HHS Public Access

Author manuscript

Semin Cell Dev Biol. Author manuscript; available in PMC 2021 April 01.

Published in final edited form as:

Semin Cell Dev Biol. 2020 April ; 100: 52–61. doi:10.1016/j.semcdb.2019.10.003.

Homeobox gene Meis1 modulates cardiovascular regeneration

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Abstract

Regeneration of cardiomyocytes, endothelial cells and vascular smooth muscle cells (three major lineages of cardiac tissues) following myocardial infarction is the critical step to recover the function of the damaged heart. Myeloid ecotropic viral integration site 1 (*Meis1*) was first discovered in leukemic mice in 1995 and its biological function has been extensively studied in leukemia, hematopoiesis, the embryonic pattering of body axis, eye development and various genetic diseases, such as restless leg syndrome. It was found that Meis1 is highly associated with *Hox* genes and their cofactors to exert its regulatory effects on multiple intracellular signaling pathways. Recently with the advent of bioinformatics, biochemical methods and advanced genetic engineering tools, new function of Meis1 has been found to be involved in the cell cycle regulation of cardiomyocytes and endothelial cells. For example, inhibition of Meis1 expression increases the proliferative capacity of neonatal mouse cardiomyocytes, whereas overexpression of Meis1 results in the reduction in the length of cardiomyocyte proliferative window. Interestingly, downregulation of one of the circular RNAs, which acts downstream of Meis1 in the cardiomyocytes, promotes angiogenesis and restores the myocardial blood supply, thus reinforcing better regeneration of the damaged heart. It appears that Meis1 may play double roles in modulating proliferation and regeneration of cardiomyocytes and endothelial cells post-myocardial infarction. In this review, we propose to summarize the major findings of Meis1 in modulating fetal development and adult abnormalities, especially focusing on the recent discoveries of Meis1 in controlling the fate of cardiomyocytes and endothelial cells.

Keywords

Homeobox gene; Meis1; cardiomyocytes; endothelial cells; regeneration; heart

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Conflict of interest

Authors disclose no conflict of interest.

1. Introduction

A successful clinical application of cardiac regeneration would save almost 3.7 million lives per year worldwide caused by myocardial infarction (MI)-induced heart failure (HF) [1]. To this end, stem cell-based therapy for heart regeneration has attracted more attention in the past years. However, current clinical trials using stem cells alone shows certain drawbacks of low cell viability and low retention following transplantation and these issues have not been overcome yet. Combinatorial approaches, such as gene-based therapy together with stem cell, may facilitate the regeneration of damaged heart post-MI [2]. In the past two decades, researchers have highlighted multiple genes, such as *Vegf* and *Angiopoietin-2* [2, 3], which are involved in the development and regeneration of the cardiovascular system, as potential therapeutic targets [4, 5]. The recent discovery of *Myeloid ecotropic viral integration site 1* (*Meis1*) in regulating cardiomyocyte (CM) cell cycle [6] is one of them, which has opened a new window for researchers to unravel its role in the regeneration of the cardiovascular system.

Homeodomain containing transcription factors are the conserved group of proteins known as the Hox proteins, which are organized into chromosomal clusters and have been established as key players in the patterning of body axis [7]. These transcription factors are known to be involved in various aspects of developmental processes during embryogenesis (reviewed in [8]). Apart from embryonic development, Hox proteins are also involved in several processes at the adult stage, such as hematopoiesis [9–13], angiogenesis [9], apoptosis [14], receptor signaling as well as in diseased conditions like oncogenesis [15–21]. Meis1 belongs to the family of highly conserved three amino-acid loop extension (TALE) homeobox class that was first identified as a common viral integration site in myeloid leukemic cells of BXH-2 mice [21].

Ever since its discovery, researchers have found the roles of Meis1 in limb development [22] and several pathological disorders, including restless leg syndrome [23, 24], eye defects [25], cardiac defects [26] *etc.*, which has drawn tremendous amount of attention to explore this protein as a therapeutic target. It was found that deficiency of Meis1 in murine results in lethality at embryonic day 14.5 with reduction of megakaryocytes and definitive hematopoietic stem cells (HSCs) along with malformed capillaries and smaller eye lenses [13, 24, 27]. Furthermore, haploinsufficiency of Meis1 causes micro-ophthalmic traits and visual impairment in adult mice, suggesting its role in varied cellular and developmental processes in a Hox-Pbx independent manner in contrast to the leukemic conditions [25].

Since majority of the previous studies have focused on leukemia, the detailed mechanism regarding *Meis1* in modulating leukemogenesis has been well studied (reviewed in [12]); however, its molecular responsibilities in normal hematopoiesis, mammalian vascular network, and cardiac regeneration remains largely unknown although clear phenotypic changes have been observed in the animal models. In this review, we have provided a comprehensive picture of this gene in regulating organogenesis during the embryonic development and the progression of various diseases, eventually leading the readers in the path of unraveling the role of *Meis1* in modulating cardiovascular system, especially CMs and endothelial cells (ECs) - the two critical cardiac components.

2. Meis1 is an established Hox co-factor

Functions of *Hox* genes depend on the interaction with their cofactors. By increasing the DNA binding efficiency and specificity of the Hox proteins, these cofactors regulate various highly complex and ordered cellular processes like hematopoiesis [10]. Among all *Hox* cofactors, *Pbx1* and *Meis1* have established themselves as key regulators of differentiation and maturation of HSCs whose expression follows a similar trend as *Hox* genes during embryonic development and at the later stages [28]. Comprehensive knowledge of the protein structure and domain organization will enable us to gain insight into Meis1's mechanism of action where it binds to the DNA as well as to other proteins in order to form hetero dimeric, trimeric or tetrameric complexes.

Meis1 generally forms dimers with its common partner Pbx1, although it can also interact with other proteins to form higher-order complexes. Through a varied combination of interactions, the transcription of each Hox-regulated gene is controlled at different developmental stages by forming a Hox regulatory protein complex mostly with Meis1, Pbx1 and Prep1 [29]. These three cofactors share a common structural organization containing a DNA-binding homeodomain towards the carboxy-terminus and two protein-protein interaction domains towards the amino-terminus [30, 31]. The characteristic feature of these proteins is the presence of the 60 residues long helix-loop-helix DNA-binding homeodomain. The loop connecting helix-1 and helix-2 is a highly conserved sequence of three amino acids, which gives this protein family its name -Three Amino acid Loop Extension (TALE) [30]. Two protein-protein interaction domains enable the accommodation of additional proteins which depends on the cell-type and developmental stage. It has been well documented through various studies demonstrating that the combination of different proteins in the Hox regulatory complex gives rise to patterning of organs in the embryo and at the later stage of development in maintaining hematopoiesis or in the progression of leukemia [14–17, 20].

