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Beyond endothelial cells: Vascular endothelial growth factors in heart, vascular anomalies and placenta

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ABSTRACT

Vascular endothelial growth factors regulate vascular and lymphatic growth. Dysregulation of VEGF signaling is connected to many pathological states, including hemangiomas, arteriovenous malformations and placental abnormalities. In heart, VEGF gene transfer induces myocardial angiogenesis. Besides vascular and lymphatic endothelial cells, VEGFs affect multiple other cell types. Understanding VEGF biology and its paracrine signaling properties will offer new targets for novel treatments of several diseases.

1. Introduction

Vascular endothelial growth factors (VEGFs) and their receptors are important for normal physiology and they participate in the pathogenesis of several different diseases. Physiological levels of VEGFs are required for the maintenance of normal endothelial function but too high concentrations lead to aberrant angiogenesis and other pathological findings. In the heart, VEGFs induce new vascular growth in ischemic myocardium. However, the effects of VEGFs extend also to other cell types beyond endothelial cells. Examples are fibroblasts, stem cells and neurons, where some unexpected findings have been observed after administration of certain VEGF family members, leading to significant side effects, such as arrhythmias.

VEGFs and their receptors play significant roles in the pathogenesis of vascular anomalies, such as hemangiomas and arteriovenous malformations. VEGFs are also crucial in maintaining normal function of the placenta, while some problems in abnormal placental function have been related to dysfunctional endothelium and angiogenic signaling. Some members of the VEGF family have also significant effects on the growth of lymphatic vessels, which open new avenues for studies related to the circulation and tissue fluid handling in various organs, such as brain, heart and skin. In this review, we summarize recent findings in the VEGF biology in areas less frequently studied in connection with VEGFs and their receptors. It is likely that further understanding of the normal physiology and pathogenesis related to these growth factors in the heart, aberrant vascular structures, placenta and lymphatic system, will offer new targets for novel treatments of several diseases.

1.1. VEGFs in the heart

VEGF therapy aimed at improving cardiac function has largely concentrated on myocardial angiogenesis and improving blood flow. However, a functional heart requires the coordinated function of multiple cell types (Fig. 1). VEGFs have been shown to have effects on all cell types found in the heart, and a broad spectrum evaluation of their effects is therefore essential to understand both therapeutic effects and potential side effects of novel therapies. The different cell phenotypes present in the heart and their role in VEGF biology is discussed below.

1.1.1. Endothelial cells

Endothelial cells are by far the most established target for VEGFs, and they are known to express all VEGFR isoforms. VEGF expression

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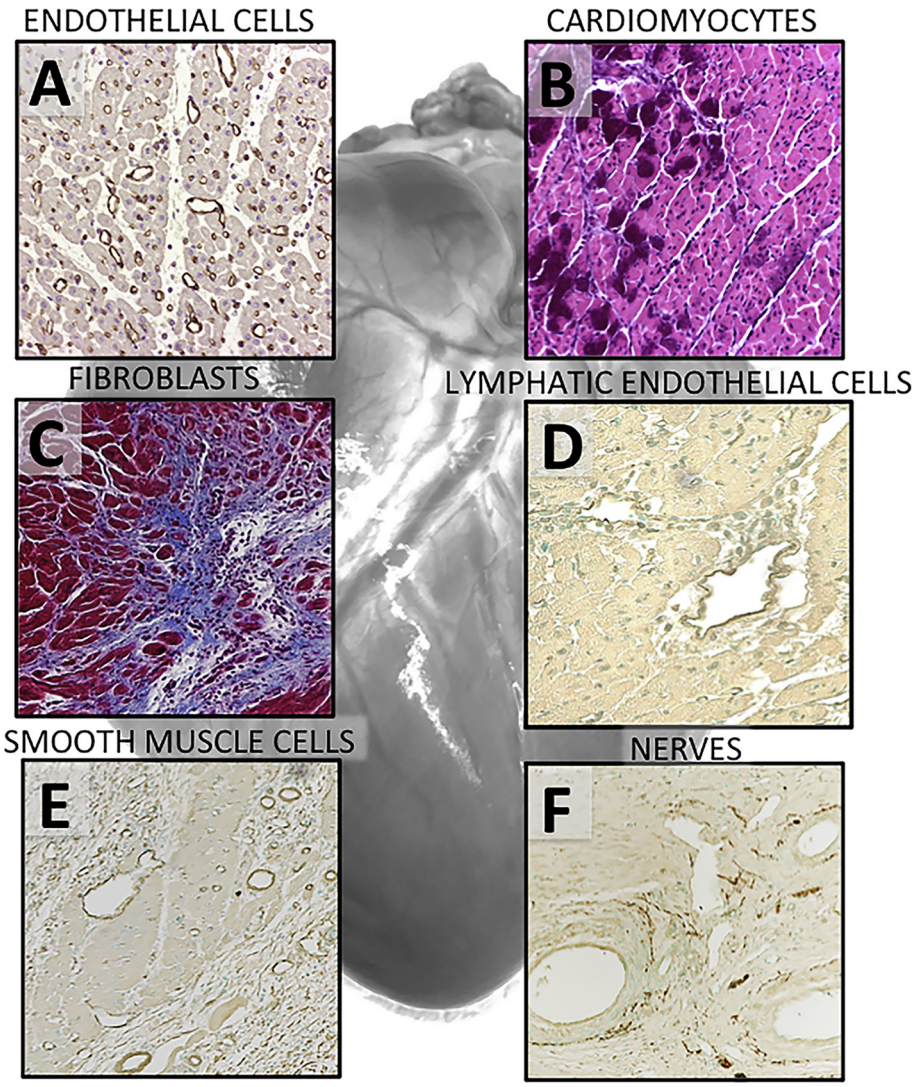

