Chapter 4
Vascular Stem Cells in Regulation of Angiogenesis

Jingwei Lu, Vincent J. Pompili, and Hiranmoy Das

Abstract Angiogenesis is the process by which new vessels are generated from the preexisting blood vessels, which is the major contributor of postnatal neovascularization process. Disruption or dysregulation of angiogenesis is involved in various pathological conditions, such as ischemia and tumor progression. Stimulation of angiogenesis was proposed to be able to restore the blood flow and contribute to the tissue recovery in ischemia, while inhibition of angiogenesis can impede tumor progression. The importance of angiogenesis has generated tremendous interest in studying the mechanisms and to find out major contributors of the process. The current stem cell research has significantly improved our understanding of angiogenesis and its possible therapeutic application. Hypoxia is the most important driving force of angiogenesis, while other factors, such as chemokines and cytokines, haptotaxis, and mechanotaxis, are also important in regulating neovascularization process. In this chapter, we will focus on the progenitor cells that contribute to the angiogenesis and the underlining mechanisms involved in this process.

Keywords Angiogenesis • Stem cells • Hypoxia • Cytokines • Hypotaxis • Mechanotaxis • Signaling molecules • MicroRNA

1 Introduction

Galen, the second-century physician, speculated that the vascular system served to carry blood and provide nutrition to the human body [1]. It is now well established that the vascular system provides the main network of channels for nutrients (such as amino acids, electrolytes, oxygen, and hormones) to all the body tissues. Disturbances in the vascular system, mainly blocking the blood supply to the

J. Lu • V.J. Pompili • H. Das (✉)
Cardiovascular Stem Cell Research Laboratory, The Dorothy M. Davis Heart and Lung Research Institute, Wexner Medical Center at The Ohio State University, 460 West 12th Avenue, BRT 394, Columbus, OH 43210, USA
e-mail: hiranmoy.das@osumc.edu

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tissues, cause a variety of circulatory diseases, from peripheral artery disease to peripheral venous disease, and include among them vascular diseases like aneurysms, renal artery stenoses, and Buerger's disease [2]. Disruptions of angiogenesis play a critical role in the pathological progression of various ischemic diseases, such as stroke, ischemic heart disease, and the multiple peripheral vascular disease syndromes, resulting in a shortage of blood supply and which eventually induce apoptosis and necrosis of cells and the tissues of the vascular system. Angiogenesis, however, plays an important role in the regeneration of such ischemic tissues. In a seemingly contradictory role to that in the ischemic diseases, angiogenesis contributes to damage caused by the progressive growth of malignant tumors. Targeting tumor growth by targeting tumor angiogenesis, as in using various drugs to reduce blood supply to the tumor, is one of the major therapeutic considerations for effective control. Rapid proliferation of tumor cells, with lack of blood supply and lack of oxygen, triggers upregulation of vascular endothelial growth factor (VEGF) secretion, which promotes the angiogenesis process. The importance of angiogenesis in pathological conditions has generated interest in studying the mechanisms and signaling pathways for angiogenesis. Various stem cells were proposed to be important for initiation of the angiogenesis process. Mesenchymal stem cells (MSCs) and hematopoietic stem cells (HSCs), which were shown to repair ischemic tissues, have great ability to promote angiogenesis via neovascularization and thereby to reduce the amount of ischemic tissue damage [3, 4]. Other cell types such as smooth muscle stem cells and vascular pericytes were also shown to be beneficial for the process of angiogenesis. In this chapter, we will focus on the role of various stem cells on the angiogenesis process and on the molecular mechanisms that promote these stem cells to form new blood vessels.

