targets genes (Table 2) involved in cell proliferation to regulate tumor growth.

Similarly, miR-145 acts as a suppressor of cell proliferation in bladder cancer. The expression of miR-145 is lower in bladder cancer samples and bladder cancer cell lines when compared to normal tissues and human uroepithelial cell lines, respectively (49). miR-145 directly targets 3'UTR of *KLF4* and the ectopic expression of miR-145 in human bladder carcinoma cell line leads to the down-regulation of *KLF4* and repression of cell proliferation (49). Another study showed that the proto-oncogene plasminogen activator inhibitor-1 (*PAI1* encoded by the *SERPINE1* locus) is upregulated and the miR-143/145 cluster is downregulated in all stages of

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Table 2 miR-145 targets in cancer that are present in cardiovascular cell types.

Target genes	Cardiovascular cell type	Type of cancer	References
OCT4 (*POU5F1*)	SMC, perivascular cells	LSCC, cervical, NSCLC	(42, 43, 48, 86, 87)
SOX2	EC	Colorectal, LSCC, cervical	(32, 42, 43, 92)
KLF4	SMC	LSCC, cervical, bladder	(8, 42, 43, 49)
NANOG	SMC	Cervical	(43, 88)
c-MYC (*MYC*)	Cardiomyocytes	Breast, prostrate, colon, NSCLC	(44, 46, 47, 103)
PAI1 (*SERPINE1*)	SMC	Bladder	(50, 84)
p70S6K1	EC	Colon	(53, 58)
DDX17 (*p72*)	Cardiomyocytes	Colon	(52, 106)
MUC1	EC	Lung	(61, 91)
SMAD3	Fibroblasts, cardiomyocytes	Nasopharyngeal	(63, 100)
HOXA1	Cardiac neural crest cells	Oral (OSCC)	(65, 104)
FSCN1	EC	Lung (NSCLC), breast	(70, 71, 90)
JAMA (*F11R*)	EC, leukocytes	Breast	(69, 72, 108)
N-RAS	EC, SMC	Breast, colorectal	(30, 54, 84, 97)
VEGFA	EC	Breast	(30, 95, 96)
PAK1	Cardiomyocytes, EC	Bladder, breast	(66, 67, 93, 94)
PAK4	Cardiomyocytes, EC	Colon	(55, 93, 94)
IRS1	EC	Colorectal	(54, 98)
N-CADHERIN	EC	Lung	(74, 75)
ADAM17	EC, SMC, fibroblasts	Renal	(59, 102)
ROCK1	SMC	Breast	(73, 85)

EC, endothelial cells; LSCC, laryngeal squamous cell carcinoma; NSCLC, non-small cell lung cancer; OSCC, oral squamous cell carcinoma; SMC, smooth muscle cells.

bladder cancer (50). PAI1 is known to enhance cancer cell proliferation and angiogenesis by inhibiting apoptosis (51). Both miR-143 and miR-145 directly bind to the 3'UTR of *PAI1* mRNA and reduce *PAI1* mRNA and protein levels in bladder cancer, cervical cancer and non-small lung carcinoma cell lines (50). Thus, miR-143/145 cluster targets the oncogene, *PAI1,* in various cancers.