2.1. Embryonic development

Embryonic patterning and development are centrally governed by homeobox-containing transcription factors whose functions are partly regulated by their co-factors, like *Meis1* and *Prep1* [29]. As mentioned earlier, the phenotype of *Meis1* deficient mice, which die at mid-gestation due to fetal hematopoietic failure, marks its importance as an essential developmental gene [13]. During organogenesis, *Meis1* is expressed and has functional roles in many organs and embryonic structures, such as in the central nervous system and sensory structures of the head, including the ears, eyes, nose [27, 32, 33] as well as in the four cardiac chambers [26], lungs and limb [22, 34]. These observations collectively underscore the molecular mechanisms determined by Hox-Pbx-Meis1 complex in the mammalian embryo, which is briefly addressed for different stages of development in the following sections.

The function of Hox-Pbx-Meis1 protein complex in limb patterning was already verified in an earlier study, which shows Meis1 cooperatively binds with Pbx1 in a unique way independent of the tryptophan residue amino-terminal to the promoter of CYP17 gene containing cAMP-responsive sequence (CRS1). This leads to promotion of growth and

differentiation via modulating c-AMP-dependent transcriptional pathway [35] [36]. Further studies on vertebrate limb growth showed that expressions of Meis1 and Pbx1 are restricted in the proximal regions of the vertebrate limb where *Meis1* controls *Pbx1* activity by promoting nuclear import of the Pbx1 protein. This process is essential for cell fate specification and differentiation patterns along the proximodistal (P-D) axis of limb giving rise to distinct limb domains during development. The mechanism was also observed in Drosophila *melanogaster*, chicken, and murine embryonic development hinting toward its conserved nature [22]. Moreover, expression analysis and functional assays in mouse myoblast cell line demonstrated that Meis1 accompanied by c-AMP responsive element binding protein (Creb) and Nuclear transcription factor Y (Nf-Y) cooperatively bind to the conserved promoter/enhancer site of *Myostatin (MSTN). MSTN* is a key player of vertebrate skeletal muscle growth during early myogenesis, which acts as a strong inhibitor of skeletal muscle growth in vertebrates [37]. Together, these observations indicate that *Meis1* not only associates with Pbx1, but also becomes an ally of other DNA binding proteins, like Creb and Nf-Y, in limb patterning and development.

In a *pbx* dependent manner, *meis1* is shown to be responsible for proper hindbrain segmentation in zebrafish where the gene is expressed in bilaterally symmetric regions of ventral telencephalon [38–40]. In mice, it was identified to act upstream of Paired box protein 6 (Pax6) and positively regulate its expression by binding to its enhancer. This interaction is essential for lens ectoderm development and brings essential specification of the eyes [32, 33]. A study of *Meis1* loss-of-function and conditional functional rescue has further provided insights into its role in series of events throughout eye development, in which Meis1 binds to Hox/Pbx independent sites during optic cup development, eye patterning, retinal proliferation and differentiation [25]. This is the first time when *Meis1* has been reported to be functional without any association of *Pbx-Hox* genes and making it as a part of the *Notch* signaling pathway. It will be interesting to find out the Hox independent action of Meis1 that can establish its multifaceted mechanisms of action.

2.2 Leukemia

Accumulation of multiple genetic alterations results in disruption of the normal growth and differentiation mechanism, which is believed to be the main cause behind many cancers, including leukemia. Studies have shown that Meis1 locus in chromosome 11 serves as a viral integration site in 15% tumors arising in BXH-2 mice, thus resulting in expression of an ecotropic murine leukemia virus that alters expression of cellular proto-oncogenes leading to myeloid leukemia [21]. Concomitant with this discovery, the MEIS1 locus 2p23-p12 in human is also subjected to chromosomal translocations, thereby altering gene expression in human leukemia. Since Meis1 is found to be frequently upregulated in human acute myeloid leukemia (AML) and acute lymphoid leukemia (ALL) [18, 41, 42], the characterization of this protein and its interacting partners have given us more insights into understanding the blood and bone marrow cancers.

It is believed that Meis1 acts as a cofactor of HoxA7 and HoxA9 in induction of AML of mouse model [21, 43, 44], suggesting its role as a Hox-cofactor to facilitate DNA binding specificity and increase the efficiency of Hox proteins. This explanation of Meis1's role

coincides with the observation from differential expression study throughout murine hematopoietic ontogeny, where the expression profiles of Meis1 and Pbx1 (already established as a Hox cofactor) closely resemble with that of Hox genes during different stages of hematopoiesis [28]. Further investigation confirms that along with forming a dimeric DNA-binding complex with HoxA9 [45], Meis1's Pbx interaction domain binds to Pbx2 and Pbx3 in order to collaborate with Hox proteins forming a Meis1-Pbx-Hox trimeric complex in myeloid progenitors [12, 16, 46].

The molecular mechanism underlying oncogenicity of Meis1 has been revealed from its association with HoxA9 and Pbx, which stabilizes the complex in order to promote transcription of *Cyclin dependent kinase 6 (Cdk6), Fms related tyrosine kinase 3 (Flt3), (Myb proto-oncogene (c-Myb)*, resulting in hyper-proliferation of myeloid leukemic cells (Figure 1) [16, 17, 20]. Meanwhile, activation of *B Lymphoma Mo-MLV Insertion Region 1* (*Bmi1*) through this complex leads to down-regulation of *Cyclin dependent kinase inhibitor 2a (Cdkn2a)* augmenting the process of cell proliferation (Figure 1) [20, 47].

2.3 Hematopoiesis

Hematopoiesis is a fundamental process of formation of blood cellular components during embryonic development and throughout life. It is well established that hematopoiesis is dependent on *Hox* genes, which are found to be preferentially expressed in HSC-enriched subpopulations followed by a downregulation through differentiation and maturation [10]. This pattern was observed at both adult and fetal stages of hematopoiesis. The trend of expression level change in *Hox* genes in HSCs is consistent with that of *Meis1*, whereas *Pbx1* follows a different expression pattern [28]. Knockout studies found that mice lacking *Meis1* show extensive hemorrhaging in the trunk and die at embryonic day 14.5 due to failure in definitive hematopoiesis. The number of HSCs in $Meis1^{-/-}$ fetal liver is found to be dramatically reduced, where the cells fail to protect themselves in irradiated mice [13, 27, 48], denoting the importance of *Meis1* mediated *Hox* regulated processes in hematopoiesis.