2 Angiogenesis and Stem Cells

The wall of blood vessels contain endothelial cells, mural cells, and extracellular matrix (ECM). The inner lining of blood vessel is the endothelium, which is a thin layer of endothelial cells. Mural cells are specified as determined by the location of the vessel; they could be pericytes, smooth muscle cells, and fibroblasts. The mural cells are embedded in the extracellular matrices [5]. The various types of cells forming blood vessels could be derived from multiple stem/progenitor cells. Circulating endothelial progenitor cells (EPCs) and HSCs could differentiate into endothelial cells thus directly contributing to the angiogenesis process. MSCs, though they may not be able to directly differentiate into endothelial cells, can secrete factors, such as VEGF, and promote the neovascularization process. Other progenitor cells, such as vascular pericytes and smooth muscle progenitor cells, can also contribute to angiogenesis (Fig. 4.1).

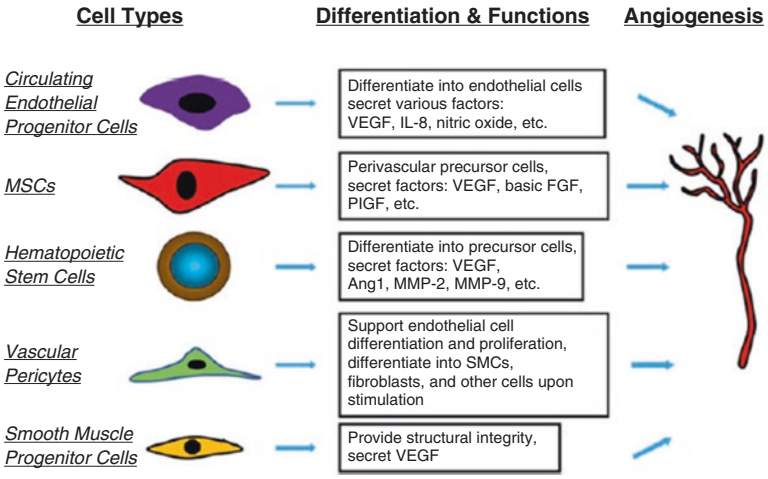

Fig. 4.1 Contribution of various stem/progenitor cells and their secretory molecules in the angiogenesis process (*MSCs* mesenchymal stem cells, *SMCs* smooth muscle cells, *VEGF* vascular endothelial growth factor, *IL-8* interleukin-8, *FGF* fibroblast growth factor, *PlGF* placental growth factor, *Ang1* angiopoietin 1, *MMP* matrix metalloproteinases)

2.1 Circulating Endothelial Progenitor Cells

The first study on putative EPCs was based on isolation of CD34+ mononuclear blood cells. The isolated cells were adhered to plastic and differentiated into endothelial cells upon culture [6]. Since the discovery of EPCs, various markers have been proposed to identify EPCs, such as CD34, CD133, expression of both CD133 and vascular endothelial growth factor receptor (VEGFR) 2, and expression of monocyte/macrophage-related molecule CD14 with minimal CD34 molecule [7]. The functional role of circulating EPCs has been actively investigated during the past few years. It was shown that higher level of VEGF may induce a rapid mobilization of HSCs and bone marrow-derived circulating endothelial precursor cells, which contribute to postnatal angiogenesis and hematopoiesis [8]. However, further study has shown that bone marrow-derived cells do not significantly contribute to tumor- or cytokine-induced angiogenesis rather tumor- or VEGF-induced angiogenesis is involved [9]. Based on their proliferation properties, two different categories of EPCs were identified in the peripheral blood, early EPCs and late EPCs. Early EPCs secrete more angiogenic cytokines, such as VEGF and interleukin (IL)-8 than do late EPCs; however, late EPCs produce more nitric oxide and incorporate more readily into human umbilical vein endothelial cell monolayers and form capillary tubes as compared to early EPCs [10].