In colorectal cancer, miR-145 and miR-143 attenuate tumor cell growth in the small intestine of $Apc^{Min/+}$ mice, which develop colorectal tumors, by inhibiting the extracellular signal regulated kinase 5 (ERK5)/c-MYC and p68/p72/B-catenin signaling pathways (52). B-catenin signaling pathway is known to be involved in tissue homeostasis and is often aberrantly activated in many cancers. In colon tumor development, DEAD-box RNA helicase subunits p68/p72 (DDX5/DDX17) are critically involved in β-catenin signaling, to activate many downstream effectors such as *c-MYC*. Moreover, miR-145 directly targets the 3'UTR of *p72,* impairing B-catenin signaling, and represses c-MYC in human colon cancer cells (52). Another study demonstrated that miR-145 inhibits tumor growth in patients with colon cancer by targeting the mammalian target of rapamycin (mTOR)/p70S6K1 signaling (53). mTOR/p70S6K1 signaling regulates various cellular functions such as cell cycle, apoptosis, cell growth and proliferation and hence is the most targeted pathway in cancer therapy. miR-145 directly inhibits *p70S6K1* by binding to its 3'UTR. It downregulates the downstream targets of p70S6K1, angiogenic factors and tumor growth effectors, hypoxia-inducible factor 1 (*HIF-1 (HIF1A)*) and vascular endothelial growth factor (*VEGF*). Thus, miR-145 inhibits tumor growth via *p70S6K1*. In addition, miR-145 inhibited *HIF1* and *VEGF* by directly targeting oncogene *N-RAS* and insulin receptor substrate, *IRS1,* thereby reducing tumor growth in colorectal cancer (54). Another study in colon cancer, demonstrated that miR-145 directly targets p21-activated kinase-4 (*PAK4*) and downregulates ERK pathway to inhibits tumor cell growth (55). PAKs are a family of protein kinases that regulate various cellular functions such as cell survival, proliferation and migration and are often hyperactivated in various cancers. Thus, miR-145 suppresses tumor growth in various cancers by targeting different signaling pathways involved in proliferation, survival and growth.

Angiogenesis and re-endothelialization are common vascular consequences in many diseases including cancer, atherosclerosis and ischemic heart disease. A key factor that influences tumor growth and metastasis is tumor angiogenesis. There are growing reports that demonstrate that ectopic expression of p70S6K1 in vascular endothelial cells and cancer cells can lead to tumor angiogenesis (56, 57, 58). miR-145 functions as a tumor suppressor by downregulating HIF1α and VEGF expression by directly

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targeting *p70S6K1*, thereby repressing tumor growth and angiogenesis (53). In another study, miR-145 was shown to inhibit tumor angiogenesis and growth by regulating *N-RAS* and *VEGF-A* in breast cancer cells and colorectal cancer (30). Thus, miR-145 plays an important role in cancer malignancy by inhibiting tumor growth and angiogenesis.

miR-145 and apoptosis

Metalloprotease a disintegrin and metalloproteinase 17 (ADAM17) expression increases with the degree of malignancy in different cancers including renal cell carcinoma (RCC). On the contrary miR-145 levels are lower in renal carcinoma cell lines and renal cancer patient tissues when compared to primary renal cell lines and normal tissue ,respectively (59). In renal carcinoma cells, miR-145 directly binds to the 3'UTR and represses *ADAM17* mRNA, leading to down-regulation of ADAM17 protein levels and decrease in tumor cell proliferation. This also leads to an increase in cells in the G_1/G_0 phase of cell cycle with fragmented nuclei, indicative of early apoptosis (59). In urothelial carcinoma cells, miR-145 overexpression strongly stimulates activated caspase dependent and independent apoptotic pathways (60). Thus, miR-145 affects the tolerance of tumor cells to apoptotic factors.

miR-145 and tumor invasion and metastasis

Malignant tumors are characterized by the local invasion of tumor cells and metastasis to other organs. miR-145 controls tumor malignancy in different cancers. miR-145 significantly inhibits cell invasion of metastatic breast cancer cells and suppresses lung metastasis in mouse models of metastasis through downregulating *MUC1* (61). *MUC1* is a highly characterized metastasis promoting gene, which is upregulated in different tumors, and is a direct target of miR-145 (61, 62). In addition, miR-145 affects the invasive and metastatic characteristics of nasopharyngeal cancer (NPC) by directly targeting *Smad3* (63). Smad3 is a known intracellular mediator of TGF-B signaling and is known to promote invasion and metastasis in many cancers (64). In oral squamous cell carcinoma (OSCC), miR-145 is poorly expressed and homeobox A1 (*HOXA1*), is highly expressed. HOXA1 is an important transcriptional factor during development and a potential activator of ERK/MAPK pathway involved in cell proliferation, apoptosis and growth. Ectopic expression of miR-145 inhibits *HOXA1* and inactivates the ERK/MAPK signaling pathway, thereby suppressing oral squamous cell carcinoma proliferation, migration, and invasion (65). In breast cancer, PAK1 is a known activator of the MAPK pathway (66). In bladder cancer, the levels of miR-145 negatively correlate with expression of *PAK1* (67). PAK1 is known to enhance invasion of cancer cells through the expression of *MMP-9*. miR-145 directly targets *PAK1*, decreases *MMP-9* expression, and thereby inhibits cell invasion of bladder cancer.