It is well known that HSCs use glycolytic metabolism to meet their energy demands [14, 49]. Inducible deletion of Meis1 in adult HSCs results in downregulation of Hif-1alpha (Hif-1α) and Hif-2alpha (Hif-2α), thereby increasing their oxygen consumption and decreasing the glycolytic flux [14]. This results in the production of higher level of Reactive Oxygen Species (ROS) associated with increased expression of proteins p16(INK4) and p19(ARF) (Figure 2). These findings imply that Meis1 acts upstream of a transcriptional network that regulates metabolism and oxidative stress response of HSCs [14].

2.4. Vascular Network

Vasculogenesis, angiogenesis and arteriogenesis are three common processes to form vertebrate blood vessel network. Vasculogenesis is defined by the aggregation of angioblasts for *in situ* differentiation and growth of blood vessels [50–52], while angiogenesis is the process where new blood vessels sprout from the existing ones [53]. Arteriogenesis refers to a process where collateral arteries are promoted by increasing radius of existing arteries to accommodate more supply of blood [54, 55].

Depending on the growth of each organ in the body, the requirement of blood to supply an adequate amount of oxygen for every single cell increases, thus maintaining normal tissue function. Angiogenesis and arteriogenesis play a big role in maintaining the health of each tissue and organ by branching into several vessels and capillaries. Unraveling the mechanism of blood vessel formation can offer us therapeutic options to improve or even cure ischemic disorders that are now one of the leading cause of mortality [56]. For example, to prevent proliferation of the cancerous cells in a solid tumor, impediment of angiogenesis could act as a therapeutic procedure, whereas the condition of patient with coronary artery disease can be ameliorated by stimulating angiogenic factors to increase either arteriogenesis or angiogenesis at the site of infarction. The basic frameworks of vasculature are conserved among vertebrates, which make it possible to assign homologies between distinct blood vessels and to directly compare the formation of these vessels in different vertebrate species [57].

2.4.1 Meis1 is a key gene regulating vertebrate vasculogenesis—Zebrafish is an ideal model to study hematopoiesis and vascular development due to their externally fertilized and transparent embryos, which enable *in vivo* visualization of early embryonic processes from birth of HSCs in the mesoderm to migration of blood cells [11, 58–60]. In addition, 70% of genetic similarities between human and zebrafish makes it advantageous for experimental and genetic analysis of vascular development [61].

Kawahara *et al.* reported, for the first time, that *meis1* plays a role in zebrafish vascular network development by regulating ECs [62]. Differentiation of ECs and the formation of vasculature, *i.e.* the *de novo* emergence of vascular network, by endothelial progenitor cells [63] in *meis1* morphants (MO) were found to be affected with abnormal patterning of pan EC markers, such as *platelet and endothelial cell adhesion molecule 1 (pecam1, also known as cd31)* and *friend leukemia integration 1* (*fli1*). Diminished expression of artery markers, such as *eph related receptor tyrosine kinase ligand B2 (ephrinB2)*, with upregulation of vein markers, such as *fms related tyrosine kinase 4 (flt4)* and *eph receptor B4 (ephB4)*, *in* presumptive arterial vessels *meis1* MO imply that *meis1* is involved in arterial vessel development. At the same time, expression of *fetal liver kinase 1(flk1, also known as kdr or vegfr)* gene was found to be significantly reduced in *meis1* morpholino injected zebrafish embryo, suggesting direct regulation of this vascular endothelial growth factor receptor *(vegfr)* in vertebrate embryonic development [62]. Epistasis analysis on zebrafish has concomitantly resulted in a similar outcome, where it is found to be involved in hemangioblast formation acting downstream of *hoxd4a* (Figure 3) [9]. Hemangioblasts are the common precursors of the blood and endothelial lineages, malformation of which directly affects the three fundamental processes in the body namely, vasculogenesis, angiogenesis, and hematopoiesis.

Meis1 global KO mice developed ocular and angiogenesis defects with failure to produce megakaryocytes and displaying extensive hemorrhaging [27, 64]. Meis1 was found to be expressed in specific and discrete locations throughout the mouse embryo, including the aorta-gonad-mesonephros (AGM) mesenchyme, in the hemogenic embryonic arterial endothelium and in the hematopoietic clusters within the arteries [13]. In all three *Meis1* expressing cell populations, impaired expression of a critical endothelial transcription factor,

Runt-related transcription factor 1 (Runx1), caused disruption of vascular patterning probably through affecting ECs [13]. A comparable phenotype in zebrafish and mice indicate that *Meis1*'s role in vascularization is conserved throughout the vertebral species.

HSC differentiation gives rise to several types of cells, of which hemogenic ECs are the ones involved in aorta formation [65]. A chemical defined hematopoietic differentiation model accompanied by whole genome gene profiling study as well as CRISPR/CAS9 gene editing technology recognized *MEIS1* acting as a regulator in generation of hemogenic endothelial cells, megakaryopoiesis and thrombopoiesis in human pluripotent stem cells through targeting endothelial cell-specific genes, *TAL1* and *FLI1* [66]. Both *TAL1* and *FLI1* are known to control angiogenic response of ECs through stimulating cell morphogenesis and angiogenic migration [67, 68]. These results give us an insight into the regulation of vascular patterning in vertebrates via maturing ECs from its progenitors through *MEIS1*. Further investigation into EC specific *MEIS1*'s role will strengthen our understanding of vasculogenesis, angiogenesis, and arteriogenesis.

2.4.2. A possible relationship between Meis1 and EC proliferation—The above discussion provides us perception that the regulation of vascular patterning in vertebrates is likely through modulation of either mature ECs of its progenitors by *Meis1*. Consequently, investigation into EC-specific role of *Meis1* will further strengthen our understanding of angiogenesis and/or arteriogenesis through EC proliferation. The reduced expression of EC markers as well as molecular players in *vegf* signaling pathway due to Meis1 downregulation in the above-mentioned studies in zebrafish and murine places this gene, which was previously known to be as a proto-oncogene followed by known to be a cardiogenic transcription factor, in the list of regulators of EC regulation.