2.2 Hematopoietic Stem Cells

HSCs and EPCs develop in close proximity to each other within the embryo. HSCs share the same ancestor with EPCs, called the hemangioblast. The existence of hemangioblasts was supported by various experimental observations, but its role during development is still controversial. Even though evidence has shown that single cell-derived colonies could produce both hematopoietic and endothelial cells in vitro, only a small portion of hematopoietic and endothelial cells were derived from hemangioblasts during development, which indicated that hemangioblasts might not be as significant as originally expected [11]. However, these studies illustrated the relationship of hematopoietic and endothelial lineage and indicated the possibility that HSCs might facilitate the angiogenesis during embryonic development and postnatal development. Indeed in acute myeloid leukemia (AML)-1-deficient embryos, which lack definitive hematopoiesis, defective angiogenesis in the head and in the pericardium was observed. The disruption in angiogenesis of para-aortic splanchnopleural (P-Sp) explant culture was rescued by addition of HSCs [12]. The recruitment of myeloid cells was found to be associated with formation of new blood vessel during pathological angiogenesis, and depletion of circulating myeloid cells significantly reduced the density of microvessels in a bioengineered human vascular implant [13]. The functional role of HSCs during angiogenesis may come from expression of proangiogenic factors such as VEGF and Ang1 and remodeling factors such as matrix metalloproteinase (MMP)-2 and MMP-9, which promote angiogenesis and guide the migration of endothelial cells [12]. It was found that hematopoietic cytokines SDF-1, induced by soluble Kit ligand, thrombopoietin, erythropoietin, and granulocyte-macrophage colony-stimulating factor (GM-CSF) released from platelets, enhanced neovascularization through mobilization of chemokine receptor (CXCR)-4+ VEGFR1+ hemangiocytes [14]. The important role of hematopoietic cells in angiogenesis has received great attention and proposed to be important target for anti-angiogenesis therapy following radiotherapy during treatment of tumor progression [15].

2.3 Mesenchymal Stem Cells

MSCs are present in many organs and function to maintain and regenerate connective tissues and replace damaged tissues following injury or inflammation. MSCs could efficiently stabilize nascent blood vessels in vivo acting as perivascular precursor cells, although differentiation of MSCs into endothelial cells was not detectable [16]. Co-implant human primary endothelial cells with human bone marrow MSCs showed enhanced formation of a network of functional, mature blood vessels accessed by in vivo whole body bioluminescence imaging in immunodeficient mice

[17]. Transplantation of MSCs was shown to be able to decrease fibrosis and myocardial scarring and improve myocardial regeneration in infarct-damaged hearts, through paracrine effects, via secretion of VEGF, basic fibroblast growth factor (bFGF), and placental growth factor (PlGF), even though MSC differentiation into ECs was not clearly demonstrated [18].

2.4 Smooth Muscle Progenitor Cells

Smooth muscle cells in the vascular system provide the structural integrity of the vessel wall. Recent study has shown that smooth muscle progenitor cells may have a potential role in angiogenesis. In a murine stroke model, it was shown that co-injection of smooth muscle progenitor cells with EPCs gave better results than administration of EPCs alone for vascular remodeling, cell proliferation, and neuroblast migration [19]. Perturbation in the signaling of transforming growth factor (TGF)-β, which is a multifunctional cytokine and plays an important role in carcinogenesis, was reported to affect endothelial and smooth muscle cell function and to contribute to tumor angiogenesis and tumor progression [20]. Smooth muscle cells can also contribute to angiogenesis by secreting mitogens, such as VEGF upon response to the hypoxia [21].

2.5 Vascular Pericytes

Pericytes are located surrounding the endothelial cells of the capillaries. Clonally isolated cells expressing pericyte markers were shown to be myogenic in culture in vivo [22]. It was proposed that pericytes derived from MSCs retain nascent stem cell properties, were recruited to the nascent microvascular wall during development and postnatal growth, and remained in a growth-arrested state until triggered to resume proliferation and differentiation later [23].

3 External Factors Regulate Angiogenesis Process

There are three distinct mechanisms, which promote cell migration during angiogenesis, chemotaxis, haptotaxis, and mechanotaxis. Chemotaxis directs cell migration toward a gradient of soluble chemoattractants, such as VEGF and bFGF. Haptotaxis attracts cells toward a gradient of immobilized ligands such as integrins binding to ECM components. Mechanotaxis promotes cell migration by mechanical forces, such as fluid shear stress [24]. Other factors including hypoxia will also be discussed here (Fig. 4.2).