Some invasive tumor cells are characterized by increased cell motility, which require actin cytoskeletal reorganization, decreased cell-cell adhesion and an increased formation of actin-based cellular protrusions called filopodia. Fascin 1 (FSCN1) is an actin binding protein involved in the formation of filopodial protrusions. It plays an important role in cytoskeletal dynamics and the regulation of cell adhesion and motility (68). Another protein important in cell-cell adhesion is JAMA (F11R), a membrane protein that is often dysregulated in cancer cells leading to increased migration and invasion (69). In non-small cell lung cancer and in breast cancer, FSCN1 promotes migration and invasion (70, 71). miR-145 expression inhibits cell migration and invasion in these cancers by directly targeting and downregulating *JAMA* and *FSCN1* (70, 71, 72). RhoA and its downstream effector, Rho-associated kinase (ROCK1), are key regulator of actin cytoskeleton reorganization. Since actin reorganization plays an important role in cancer cell migration and invasion, ROCK is a positive regulator of cancer cell invasion. Overexpression of miR-145 directly targets and represses *ROCK1* and greatly reduces the invasive ability of glioma cells (73).

Tumor metastasis is a multi-step process that also includes epithelial to mesenchymal transition (EMT). Loss of E-cadherin with increased N-cadherin expression is an important step during EMT and an important characteristic of metastatic cells. Studies have shown that overexpression of miR-145 directly targets and reduces N-cadherin expression, inhibiting invasion, and metastasis of a lung adenocarcinoma cell line (74). In non-small-cell lung cancer cell lines, miR-145 expression is low and ectopic expression of miR-145 inhibits TGF-β-induced epithelial to mesenchymal transition (EMT) and suppresses cancer cell invasion and migration (75).

In summary, miR-145 plays an important role in various stages of cancer development from cancer stem cells, tumor growth, angiogenesis, invasion and metastasis (Fig. 1).

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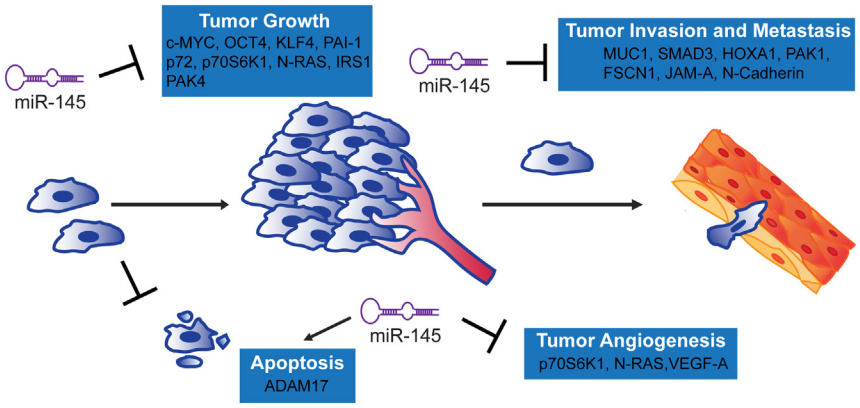

Figure 1
Tumor progression is a multi-step process and miR-145 regulates various targets at different stages of tumorigenesis. miR-145 inhibits tumors growth and angiogenesis and increases apoptosis by directly suppressing various genes. miR-145 inhibits cell invasion and tumor metastasis in various cancers.

miR-145 in the cardiovascular system. Is there a link to cancer?

During embryogenesis, miR-145 is highly expressed in the heart and blood vessels of the developing embryo (6, 10). Postnatally, miR-145 expression is reduced in the heart and is abundantly expressed in vascular smooth muscle cells (vSMC) of the aorta, pulmonary artery, and coronary vessels (6, 7, 10). miR-145 is found in lung myofibroblasts (76) and cardiac fibroblasts (14) and is present in endothelial cells (15). Moreover, evidence reveals that it is detectable in plasma and can be secreted by both endothelial and smooth muscle cells (77, 78). These data indicate that miR-145 has diverse functions within the cardiovascular system.