Interestingly, Meis1's role in the regulation of hemogenic ECs that are required for aorta formation by acting upstream of endothelial-specific proteins, TAL1 and FLI1, demonstrate that Meis1 is a crucial factor in EC lineage specification and maintenance. Both TAL1 and FLI1 are known to control angiogenic response of ECs by stimulating cell morphogenesis and angiogenic migration [67, 68]. The study on cardiac super-enhancers [69] show injection of Nfix shRNA at the infarcted site of adult heart results in comparatively higher blood vessels through angiogenesis than those in the heart of wild-type (WT) animal. As it is already established by the study that Meis1 positively regulates expression of Nfix by binding to its super-enhancer region, downregulation of this circular RNA leads to better recovery from myocardial infarction [69]. Collectively, these data suggest a inhibitory role of Meis1 in the process of angiogenesis.

2.5 Other genetic diseases

Other than leukemia, Meis1 is known to be associated with restless leg syndrome (RLS), in which several non-synonymous variants in Meis1 have been identified in RLS patients [23, 70]. Meis1 locus is the most important RLS susceptibility gene [71]. Similar to RLS, Meis1 is also involved in locomotor hyperactivity [72]. It was found that Meis1 is strongly expressed in dopaminergic neurons of the substantia nigra, in the spinal cord and, in the red nucleus [73]. As a part of a transcriptional regulatory network governing motor neurons, it is

believed that Meis1 plays a critical link to the pathophysiology of RLS. The involvement of Meis1 in eye development mentioned early suggests its role in microphthalmia and genetic eye defects [25].

3. Meis1 in the cardiovascular system

Human cardiac tissue is probably the most difficult part in the body to regenerate following ischemic damage (*i.e.*, MI). The underlying principle that inhibits cardiac regeneration is the fact that adult CMs quickly lose their ability to proliferate after birth and remains an extreme low ability of regeneration in contrast to the hearts of amphibians and zebrafish [74]. However, the most important discovery in the field of cardiac regeneration is that neonatal mouse heart can efficiently regenerate until 7 days through proliferation of CMs, which lays a foundation for researchers to study the genetic and epigenetic markers that can potentially preserve this property of CMs beyond this proliferative window even at the adult stage [75].

3.1 Cardiac development

Meis1 has previously been found to play vital roles in cardiogenesis and heart development in zebrafish and murine [26, 76], suggesting it is a conserved cardiac-specific gene across species; however, its expression level in heart changes dramatically from the embryonic stage to the adult stage with very low level of Meis1 protein in adult heart [77]. The implication of stage-dependent Meis1 expression remains largely unknown in mammalian heart development and maintenance (Table 1).

3.1.1 Embryonic stage—Studies found that Meis1 homozygous deletion results in lethality in mice with death occurring between embryonic days 11.5 and 14.5 due to internal hemorrhages and hematopoietic defects [13, 27], suggesting Meis1 as an important molecular player in embryonic development. By knockdown (KD) of *Meis1* by anti-sense mRNA, meis1 morphants exhibit heart edema and malformed heart loop, along with weak heartbeats and abnormal circulation of blood cells in zebrafish [11, 62]. Disruption of Meis1 also leads to malformed cardiac outflow tracts with overriding the aorta and ventricular septal defect in mice [26]. These findings signify that Mei1 exerts important regulatory impacts on CMs, ECs, and/or vascular smooth muscle cells (VSMCs) during embryonic development.

Heart development is a complex process with a range of differentiation steps where cardiogenic mesoderm differentiates into endoderm and myocardium [78]. Generation of myocardium involves three sequential transitions from cardiac precursors to primitive CMs and finally definitive CMs [79]. Although it is recognized that Meis1 is an important cardiac-related gene, based on phenotypic changes in the animal model, its mechanism in modulating cardiogenesis at different stages is not explored to a large extent; however, a few studies that have resulted in bridging the gap of knowledge in this regard draw a clear and interesting picture. Investigation of epigenetic regulation of cardiac lineage development showed MEIS1HOXA9 motif was enriched at a subset of enhancers at the cardiac precursor stage. Enhancer-based gene network analysis showed Meis1 along with Gata4 activate the enhancer of *Myocd*, an essential gene in differentiation and proliferation of VSMCs [80].

This therefore validates the proposal by Stankunas *et al.* that Hox proteins are functionally associated with Meis1 partners during heart development at the molecular level [26].

Series of events during cardiogenesis takes place through controlled expression of several temporal genes that result in chromatin and histone modifications [81]. At the embryonic stage, histone chaperone HIRA modulates gene expression through deposition of histone variant H3.3, which is an euchromatic marker of gene activity [82]. Conditional KO of HIRA in embryonic mouse hearts leads to cardiac septal defects accompanied by differential down-regulation of *Meis1*, *Tbx2* and *Gata6* [83]. These data suggest that HIRA directly binds the enhancers of *Meis1* and *Gata6* and the promoter of *Tbx2* activate their expression (Figure 4). A combination of ChIP-Seq and RNA-Seq studies in cardiac development has found that Meis1 and Nkx2–5 sequential binds to the enhancers of several cardiac regulatory genes, like *Popeye domain-containing protein 2 (Popdc2)* [84]. Together, these studies give us a partial picture of upstream and downstream genes of Meis1, where HIRA binds to the enhancer of Meis1 and activates its expression, which then triggers activation of several down-stream cardiac-specific genes during myocardial differentiation.

3.1.2 Neonatal and adult stage—As mentioned earlier, *Meis1* regulates cell cycle exit and regenerative capacity of neonatal CMs [6]. *Meis1* deletion in neonatal mouse heart extended CMs proliferative window from 7 to 14 days. Additionally, by deleting *Meis1* in adult CMs, cyclin-dependent kinase inhibitors were down-regulated, which were usually up-regulated in the postnatal stage, resulting in cell cycle re-entry and promoting CMs proliferation without cell hypertrophy or deleterious effect on cardiac function[6]. In contrast, overexpression of *Meis1* in neonatal CMs reduced CMs proliferation and inhibited the heart regeneration response[6]. Xiang *et al* [85] found Meis1 was directly bound and repressed by Tbx20 (a cardiac-specific gene), specifically in adult CM, to promote adult CM proliferation and preserve cardiac performance after myocardial infarction (Figure 4).