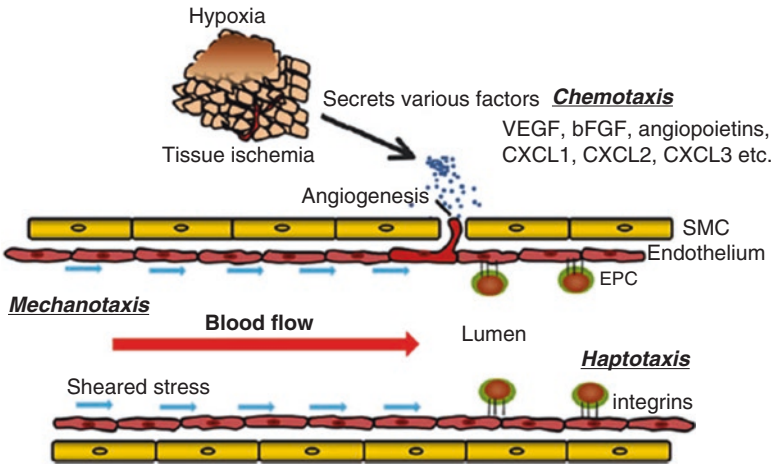

Fig. 4.2 Factors regulating angiogenesis process. Hypoxia, chemokines and cytokines, hypotaxis, and mechanotaxis are the major factors induce and regulate angiogenesis process (*EPCs* endothelial progenitor cells, *SMC* smooth muscle cells, *VEGF* vascular endothelial growth factor, *bFGF* basic fibroblast growth factor, *CXCL* chemokine (CXC-motif) ligand, *MMP* matrix metalloproteinases)

3.1 Chemotaxis: Cytokines, Chemokines, and Growth Factors

Various cytokines and soluble proteins, such as VEGF, bFGF, angiopoietins, FGF-2, hepatocyte growth factor (HGF), platelet-derived growth factor (PDGF), epidermal growth factor (EGF), TGF-β, interleukins, and tumor necrosis factor (TNF)-α, promote the migration of endothelial cells during angiogenesis. VEGF is a major factor that regulates angiogenesis. Various factors can induce the production of VEGF, and hypoxia was reported to be one of them. Hypoxia is able to enhance the production of VEGF and its receptors [25]. Production of reactive oxygen species (ROS), for example, hydrogen peroxide (H_2O_2), also upregulates the gene expression of VEGF in endothelial cells [26]. VEGF was also found to be expressed by almost all solid tumor as an angiogenic mitogen and so is now targeted for anti-angiogenesis therapy for tumor metastasis [27]. VEGF and its family members stimulate cellular responses by binding to the tyrosine kinase receptors called VEGFRs. VEGFR1 (Flt-1) is required for the recruitment of hematopoietic precursors and migration of monocytes and macrophages [28]. VEGFR1-deficient mice die in utero between 8.5 and 9.5 days post-coitum due to early defects in the development of hematopoietic and endothelial cells [29]. The functional role of VEGFR2 (KDR/Flk-1) has been linked with proliferation, migration survival, and increased permeability, all of which contributes to the angiogenesis process [30].

VEGF plays critical roles in endothelial differentiation, in acquisition of arterial endothelial cell identity, and in the vascular patterning of vertebrate embryos. VEGF ligands and receptors such as VEGF-A, the prototype of VEGF ligand, VEGFR1, VEGFR2, and VEGFR3 regulate vasculogenesis and angiogenesis during various

stages of growth [31]. By studying a series of nerve-specific Cre lines, it was shown that peripheral nerve-derived VEGF promotes arterial differentiation through the $VEGF^{164}$-NRP1 positive-feedback loop [32]. It was further demonstrated that VEGF acted downstream of sonic hedgehog (Shh) and upstream of Notch pathway in the differentiation of endothelial cells to arterial fate [33].