Fig 1. VEGFs have effects on multiple cell types in the heart. VEGF receptors are widely expressed in the heart and VEGF over-expression has effects on all cell types. VEGFs have unique effects depending on their receptor binding profile and solubility. (A) Endothelial cells express VEGFR-1 and VEGFR-2. VEGFR-2 expression is upregulated in angiogenic capillaries and in response to ischemia. Both VEGFR-1 and VEGFR-2 ligands induce angiogenesis in the heart. (B) Cardiomyocytes express both soluble and a cell membrane bound form of VEGFR-1. Its expression has been shown to be upregulated in ischemia but the function is yet unknown. Long-term over-expression of VEGFR-1 ligands leads to cardiac hypertrophy and metabolic changes in cardiomyocytes. (C) Fibroblasts express low levels of all VEGFRs. However, overexpression of VEGFs often leads to fibroblast proliferation and activation. This may be either a direct effect, or a result of interstitial edema and infiltration of inflammatory cells. VEGFR-1 ligands promote α-SMA expression and transformation to myofibroblast phenotype. Myofibroblasts express VEGFR-1, -2, -3 and VEGF-A, and play an important role in wound healing after myocardial infarction. (D) Cardiac lymphatic vessels are mainly located on the epicardial surface and express VEGFR-3. VEGFR-3 ligands induce lymphangiogenesis in the heart. Cardiac lymphatics are important in repair processes after myocardial infarction, and may be utilized to reduce the side effects of angiogenic therapies. (E) Smooth muscle cells express both VEGFR-1 and VEGFR-2. Smooth muscle cells play an important role in both growth of larger collateral vessels and in stabilization of neovascularization. In coronary vessels excessive smooth muscle cell proliferation promotes plaque formation and restricts blood flow. Both VEGFR-1 and -2 ligands induce smooth muscle cell proliferation. In therapeutic applications direct stimulation of smooth muscle cells may not be required, as blood flow and increased shear stress in the vascular wall, appear to be sufficient to promote arterial growth. (F) Neurons express VEGFR-1, -2 and Nrp-1. Both activation of VEGFR-1 and inhibitory signaling through Nrp-1 are suggested to effect nerve growth and regeneration.

and activity is induced in response to ischemia [1–3], resulting in the stimulation of microvessel dilation and growth [4], thus inducing myocardial angiogenesis [5,6]. Although the VEGF family is large, each VEGF has a unique profile, for example, VEGF-B appears to be myocardium-specific, at least in larger mammals [6]. VEGFs promote essentially all processes required for neovessel formation: nitric oxide mediated vasodilation, endothelial cell detachment, cell survival, proliferation, migration and tube formation [7]. Solubility of VEGFs determine the angiogenic pattern and the spread of vessels within the ischemic tissue [8]. More soluble factors like the proteolytically processed form of VEGF-D are distributed distant from the producing cells, while heparin sulphate binding factors like VEGF-A_{165} are retained in the vicinity of the producing cells [5]. In the heart, myocardial architecture guides vessel growth and limits diffusion of growth factors, causing angiogenesis to be more vessel dilation-related and associated with growth of pre-existing capillaries rather than neovessel sprouting [9]. In endogenous angiogenesis, growth factor gradient guide the formation of vessels in the ischemic areas [10]. In the infarction area, organized myocardial structure is replaced with a fibrous scar, and neovessel sprouting is observed [11,12].

Both vasodilation and angiogenesis mediated by VEGFR-1 and -2 lead to increased vascular permeability [13–15]. Increased endothelial cell surface area and immature neovessel structure result in increased leakage of proteins to the interstitial space. In the heart, edema can weaken mechanical properties of the myocardial wall by stiffening the muscle and hindering diastolic function. The most severe outcome is accumulation of pericardial effusion leading to diminished cardiac output and even tamponade [4,5]. Attempts to uncouple vascular permeability from angiogenesis have so far been unsuccessful (unpublished observations), and therefore choosing the growth factor with the best benefit/side effect ratio remains the best strategy. Simultaneous improvement of lymphatic clearance is another approach to minimize fluid accumulation in the heart.