In addition, Meis1 was also found to inhibit angiotensin II-induced CMs hypertrophy by binding to *poly (rC)-binding protein 2 (PCBP2)* promoter and promoting its expression [86]. Expression of Meis1 is dramatically down-regulated in human failing heart tissues and murine hypertrophic heart tissues. Meanwhile, angiotensin II-induced hypertrophic is significantly inhibited by *Meis1* overexpression or promoted by *Meis1* knockdown [86]. *Meis1* inactivation in the mouse neural crest leads to altered sympatho-vagal regulation of cardiac rhythmicity in adults, which is characterized by chronotropic incompetence and cardiac conduction defects, thus increasing susceptibility to sudden cardiac death [73]. The role of *Meis1* as an anti-cardiomyocyte proliferative gene was again validated by Pandey *et al.* when they found translational inhibition of Meis1 by three micro RNAs (miRNA), miR-548c-3p, miR-509–3p, and miR-23b-3p increases proliferation of cardiomyocytes significantly [87] (Figure 5).

In the past decade, non-coding RNAs have emerged as vital molecular players in maintaining cardiac health by regulating gene network either in cell cycle progression of cardiomyocytes or induction of certain molecular phenomenon in the heart under healthy as well as diseased conditions. A recent study by Huang *et al.* has discovered that one of the crucial non-coding circular RNAs implicated in the cardiovascular system is regulated by

Meis1 via binding to its super-enhancer region [69]. Silencing of this conserved mammalian circular RNA Nfix has a similar phenotype as that of cardiomyocyte-specific *Meis1* KO, while cardiac regeneration of post-MI is greatly enhanced in circRNA Nfix KD adult mice in comparison to that of WT mice, in which enhancing angiogenesis is resulted by ubiquitin-dependent degradation of Ybx1 and rescuing miRNAmiR-214. In contrast, overexpression of this circular RNA inhibited the neonatal cardiac regeneration post-MI [69].

The mammalian heart undergoes a series of coordinated metabolic changes from the embryonic stage to the postnatal stage [88]. Maturation of the fetal heart is accompanied by higher myocardial metabolic activity switching from glycolysis to oxidative phosphorylation in order to produce ATP in an efficient manner [88]. This transition of mitochondrial activity is closely linked with cell proliferation, where higher metabolic activity reinforces the event of cell division [89]. A recent study on fetal, neonatal, and adult sheep CMs by Drake *et al.* has revealed that Meis1 is a key regulator of metabolic activity switch at birth corroborating with the loss of proliferative capacity of post-natal mammalian CMs. Suppression of Meis1 expression leads to increased oxygen consumption in fetal CMs, presumably mimicking the characteristics of adult stage, but no effect was found in the CMs isolated from neonatal sheep in contrast to the results by Mahmoud *et al.* [6], which is probably due to the difference in the nature of CMs at the post-natal stage between murine and sheep. siRNA mediated Meis1 knockdown resulted in downregulation of important metabolic genes glycolytic genes *aldolase (ALDO), enolase (ENO)* and *post-glycolytic gene lactate dehydrogenase B (LDHB)*, which may underscore its role as CM metabolic regulator [77]. This observation implicates a normal down-regulation of Meis1 with age to provide a switch in oxidative metabolism [77].

The change in metabolic activity of neonatal and adult CMs explains the sudden change of expression of Meis1 between two stages (neonatal vs adult). It is important to understand what makes Meis1 as a negative regulator of cardiac regeneration post-MI even though it is expressed minimally at the post-natal stage. The previous results indicate an interaction between metabolic activity of CMs and cell cycle, which is regulated through Meis1 likely under different mechanisms, depending on the age of CM.

3.2. Cardiac regeneration through augmented vascularization

As stated above, exogenous stem cell therapy for infarcted heart still faces several challenges in terms of techniques and logistics, such as lack of availability of blood supplies to the newly transplanted cells sitting on the scar tissue, efficiency of the delivery system and low cell viability and low retention. In order to increase the efficiency of cardiac regeneration or survivability, the newly transplanted CMs located in the post-infarct environment must be supplied for a sufficient supply of oxygenated blood to meet the required myocardial oxygen demand. To this end, angiogenic pre-treatment in the scar tissue by stimulating endothelial growth or vascular proliferative genes would initiate angiogenesis or arteriogenesis [90].

Animal study shows that EC angiogenesis play an essential role in CM regeneration post-MI by supplying blood perfusion to the infarcted heart and guiding CM migration [3], thus defining the important sequence of events that has to take place in order to promote myocardial regeneration or repair, *i.e.*, angiogenesis precedes cardiomyocyte migration. Any

stimulus that enhances EC or VSMC proliferation to form collateral blood vessels or drives the existing branches into small capillaries can be used as the first step in cardiac regenerative therapy. Under such pre-vascularization condition, the implanted stem cell or CMs or *in situ* programmed existing cells will have a fair chance to survive in the damaged cardiac tissue.

Importantly, adult mouse heart appeared to be promisingly recovered post-silencing of circRNA Nfix in the periphery of the infarcted region [69]. This improvement in the prognosis is brought about by a combination of restoration of CMs and angiogenesis. Thus, it provides a promise to use Meis1 transcript factor as a potential therapeutic target in modulating ECs and CMs, thereby increasing vascularization or cardiomyogenesis in the infarcted heart as it acts immediately upstream of Nfix by binding and activating its enhancer; however, how Meis1 modulates ECs or CMs as an upstream element of endothelial-specific genes, such as *Tal1*, *Fli1, Runx1, Scl1, etc*, [9, 66] remain not clear.

3.3. Meis1, inflammation, and cardiac repair

Cardiac tissue repair does not only involve CMs and ECs, but also dependent on the immune cells, like macrophages that are recruited immediately after tissue injury. The inflammatory response after tissue injury is deleterious yet essential for tissue repair, where macrophages play a critical role in the initiation, maintenance and shut down the process [91]. The remarkable capacity of neonatal mouse heart to repair itself after apical resection or myocardial infarction is also attributed to the type of macrophage population residing in the cardiac tissue [92]. Calvo *et al.* showed that neonatal cardiac macrophage composition differs from that of adult heart. The interesting findings from this study underscore the important distinction between the resident macrophage (embryonic origin) and the monocyte derived macrophage with respect to cardiac tissue injury. Neonatal heart selectively expands the number of resident macrophages which is reparative and produces minimal inflation whereas adult heart recruits more monocyte derived macrophages leading to prolonged inflammation [92].