Other factors also play important roles in promoting angiogenesis including bFGF, angiopoietins, HGF, PDGF, EGF, TGF-β, TNF-α, etc. Slow release of bFGF (using gelatin hydrogels) can promote new blood vessel formation compared with a control group in a murine limb ischemia model [34]. Angiopoietin was required for endothelial development from progenitors circulating in human cord blood. More specifically, endogenous angiopoietin-1 regulates initial endothelial cell commitment, while angiopoietin-2 improves expansion of the endothelial cell progeny [35]. Angiopoietin-1 and angiopoiein-2 may also play important role in regulating recruitment of mural cells during angiogenesis [36]. It was shown that overexpression of HGF in smooth muscle cells can be beneficial in EPC differentiation, proliferation, and migration [37]. Further study has shown that HGF stimulates migration and tube formation of human umbilical vein endothelial cells in a Nox2-dependent manner [38]. However, transplantation of bone-derived MSCs showed no significant differences in promoting angiogenesis with or without HGF, which indicated that further study is needed to investigate the interplay between HGF and MSCs [39].

Chemokines are a family of small chemotactic cytokines and are classified by the presence of four cysteine residues in conserved locations. Members of the chemokine family are divided into four groups CC chemokines, CXC chemokines, C chemokines, and CX_3C chemokines. Many chemokines were proven to be angiogenic such as CXCL1, CXCL2, and CXCL3. These chemokines activate endothelial cells upon binding with their receptors. It was reported that functional differences among endothelial cells is dependent on the level of expression of CXC chemokine receptors [40]. It was also proposed that CXC chemokine IL-8; growth-related oncogenes alpha, beta, and gamma; granulocyte chemotactic protein 2; and epithelial neutrophil-activating protein-78 mediate angiogenesis in the absence of preceding inflammation partially through interaction with CXC chemokine receptor 2 (CXCR2) [41]. CXCR2 is a member of the G-protein-coupled receptor family and is expressed in endothelial cells. CXCR2 knockout mice exhibited defective neutrophil recruitment, an altered temporal pattern of monocyte recruitment, significant delay in epithelialization, and decreased neovascularization in wound-healing processes [42]. It was shown that upon binding to IL-8, CXCR2 activates the Rac pathway, which leads to cell retraction and formation of gaps between neighboring cells. Translocation of Rac into the plasma membrane eventually results in endothelial activation [43]. These experiments suggest that CXCR2 plays an important role in the recruitment of cells and promoting angiogenesis. Other than CXCR2, VEGF- and bFGF-activated angiogeneses were also partially mediated through CXCR4. Stimulation of human umbilical vein endothelial cells with VEGF or bFGF was shown to be able to induce upregulation of CXCR4. It was further shown that chemokine SDF-1α, which specifically bind CXCR4, is a potent chemoattractant for endothelial cells and participates in angiogenesis stimulated by VEGF and bFGF [44].

3.2 Haptotaxis

Haptotaxis is the directional motility of cells by the ligands typically presented in the ECM. Exposure of ECM and binding to integrin help homing and recruitment of the immune cells during the angiogenesis process. These ECM and integrin molecules are also critical for homing of transplanted HSCs to the bone marrow and the recruitment of inflammatory cells to the sites of inflammation [45]. It was shown that hematopoietic progenitor cells of β_2 integrin-deficient mice are less capable of homing to the ischemic site and that improving neovascularization and preactivation of the β_2 integrins expressed on EPCs augmented the EPC-induced neovascularization [46]. Antagonists of integrin $\alpha_4\beta_1$ were shown to be able to block the adhesion of monocytes to endothelium and prevented monocyte stimulation during angiogenesis [47]. It was further shown that administration of α_4 integrin antibody resulted in increased numbers of circulating EPCs in vivo and systemic administration of anti-α_4 integrin antibody increased recruitment and the incorporation of bone marrow EPCs in newly formed vasculature of hind-limb ischemia and myocardial infarction models [48]. Integrin-dependent homing of progenitor cells can be enhanced by various factors. It was reported that high-mobility group box 1 (HMGB1) activated EPC migration in a RAGE (HMGB1 receptor expressed on EPCs)-dependent manner and was inhibited by β_1 and β_2 integrin inhibition. HMGB1 could rapidly increase the affinity of integrin and induce polarization of integrin, which might be related to the corresponding enhanced adhesion capability of EPCs [49]. Pharmacologic activation of Epac1, a nucleotide-exchange protein for Rap1, could increase Rap1 activity and stimulate the adhesion of various human progenitor cells. EPCs, CD34+ hematopoietic progenitor cells, and MSCs are activated through increased β_2 and β_1 integrin-dependent adhesion and activated progenitor cells home to the ischemic muscles in an increased amount as a result, neovascularization occurs [50].