In vascular smooth muscle cells (vSMCs)

miR-145 is abundantly expressed in vascular smooth muscle cells and its function in vascular smooth muscle cells has been extensively examined (6, 7, 8, 9, 10, 11). The expression of miR-143/145 in development mirrors that of classic smooth muscle-specific genes, where it first appears in the heart and developing somites, and over time becomes restricted to the forming vasculature (6, 10). Its expression pattern alone hints at a regulatory role in vascular smooth muscle differentiation. Indeed, miR-143/145 transcript has been shown to be regulated by the serum responsive factor (SRF)/Myocardin (Myocd) complex, which is a critical activator of vascular smooth muscle cells differentiation (Fig. 2) (79). This complex binds to the enhancer region CArG-box in the promoter of miR-143/145 to induce tissue- specific expression in the heart and vasculature (8, 9, 80). Functionally, miR-145 suppresses the expression of *Klf4*, a positive regulator of vascular smooth muscle cells proliferation and phenotypic switch, thus promoting differentiation (8). In addition, miR-145 maintains the vascular smooth muscle cells contractile phenotype by increasing the expression of *Myocd* which in turn induces vascular smooth muscle cell differentiation and contractility (8). Overexpression of miR-145 leads to the upregulation of vascular smooth muscle cells differentiation genes such as α-smooth muscle actin (*Acta2*), calponin (*Cnn1*), and smooth muscle myosin heavy chain (*Myh11*) (7). miR-145 regulation of *Klf4* to control vascular smooth muscle cells differentiation is reminiscent of the suppression of *KLF4* in promoting differentiation of embryonic stem cells as well as cancer stem cells. Thus, in the context of smooth muscle cell fate in the vasculature and in the progression of cancer, miR-145 plays a pivotal role in the suppression of proliferation by directly targeting *Klf4*.

In addition to the SRF/Myocd pathways, miR-143/145 expression is controlled by the transforming growth factor (TGF-B1) pathway, a known stimulus of smooth muscle cell differentiation. (In cancer, TGF-B1 is often upregulated during tumorigenesis and mainly acts via the SMAD factors. It initially suppresses tumorigenesis and later drives cancer metastasis by inducing epithelial to mesenchymal transition (EMT), cell motility and invasion (64, 81)). In coronary artery smooth muscle cells, (TGF-B) induces miR-145 expression through two TGF-B signaling pathways (p38MAPK and SMAD) that act on upstream enhancers of miR-145 (Smad binding element (SBE) and CArG box), leading to the transcription of miR-143/145 (82, 83). Ectopic expression of miR-145, induces expression of *Cnn1* and *Acta2*, while anti-miR-145 reduced TGF-B1 induced expression of *Cnn1* and *Acta2*. Thus, TGF-B1 induces smooth muscle cell differentiation through miR-143/145 expression. Another study revealed that miR-145 is induced in vascular smooth muscle cells by endothelial cells through Notch signaling (84). In smooth muscle cells, miR-145 directly suppressed TGF-B receptor II (*TGF-BR2*) expression and blocked the

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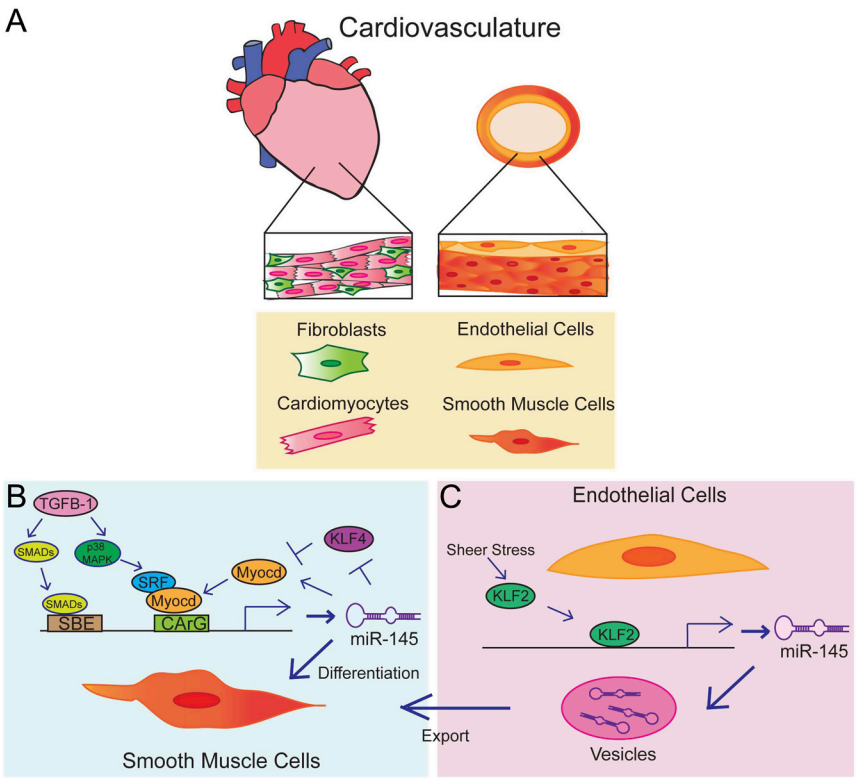