Meis1 is essential for macrophage differentiation of myeloid progenitors in cooperation with Hoxa9 [93]. *Meis1* is downregulated during monocyte-macrophage terminal differentiation along with most of the *Hoxa* genes [16]. This suggests the monocyte-derived macrophages result from downregulation of *Meis1*, but it will be interesting to see the level of Meis1 in the resident macrophages. This link between *Meis1* and the cardiac macrophages will uncover many puzzles associated with inflammation and cardiac regeneration.

Conclusion

In summary, the discovery of Meis1 in leukemic pathogenesis has been beneficial in understanding of its biological roles in cancer and HSCs, it also gives us an opportunity in grasping some of the vital mechanisms in cardiac development and regeneration. It is commonly agreed that Meis1 plays critical roles in disease progression, including in MI, apart from being an important developmental gene.

Gene therapy is a promising strategy in this era of genomics and transcriptomics. Multiple roles of Meis1 in fetal development and adult disease conditions of the mammalians gives us hope to utilize the known information to ameliorate the impairment caused by this gene through gene therapy. Meanwhile, stem cell-based therapy has caught a lot of attention to serve as an alternative promising strategy to reinstate the damaged heart, which can overcome the fundamental challenge of rare or non-proliferative nature of adult CMs. Yet, this faces another obstruction in which the newly generated CMs located on the ischemic and fibrotic-scarred tissue is unable to obtain sufficient supply of blood to ensure their survival and proliferation. In other words, myocardial regeneration and survival after transplantation largely surely depend on suitable angiogenesis or arteriogenesis to restore blood flow in the ischemic territory in the heart. Based on the literature evidence (Table 1), modulation of Meis1 in CMs, ECs and VSMCs may offer a new approach to improve the outcomes of stem cell-based therapy.

Acknowledgment

This work was supported by the National Institute of Health (1R15HL140528) and the Seed Grant from the College of Veterinary Medicine at Virginia Tech. The funding sources was not involved in preparing and submitting this manuscript. We would like to thank the online image software Biorender (https://biorender.com/) for making it helpful to create the figures.

Abbreviations:

AGM	Aorta gonad mesenchyme
ALDO	Aldolase
ALL	Acute lymphoid leukemia
AML	Acute myeloid leukemia
Bmi1	B lymphoma mo-mlv insertion region 1
CMs	Cardiomyocytes
Cdk6	Cyclin Dependent kinase 6
Cdkn2a	Cyclin Dependent kinase inhibitor 2a
C-Myb	Myb-like DNA-binding gene
Creb	Camp response element binding
ECs	endothelial cells
ENO	Enolase
Fli1	Friend leukemia integration 1
Flk1	Fetal liver kinase 1 (Aliases: Kdr, Vegfr2)
Flt3	FMS related tyrosine kinase 3 (Aliases: CD135)

HF	heart failure
Hif1α or 2α	Hypoxia inducible factor 1α or 2α
Hox	Homeobox
HSCs	Hematopoietic stem cells
KDR	Kinase insert domain receptor (Aliases: Vegfr2)
LDHB	Lactate dehydrogenase b
Meis1	Myeloid ecotropic viral integration site 1
MI	Myocardial infarction
MSTN	Myostatin
Myocd	Myocardin
Nf-Y	Nuclear transcription factor Y
Nkx2.5	NK2 homeobox 5
Pbx	Pre-b-cell leukemia homeobox
PCBP2	Poly (rc)-binding protein 2
P-D axis	Proximo-Distal axis
PECAM1	Platelet and Endothelial Cell Adhesion Molecule-1 (Aliases: CD31)
Popdc2	Popeye domain-containing protein 2
Prep1	Pbx regulating protein 1
ROS	Reactive Oxygen Species
RLS	Restless leg syndrome
Runx1	Runt related transcription factor 1
SCL	Stem cell leukemia (Aliases: Tal1)
shRNA	Short hairpin RNA
Tal1	Tal bhlh transcription factor 1 (Aliases: SCL)
TALE	Three amino acid loop extension
Tbx2	box 2
Vegfr	Vascular endothelial growth factor receptor
VSMCs	vascular smooth muscle cells

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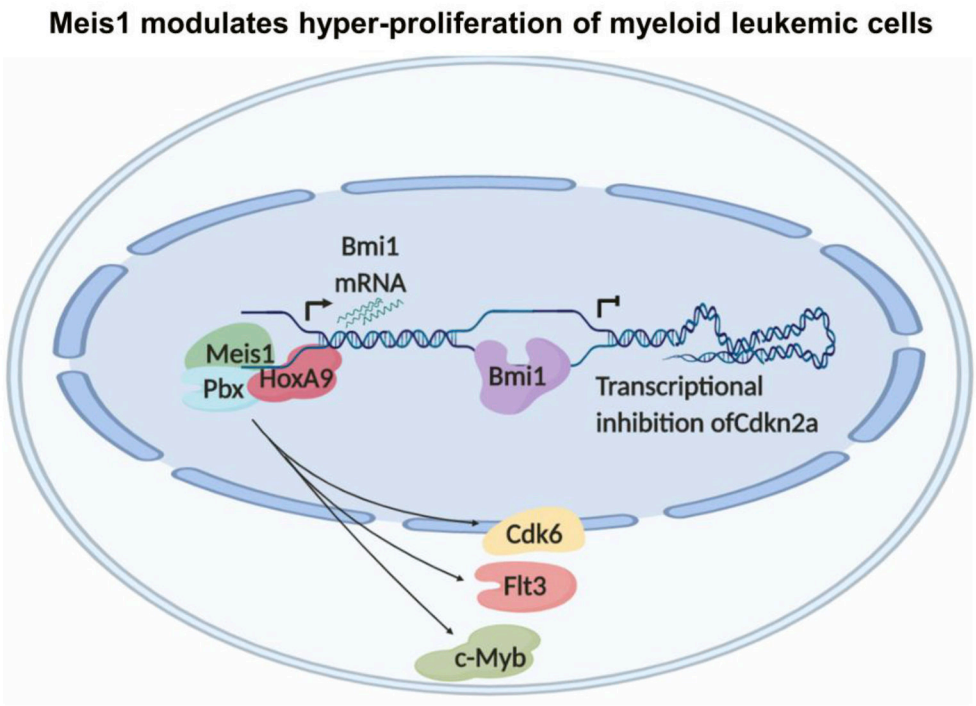

Fig 1. Illustration of Meis1 as a Hox cofactor in myeloid leukemic cells.