3.3 Mechanotaxis

Mechanotaxis is the directed movement of cells by mechanical cues, such as fluidic shear stress and stiffness of substrate. Endothelial cells which make up the inner lining of blood vessels are constantly under fluid-mediated shear stress in vivo, and it was shown that this mechanical stress-mediated signaling contributes to each step of endothelial migration, cell-ECM adhesion, and cell–cell adhesion processes [51]. Shear stresses were reported able to induce changes in the shape of endothelial cells and partial disassembly of adherent junctions [52]. It was shown that endothelial cells, cultured on type I collagen-coated coverslip and wounded later, enhanced wound healing under higher shear stress [53]. The endothelial cell alignments induced by fluid shear stress were proposed to act through the p38/mitogen-activated protein (MAP) kinase-activated protein kinase 2 (MAPKAP kinase 2)/heat shock

protein (HSP) 25/27 pathway due to its critical role in actin dynamics. It was shown that by inhibiting p38 signaling, endothelial elongation and alignment were blocked in the direction of flow, elicited by shear stress [54]. Other mechanisms involving G protein have also been studied. It was shown that shear stress-induced cytoskeletal reorientation was abolished in cells overexpressing dominant negative Rac 1. This indicated that the Rac GTPase might play a role in regulating endothelial cytoskeleton by shear stress [55]. The endothelial cell reorientation in response to shear stress was further studied and was proposed to follow a two-step process involving Rho-induced depolarization, followed by Rho−/Rac-mediated polarization and migration in the direction of flow [56].

3.4 Hypoxia

Hypoxia plays a critical role in neovascularization, both in embryonic development and in postnatal development. During embryonic development, the vascular system is stimulated by an inadequate supply of oxygen, which is caused by rapid expansion of embryonic tissues. In adult tissues, the blood vessels do not undergo significant growth, and the oxygen concentrations remain relatively constant between 30 and 50 mm of Hg. In pathological conditions, however, as in ischemia, hypoxia is created by the lack of blood, which is the main carrier of oxygen, and reduction of the oxygen level triggers angiogenesis. Important molecules involved in the hypoxia response include prolyl hydroxylase domain-containing proteins (PHDs) and hypoxia-inducible factors (HIFs). PHDs play an important role in oxygen sensing by inhibiting HIFs expression and by promoting HIFs degradation. HIF is a key transcription factor governing a large set of gene expressions for hypoxia adaptation, for example, the inhibition of PHD suppressed lipopolysaccharide-induced TNF-α expression. Reducing oxygen will lead to poor hydroxylation activities by PHDs and thus lead to accumulation of HIF-α. Hundreds of proteins were regulated by HIFs in response to hypoxia. It was shown that hypoxia, by regulating HIF, stimulates the production of various angiogenic cytokines such as VEGF and angiopoietin-1 and promotes proliferation of embryonic hemangioblasts [57]. Hypoxia can also promote recruitment of bone marrow-derived vascular modulatory cells through HIF-1α, which enhances the synthesis and secretion of endothelial molecules on vascular progenitor cells, such as CD31, VEGFR2, and endothelial NO synthase (eNOS) [58]. Even though hypoxia has been demonstrated to be useful in maintaining undifferentiated stem cells, researchers have found that hypoxia can also stimulate differentiation of stem cells in certain condition [59]. Hypoxia may stimulate adipose stromal cells (ASCs) into endothelial-like cells. It was shown that secretion of VEGF correlates inversely with oxygen concentration, and ASCs assumed an endothelial phenotype characterized by their ability to form tubes when seeded with differentiated endothelial cells on Matrigel assays [60]. ASCs were reported to be able to express endothelial markers when cultured with VEGF and