Figure 2
Roles of miR-145 in different cardiovascular cell types. (A) In the cardiovascular system, miR-145 plays various roles in smooth muscle cells (SMCs), endothelial cells, fibroblasts, and cardiomyocytes. (B and C) The role of miR-145 in SMCs and endothelial cells is highlighted in detail. In SMCs, miR-145 expression is regulated by TGFβ-1 signaling and SRF/Myocd complex. It represses KLF4 and promotes the differentiation of SMCs. In endothelial cells, miR-145 is induced by KLF2 in response to sheer stress. Subsequently, miR-145 is exported in exosome-like vesicles to SMCs to regulate SMC phenotypes. SRF, serum response factor; KLF4, Kruppel-like factor 4; SBE, SMADs binding element.

expression of extra cellular matrix genes, while allowing the expression of smooth muscle specific differentiation genes (84). Overexpression of miR-145 decreases serpine1 (*PAI1*), *N-RAS* and *SMAD7*, which are downstream genes of the TGF-B pathway. TGF-B1 is also known to activate the Rho GTPase, which is an important intermediate in the expression of smooth muscle differentiation genes *Acta2* and *Tagln*. The downstream effector of RhoA and a known direct target of miR-145 in cancer, ROCK, is known to modulate the expression of several genes such as *PAI-1* (85). Taken together, TGF-B1 is a well characterized inducer of the miR-143/145 cluster in the vasculature and miR-145 suppresses different TGF-B pathway genes. Comparing its role in cancer and cardiovascular system, miR-145 facilitates the maintenance of vascular smooth muscle cell differentiation and contractility and inhibits cancer cell invasion and migration by regulating various targets in the TGF-B pathway.

Since, miR-143/145 gene cluster is critical in regulation of vascular smooth muscle cell contractility and differentiation, it is not surprising that they play an important role in smooth muscle cell driven pathologies of cardiovascular diseases. miR-143/145 is not essential for cardiovascular development *in vivo*, as miR-143/145 knockout mice are viable. However, smooth muscle cells from miR-143/145 null mice acquire a synthetic phenotype (less contractile) resulting in thin and distended vessels (6, 9). These mice displayed decreased vascular tone and reduced blood pressure. Proliferative vascular smooth muscle cell driven neointima lesion formation is an important step after vascular damage. miR-143/145 double knock out mice had neointima formation in the femoral artery compared to wild type mice even without any injury. This suggests that these miRNAs play an important role in vascular smooth muscle cell phenotypic switch during injury and disease (6). TGF-B1 activates multiple intermediate signaling molecules such as SMAD, MAPK, Rho/ROCK that control genes involved in various cardiovascular pathologies such as fibrosis, atherosclerosis, and thrombosis. miR-145 but not miR-143 plays a pivotal role in suppressing cardiac and perivascular fibrosis by attenuating TGF-B1 by directly targeting *Tgf-br2* (84). This study showed that miR-145 overexpression decreases *PAI-1*, a known prominent player in arteriosclerosis and perivascular fibrosis. This study also demonstrated that miR-145 null mice show an increase in TGF-B1 signaling with a marked increase in ACTA2 expression and increase in phosphorylation of p38 MAPK in aortic vascular smooth muscle cells, thus, highlighting the important role of miR-145 is controlling TGF-B1 signaling (84). In the context of cancer, miR-145 directly targets multiple players in the TGF-B1 and MAPK

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pathway to suppress cell proliferation, angiogenesis and migration. Thus, by drawing commonalities to its role in cancer, miR-145 most likely targets multiple downstream players in the TGF-B1 pathway, in vascular smooth muscle cells to regulate vascular diseases such as fibrosis and atherosclerosis.