Meis1, as a part of Pbx-HoxA9- Meis1 trimeric complex, regulates the expression of cell cycle regulators like Cdk6, c-Myb, Flt3 etc. resulting in hyper-proliferation. Meis1: Myeloid Ecotropic Insertion Site 1; Cdk6: Cyclin Dependent kinase 6; C-Myb: Myb-like DNA-binding gene; Pbx: Pre-B cell regulating protein 1; Flt3: FMS related tyrosine kinase 3.

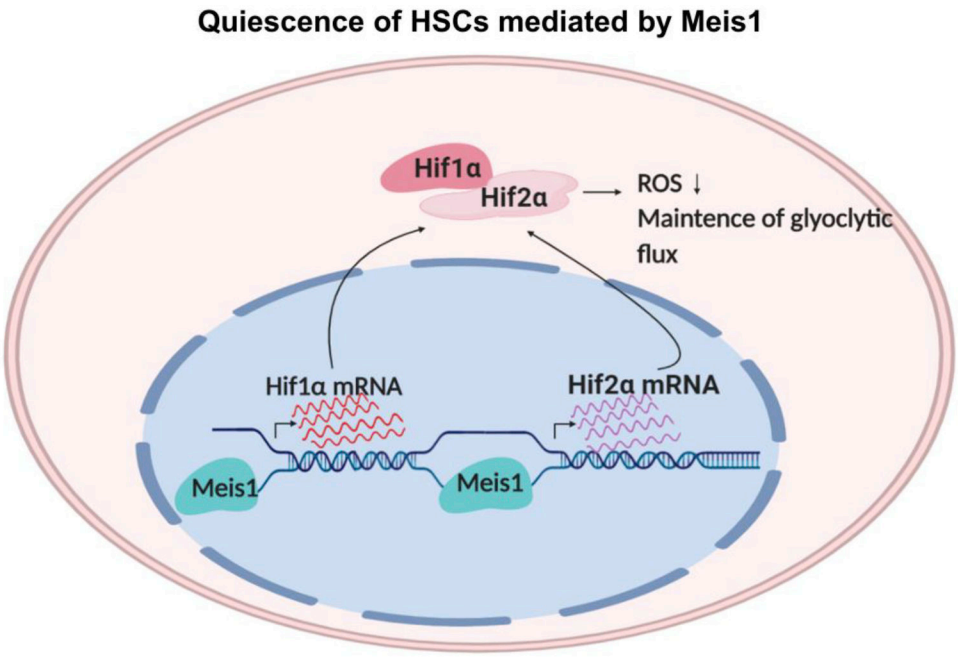

Fig 2. Meis1 as a regulator of quiescence of HSCs.

Positive regulation of hypoxia inducing factors 1α and 2α by Meis1 leads to reduction in the level of ROS and self-renewal of HSCs is restored.

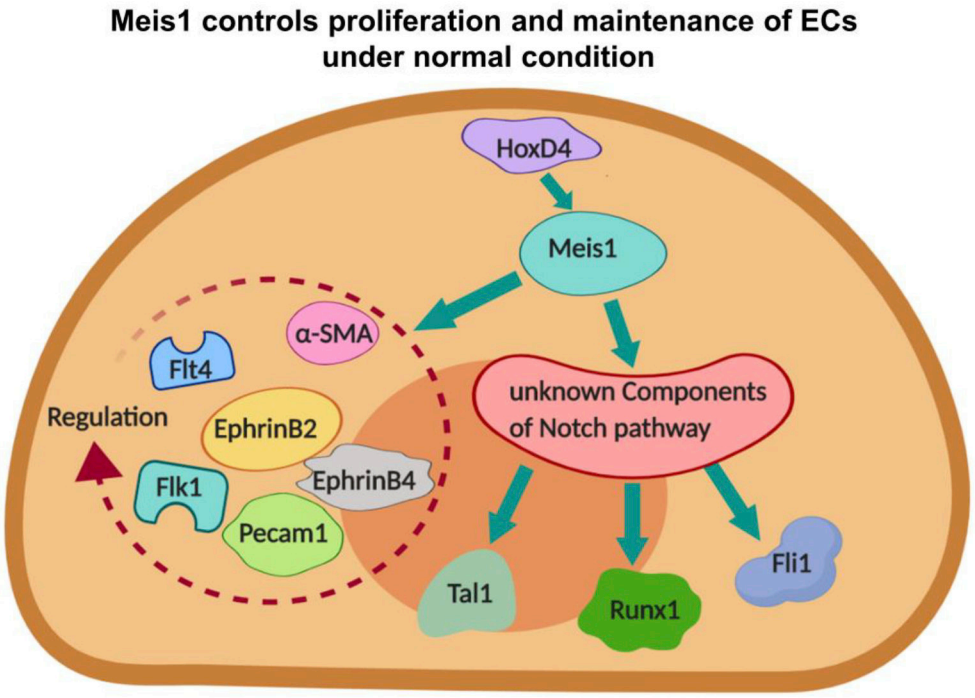

Fig 3. Illustration of EC specific role of Meis1.

HoxD4 acts upstream of Meis1 which regulates several EC genes like Tal1, Runx1, Fli1 etc. through various intermediates proteins. Normal proliferation and maintenance of ECs is widely regulated by Meis1 acting upstream of several genes. ECs: endothelial cells; Tal: Tal bhlh transcription factor 1; Runx1: Runt related transcription factor 1; Fli1: Friend leukemic integration 1.