differentiated in response to local cues into endothelial cells, which contributed to neoangiogenesis in a hind-limb ischemic model [61]. HIF-α, in response to hypoxia, regulates a variety of genes such as uPAR, collagen prolyl 4-hydroxylases, matrix metalloproteinases, and tissue inhibitors of matrix metalloproteinases, which were proposed to facilitate endothelial transition from a stable growth-arrested state to a plastic proliferative phenotype [62].

4 Signaling Molecules Involved in Angiogenesis

Several complex signaling pathways are involved in angiogenesis. However, two major signaling pathways play critical roles in angiogenesis, the Notch-signaling pathway and the hedgehog-signaling pathway, and these will be discussed here. We shall also discuss miRNAs, which are involved in the angiogenesis process (Fig. 4.3).

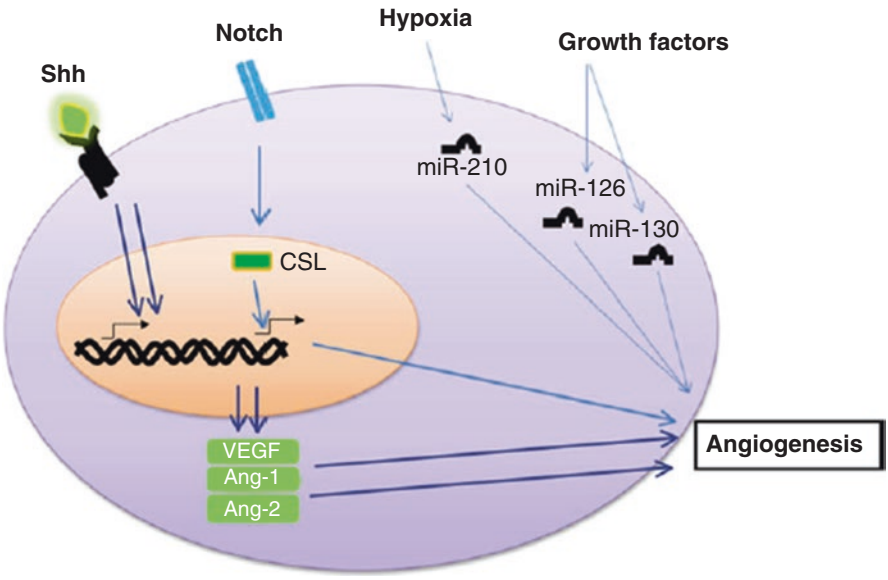

Fig. 4.3 Signaling molecules involved in cellular angiogenesis. Various cellular signaling molecules are involved in the angiogenesis process includes notch pathway, hedgehog pathway, hypoxia, and growth factors. MicroRNAs are also participating in the regulation of angiogenesis process (*Shh* sonic hedgehog, *CSL* combination of three proteins CBF1, Su (H), and Lag-2, *miR* microRNA, *VEGF* vascular endothelial growth factor, *Ang* angiopoietin)