It is known that during the development of atherosclerosis, vascular smooth muscle cells undergo de-differentiation (or phenotypic switching) resulting in loss of smooth muscle cell marker genes and increase in proliferation and migration. The embryonic stem cell core factor OCT4 plays an important role in modulating the de-differentiation of vascular smooth muscle cells during atherosclerosis. OCT4 acts as an atheroprotective agent during plague development in mouse model of atherosclerosis (Apoe$^{-/-}$ mice) by regulating smooth muscle cell phenotype (86). Another study showed that OCT4 plays an important role in perivascular cell migration during angiogenesis. Perivascular cell knockout of *Oct4* significantly reduced perivascular cell migration, delayed endothelial cell migration and thus decreased angiogenesis following injury (87). *OCT4* has already been established as a miR-145 target in embryonic stem cells and cancer stem cells and therefore, could potentially be regulated by miR-145 to control smooth muscle cells differentiation during atherosclerosis. Another pluripotency factor, NANOG, is highly expressed in vascular smooth muscle cells and in aortic wall during thoracic aortic dissections. NANOG enhances the proliferation and migration of vascular smooth muscle cells (88). Knowing that *NANOG* is a target of miR-145 and that miR-145 plays an anti-proliferative role in cancer stem cells, it would be interesting to decipher the interplay between miR-145 and NANOG during aortic dissections.

In endothelial cells

miR-145 is upregulated in endothelial cells in response to shear stress and is exported to regulate vascular smooth muscle cell phenotype to combat atherosclerosis (15). In human aortic arterial endothelial cells, shear-responsive transcription factor Krüppel-like factor 2 (KLF2) induces expression of miR-143/145 in response to shear stress. Subsequently, both miR-145-5p and 3p decrease the expression of junctional adhesion molecule-A (*JamA*), thereby reducing the monocyte recruitment into arterial wall and limiting atherosclerotic lesion formation in atherosclerosis mouse models (15, 89). miR-143/145 can transfer from endothelial cells to vascular smooth muscle cells through exosome-like vesicles (15). In addition to extracellular vesicles, the transfer of miR-143/145 is also mediated by membrane protrusions between smooth muscle cells and endothelial cells (15). miR-145 targets *JamA* to prevent migration of monocytes in atherosclerosis mouse models. In addition, *JAMA* and *FSCN1*, direct targets of miR-145, also promote migration and invasion in breast cancer (71). *FSCN1,* an important cell adhesion regulator, is highly expressed in endothelial cells (90). *MUC1* is a well-established cancer metastasis gene and a target of miR-145 in lung cancer (61). It is also expressed in vascular endothelial cells (91). Thus, in the context of endothelial cells, it is possible that miR-145 may target these genes, *JAMA*, *FSCN1*, and *MUC1* and control the migration of endothelial cells. The pluripotency factor SOX2, which is a target of miR-145 in cancer stem cells, induces endothelial mesenchymal transition that contributes to vascular calcification in atherosclerotic mice (92). Taken together, miR-145 may target *Sox2* in endothelial cells to prevent calcification.

In the context of cancer, miR-145 inhibits cell proliferation and tumor growth by inhibiting the activation of RAS/ERK/MAPK pathway. In cancer, miR-145 directly targets *PAK*s, which are known to activate ERK and p38 MAPK pathways (55, 67). Paks play an important role in the cardiovascular development and function and are expressed in endothelial cells and cardiomyocytes (93, 94). PAK1 has been linked to regulating contractility and Ca^{2+} entry during heart development. It also regulates endothelial cell adhesion in blood vessels. PAK4 plays a major role in heart development during embryogenesis. *Pak4* deletion in mice is embryonically lethal with most embryos dying by E11.5, mostly due to heart defects. *Pak4* null mice display larger blood vessel with lesser branching (93). Thus, it seems plausible that similar to miR-145 regulating *PAK*s in cancer, *PAK*s could be targeted by miR-145 in the cardiovascular system to regulate heart development.