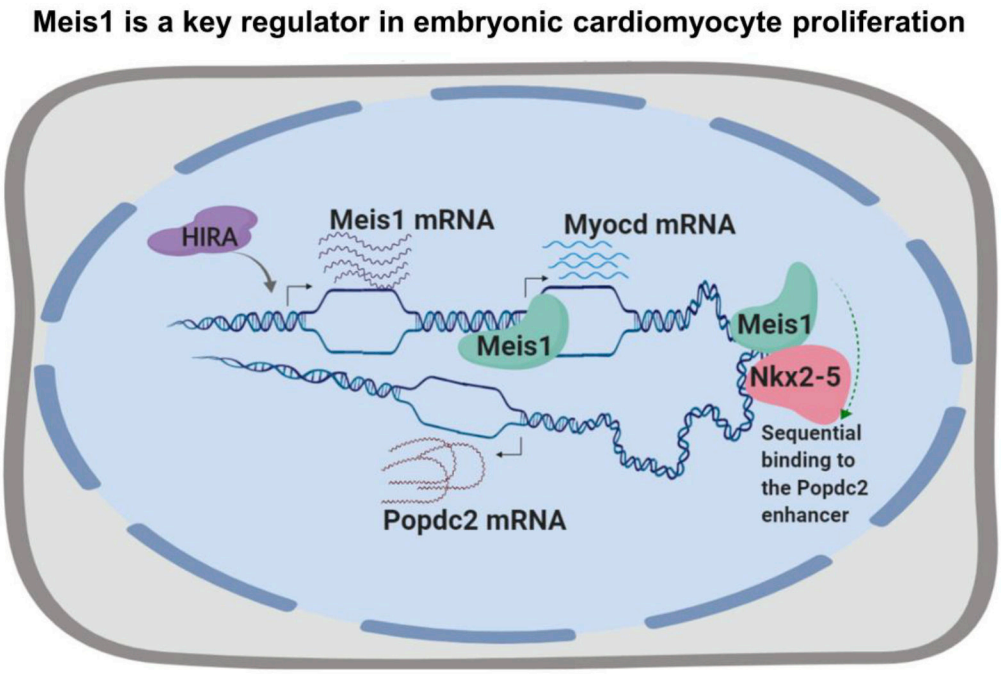

Fig4. Meis1 is a key regulator of cardiogenesis by modulating embryonic CMs.

In embryonic CM, HIRA positively regulates *Meis1* transcription. Meis1 acts as transcriptional regulators of cardiac genes, *Myocd* and *Popdc2*, by binding to their promoters and enhancers respectively. Normal expression of Meis1 at embryonic stage is required for cardiogenesis and formation of distal outflow tract. CMs, cardiomyocytes; Myocd: Myocardin; Popdc2: Popeye domain-containing protein 2.

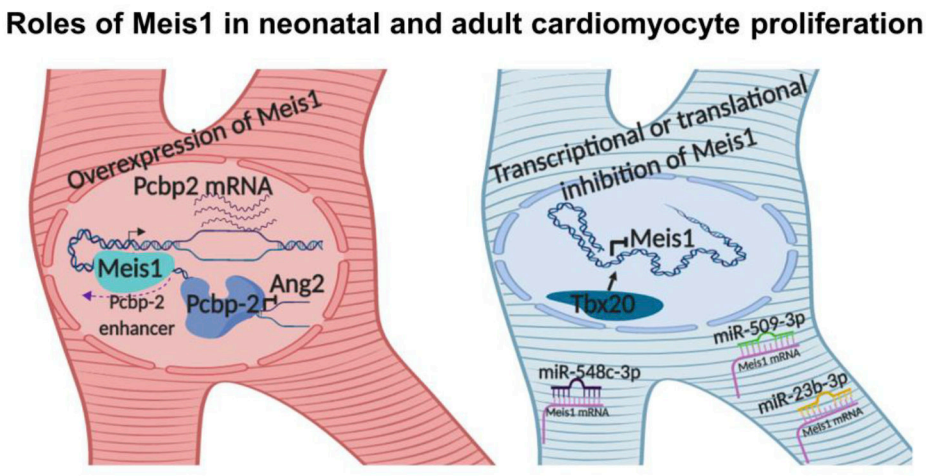

Fig 5: Illustration of the role of Meis1 in neonatal/ adult CM.

Over expression of Meis1 in neonatal cardiomyocytes is accompanied by Pcbp-2 overexpression, which inhibits the transcription of *Ang2*. This causes neonatal CMs in premature stage via cell cycle arrest, inhibitions of both neonatal heart regeneration and CM hypertrophy. On the other hand, transcriptional inhibition of Meis1 by overexpressing Tbx20- or miRNA-mediated translational inhibition leads to increased proliferation of CMS at neonatal and adult (?) stage and preserves cardiac function of neonatal heart post-MI. CMs: cardiomyocytes; Pcbp-2: Poly (rc)-binding protein 2; Ang2: angiotensin 2; Tbx20: T-box 20.

Table 1.

Summary of Meis1 in Modulating Cardiovascular System *(see footnote for* abbreviations*)*

Genetic alteration/technique used	*in-vitro* or *in-vivo* model	Functional effect/Observations	Reference
Targeted disruption of *Meis1* (KO)	Mouse	Embryonic lethality at mid-gestation day 14.5; abnormal vascular patterning	[13, 27]
Morpholino injected KD	Zebrafish	Malformed heart and disrupted vessels	[11, 57]
Targeted disruption of *Meis1* (KO)	C57BL/6 mouse	*Meis1*-null embryos display an overriding aorta and ventricular septal defect (Congenital abnormalities)	[26]
Cardiomyocyte specific *Meis1* KO	C57BL/6 mouse	Cardiomyocyte cell cycle arrest	[6]
Chip-Seq analysis of embryonic heart	Mouse	Meis1 is involved in spatio-tempral regulation during cardiogenesis.	[81]
Overexpression of Tbx20	Mouse differentiated cardiomyocytes	Overexpression of Tbx20, an inhibitor Meis1 promotes proliferation and improves cardiac function after Ml.	[82]
Induction of hypertrophy in mice	C57BL/6 mouse, Human heart samples, neonatal rat cardiomyocytes	*Meis1* promotes *PCBP2* expression in cardiomyocytes and inhibits *Angll* induced cardiac hypertrophy	[83]
miRNA overexpression in cardiomyocytes resulting in functional inhibition of Meis1	Isolated rat cardiomyocytes	Functional inhibition of Meis1 in adult cardiomyocytes, increases proliferation.	[84]
RNA-Seq and ChlP-Seq analysis of WT and histone chaperon *Hira* null cells	Mouse embryonic stem cells and early cardiomyocyte progenitors	*Meis1* is directly regulated by Hira iduring early cardiogenesis	[80]
Loss of function assay	Mouse	Circular RNA Nfix acts downstream of Meis1 and loss of which promotes angiogenesis and better heart regeneration post-MI,	[66]
siRNA mediated knock-down	Isolated sheep cardiomyocytes	Meis1 down regulation promotes oxidative phosphorylation in perinatal cardiomyocytes	[74]