4.1 Notch and Delta Signaling

Notch-signaling pathway is highly conserved with four different Notch receptors, NOTCH1, NOTCH2, NOTCH3, and NOTCH4, and five ligands from the jagged (Jagged-1 and Jagged-2) and Delta (Delta-like 1, Delta-like 3, and Delta-like 4) families plus modifier proteins from the Fringe family (lunatic, manic, and radical fringe) [63]. Notch proteins play critical role throughout embryonic development, such as cell survival, self-renewal for stem cells, and lineage determination for developing cells. Upon ligand activation, the cytoplasmic domain of Notch is proteolytically released, translocates into the nucleus, activates CSL [CBF1, Su (H), Lag-2], and converts them to transcriptional activators. The Notch/CSL-dependent signaling directly targets HERP families of transcriptional repressors, which are involved in multiple aspects of vascular development including muscle differentiation, angiogenic processes, arterial-venous cell fate determination, and vascular morphogenesis in mice [64]. The Delta-Notch-signaling pathway also targets members of the Hey family, the loss of which led to global lack of vascular remodeling and massive hemorrhage [65]. It was also shown that the differentiation-associated growth arrest in endothelial cells activated by Notch pathway was mediated by mitogen-activated protein kinase (MAPK) and phosphatidylinositol 3-kinase (PI3K)/Akt pathway [66].

4.2 Hedgehog Signaling

Hedgehogs interact with heparin on the cell surface through N-terminal basic domains. The molecular weight of this class is around 19 kDa. The role of hedgehog signaling in angiogenesis was brought to attention a decade ago. It was shown that Shh, a hedgehog homolog in mammals, can induce expression of two families of angiogenic cytokines, including all three VEGF-1 isoforms and angiopoietins-1 and -2 in interstitial mesenchymal cells. Shh was able to induce robust angiogenesis and augment blood flow recovery and limb salvage in an induced hind-limb ischemia model of aged mice [67]. By studying murine brain capillary endothelial cells (IBE cells) and human umbilical endothelial cells, it was shown that Shh-induced capillary morphogenesis through stimulating PI3-kinase activity [68]. During development, it was demonstrated that hedgehog proteins participate in the embryonic endothelial and fibroblast cell migration and play a role in the angiogenesis process [69]. In a diabetic wound-healing murine model, gene therapy of Shh together with bone marrow transplantation resulted in accelerated wound recovery partially by enhanced recruitment of bone marrow-derived progenitor cells and promoting production of angiogenic cytokines [70].

4.3 MicroRNA

In recent years it was found that microRNAs play an important role in regulating endothelial differentiation and in promoting angiogenesis. By studying zebra fish embryos, it was found that mechano-sensitive zinc finger transcription factor klf2 activates the VEGF-signaling pathway by inducing expression of endothelial-specific microRNA mir-126 [71]. Dicer is key enzyme, which contributes to the maturation of microRNA. Specific silencing of Dicer using siRNA has led to altered expression of key regulators of angiogenesis such as TEK/Tie-2, KDR/VEGFR2, Tie-1, endothelial nitric oxide synthase, and IL-8 in endothelial cells [72]. Furthermore, reduction of endothelial microRNAs by inactivation of Dicer reduces postnatal angiogenic response to exogenous VEGF, tumors, limb ischemia, and wound-healing models [73]. These findings indicate that microRNAs play important roles in regulating endothelial cells during the angiogenesis process. Multiple microRNAs have been found to influence the angiogenesis process including microRNA-17, 92, 23, 27, 24, 130a, 181a, and 210. Till recently, few microRNAs have been identified to regulated endothelial differentiation, and microRNA-mediated control of endothelial differentiation remains to be explored [74].

5 Conclusions and Future Directions

Major efforts were given in studying the mechanisms of angiogenesis in various pathological conditions. These efforts will significantly improve our understanding of therapeutic angiogenesis. Various regulating factors including microRNAs were found to be important during angiogenesis. Numerous treatments are under development targeting appropriate regulatory factors of angiogenesis in the context of pathological condition of the disease. Results are now available from many clinical trials using various stem cells for the treatment of ischemia [3, 75, 76]. It was shown that HSCs and MSCs were indeed able to improve the vascularization process in ischemic tissues and to improve clinical outcomes in both animal model and in clinical use [77]. Nevertheless, the future role of stem cell treatment compared to current pharmacologic treatment remains undetermined. Moreover, the best timing for the possible administration of stem cells is still unknown. As we learn more about the molecular mechanisms of angiogenesis, we are likely to find an effective window for future stem cell therapy to improve the outlook for the recovery of ischemic tissues.

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