Angiogenesis is a complex process in which cytokines, growth factors, etc. control endothelial cell migration and proliferation. When endothelial-lined tubes are formed, the structures are stabilized by smooth muscle cells and pericytes. miR-143/145 cluster regulates endothelial growth properties upon direct contact with smooth muscle cells during vessel formation. *In vitro* and *in vivo* studies showed that miR-143 and miR-145 can transfer from smooth muscle cells to endothelial cells to modulate endothelial cell angiogenesis and proliferation by directly binding hexokinase II and integrin-B 8, respectively (83). VEGFA and its receptors are required to maintain vascular homeostasis, regulate endothelial

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cell function and are important for developmental and pathological angiogenesis (95, 96). In the cancer context, *VEGFA* is a direct target of miR-145 (30), and regulates angiogenesis. Another study showed that miR-145 regulates VEGF signaling by directly targeting *N-RAS* and *IRS1* in colorectal cancer (54). RAS signaling is important in endothelial cell specification during development (97). IRS1 overexpression in endothelial cells leads to expression of VEGF and an improvement in angiogenesis and wound healing in diabetic mice (98).

Collectively these data suggest that miR-145 may regulate angiogenesis during vascular development and cancer progression through VEGF and RAS pathways.

In cardiomyocytes and fibroblasts

Similar to its role in differentiation of vascular smooth muscle cells, miR-145 plays a role in differentiation of lung fibroblasts and cardiac fibroblasts (14, 76). After injury to the heart, fibroblasts cells get activated to myofibroblasts and deposit extracellular matrix proteins such as collagens and contribute to scar tissue formation called fibrosis (99). miR-145 is expressed in cardiac fibroblast cells (14). Absence of miR-145 leads to an increase in TGF-B-associated cardiovascular fibrosis *in vivo* (84). SMAD3 is a well-known mediator of intracellular signaling of TGF-B. Smad3 activation in myofibroblasts in the heart plays a pivotal role in repair after myocardial infarction while SMAD3 signaling in cardiomyocytes after injury, triggers nitrosative stress and activates remodeling of myocardium and promotes cardiomyocyte death (100, 101). *SMAD3* is a direct target of miR-145 in the regulation of invasiveness of nasopharyngeal cancer (63) and could potentially be a target of miR-145 in cardiomyocytes or fibroblast cells. Thus, during cardiovascular diseases there is an interplay between miR-145 and TGF-β pathway which is reminiscent of miR-145 and TGF-β interaction during tumorigenesis (75).

ADAM17 is expressed in endothelial cells, smooth muscle cells and fibroblasts. It has been found to be overexpressed in ruptured coronary plaques from infarcted patients and in atherosclerotic plaques in mouse models (102). Being a direct target of miR-145 in cancer, it could be interesting to study the potential interplay between ADAM17 and miR-145 in the vascular system during vascular disease progression.

c-Myc is upregulated in cardiomyocytes in response to hypertrophic signals. Inhibition of c-MYC alleviates cardiac hypertrophy in rat hearts (103). miR-145 targets *c-MYC* in a variety of cancers and inhibits tumor growth (47). Thus, miR-145 could possibly target *c-Myc* in cardiomyocytes in order to regulate myocyte size.

Hoxa1 is expressed in precursors of cardiac neural crest cells (NCCs) which eventually populate the heart. *Hoxa1* null mice have major defects in the aortic arch, subclavian artery and demonstrate Tetralogy of Fallot, highlighting the requirement for Hoxa1 in early embryogenesis for patterning of arteries and the outflow tract (104). Since *Hoxa1* is a direct target of miR-145 during cancer migration and invasion, it may also be targeted by miR-145 early in embryogenesis to control vascular development.

Conclusions

miR-145 has been extensively studied in the context of cancer, with multiple gene targets being identified that regulate tumor progression in an array of cancer cell types (Fig. 1 and Table 1, 2). miR-145 is downregulated in various cancers and the overexpression of miR-145 in different cancer cells inhibits tumor growth, angiogenesis, invasion and metastasis. miR-145 directly targets core pluripotency factors thereby controlling stem cell characteristics of embryonic stem cells and cancer stem cells. miR-145 regulates many direct targets in several cellular pathways (16). Overall, miR-145 acts as an important modulator of these well-documented pathways such as the ERK/MAPK, mTOR/p70S6K1 and TGF-β which are often disrupted in cancer. Thus, in summary, miR-145 serves as a potent tumor suppressor in the progression of various cancers.

Fibroblasts in the tumor microenvironment get signals from localized tissue such as TGF-B1 and transdifferentiate to a heterogenous cell type called cancer-associated fibroblasts (CAFs) which have characteristics of tumor cell invasion and metastasis. When normal human fibroblasts are exposed to TGF-B1, they acquired a myofibroblast CAF-like phenotype. Overexpression of miR-145 inhibited the induced myofibroblastic differentiation and reverted the cancer-associated fibroblasts to a more normal fibroblast phenotype (105). miR-145 acts by downregulating numerous target genes induced by TGF-B1 such as *ACTA2* thereby inhibiting the development of cancer- associated myofibroblast phenotype. However, miR-145 is known to promote differentiation in smooth muscle cells and embryonic stem cells. These examples highlight the tissue-specific role of miR-145 and its targets. The type of cancer, stage of cancer dictates the function miR-145 in cancer and adds a layer of complexity. Thus, in depth study of the tissue-specific roles of miR-145 and its interaction with targets is crucial for the development of miR-145 therapeutics.

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In the cardiovascular system, miR-145 is expressed in vascular smooth muscle cells and plays an important role in smooth muscle cell differentiation (8, 9). miR-145 is also expressed in fibroblasts, endothelial cells, and plasma, thus, highlighting the diverse functions of miR-145 in the cardiovascular system. miR-145 has unique targets that have been identified in the cardiovascular system such as angiotensin (*ACE*), myocardin related transcription factor-B (*MRTF*), slit-Robo GTPase-activating protein 1 (*Srgap1*), *Srgap2* (9). However, due to the limited number of studies carried out in the cardiovascular system, there is potential for many other cardiovascular targets of miR-145 to be discovered. Some of the direct targets of miR-145 in cancer summarized in Tables 1 and 2, are also expressed in different cell types of the cardiovascular system. The action of miR-145 on smooth muscle phenotypic modulation is through the inhibition of *KLF4* while in cancer miR-145 targets *KLF4* to regulating tumor growth (8, 42). Other pluripotency factors SOX2 and OCT4 are known miR-145 targets in cancer. They are expressed in endothelial and smooth muscle cells and have the potential of being miR-145 targets in the cardiovascular system. The major pathways regulated by miR-145 in cancer such as the RAS/MAPK and TGF-β pathways also play crucial roles in cardiovascular function and disease (84, 93). Thus, there is a strong possibility that miR-145 has multiple targets in these pathways in the cardiovascular system. miR-145 regulates tumor growth and metastasis by targeting *PAI1* and *JAMA1* in cancer (50, 72). During cardiovascular diseases, miR-145 regulates *JAMA* during atherosclerosis and *PAI1* during cardiovascular fibrosis (15, 84), highlighting some common targets in cancer and cardiovascular system. Not surprisingly, genes and pathways that are targets of miR-145 are shared between these biological systems and many other targets are likely yet to be discovered.

miR-145 is widely described as a tumor-suppressor and is highly expressed in the heart and blood vessels. It is interesting to speculate whether the high expression of miR-145 contributes to the low incidence of cancer in the heart and vascular walls. The heart being resistant to tumor formation is most likely due the cardiomyocytes being terminally differentiated. On one hand, they cannot reenter the cell cycle and repair damaged heart tissue and thus avoid any cell cycle related mutations. This property may make them more resistant to tumor formation. miR-145 is protective against sheer stress on endothelial cells and is required for maintenance of the smooth muscle cell phenotype. There is no doubt that miR-145 has important functions in blood vessels. However, based on the limited clinical studies and almost no mechanistic studies on angiosarcoma (cancer of blood vessels), any role miR-145 may play in preventing cancer of the blood vessels is purely speculative.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of this review.

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Author contribution statement

D Sawant and B Lilly wrote, edited and had intellectual inputs to the manuscript.

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