1.1.2. Lymphatic endothelial cells

Fluid balance in various organs is very important in maintaining normal physiology. Myocardial lymphatic vessels were only recently brought into the spotlight and recognized to play a role in cardiac function and pathologic processes [16,17]. Lymphatic vessels are mainly located on the epicardial surface of the heart and they drain lymph from the myocardium and pericardial sac [18]. The lymphatic vasculature participates in repair processes after myocardial ischemia [19], prevent adverse remodeling [20], and impaired lymphatic vessels lead to poor outcome and more severe functional defects after myocardial infarction. In addition, stimulation of lymphatic vessel growth alleviates edema and accumulation of pericardial fluid, an inevitable

side effect of neovessel formation. VEGF-C and VEGF-D, together with VEGF-R3, are key drivers of lymphatic vessel growth. VEGFR-3 is expressed on cardiac lymphatic endothelium and over-expression of its ligands VEGF-C and VEGF-D rapidly stimulate lymphangiogenesis [5,20].

Previously, the role of lymphatics in metastatic spread of cancers and association to lymphedema and filariasis have been reported by several groups. However, disturbances in lymphatic flow and properties in several other pathological conditions have only recently been brought to the stage, thanks to the development of new diagnostic tools and specific reagents. Examples are the detection of lymphatic vessels in brain meninges [21,22]. Unexpectedly, blocking of VEGF-R3 activity leads to very high cholesterol and triglyceride levels in mice [17,23]. VEGFs also seem to affect lipid absorption in the gut, normal structure and function of intestine and several properties of subcutaneous tissue. Edema in the myocardium can be reduced by gene transfer of lymphatic growth factors into the myocardium. In addition, the role of lymphatic growth factors in regulating eye pressure and glaucoma has been described [24]. Therefore, lymphatic biology is becoming an integral part of vascular and VEGF biology and its role in various pathological conditions is emerging.

1.1.3. Vascular smooth muscle cells and pericytes

Smooth muscle cell activation and proliferation can be either beneficial or detrimental, depending on the context and location. In larger coronary vessels, smooth muscle cell proliferation contributes to arterial obstruction and plaque formation. In contrast, in growing collateral vessels and in neovessel formation, smooth muscle cell proliferation is essential for vessel stabilization and arterialization. Smooth muscle cells are a heterogeneous cell population, and VEGFR expression varies, for example between arterial and venous smooth muscle [25]. VEGF has been shown to induce smooth muscle cell migration but not proliferation *in vitro* [26,27]. In microvessels, a continuous smooth muscle cell layer is substituted by pericytes, which play an active role in angiogenesis and vessel maturation. In the early phases of angiogenesis, mural cell detachment is required to allow endothelial cell proliferation and migration, while in later stages, pericyte coverage is needed to prevent vessel regression and to provide mechanical support and functional regulation. VEGFs are thought to mainly promote the initiation and proliferative phase of angiogenesis, while later stabilization of vessels is regulated by other factors, such as Angiopoietins and Platelet-Derived Growth Factors [28,29]. Although soluble sVEGFR-1 and sVEGFR-2, are known to act as anti-VEGF agents, they can also be beneficial and to promote vascular maturation [30]. Pericytes respond differently to VEGFs, depending on their receptor binding profiles. While VEGF-A and placental growth factor (PlGF) induce mainly proliferation of endothelial cells and vasodilation, VEGF-B also stimulates proliferation of α-smooth muscle actin positive cells and more stable neovessels are formed [6]. However, when neovessels are needed in the ischemic environment, blood flow and increased shear stress are likely to be sufficient to stabilize newly formed vessel structures and induce collateral growth. In larger coronary vessels, VEGF responses are more controversial. VEGFs stimulate smooth muscle cell proliferation *in vitro,* and interestingly, vascular injury appears to make vascular smooth muscle cells more responsive to VEGF, by up-regulating VEGFR-1 expression [31]. Vascular injury has also been shown to increase VEGF-A and VEGFR-2 expression in neointimal smooth muscle cells [32,33]. VEGFR-1-mediated signaling can also promote inflammatory changes in the vascular wall, thus aggravating neointima formation [34]. However, in *in vivo* settings the net effect of VEGF expression may be beneficial, as VEGF-induced reendothelialization improves vascular function and prevents neointima formation [35] and VEGF over-expression has been shown to inhibit intimal thickening and macrophage accumulation [32,36,37].

1.1.4. Cardiomyocytes

Loss of cardiomyocyte function and cell death are important hallmarks of myocardial ischemia and cardiotoxic reactions. Over-expression of VEGFs has been shown to protect cardiomyocytes from apoptosis [38]. The protective effect is likely mediated via multiple mechanisms. VEGFs directly up-regulate several anti-apoptotic pathways. In addition, vasodilation and neovessel growth improve local blood flow and nourish surrounding cardiomyocytes, thus preventing tissue damage.

Long term over-expression of VEGF-A and VEGF-B has been shown to induce cardiomyocyte hypertrophy [39–42]. In some settings, hypertrophy has been described as a physiological event, triggered to benefit function, while in others, hypertrophy has led to a pathologic phenotype and loss of function. Hypoxia up-regulates expression of VEGF-A and -C in myocardial ischemia and reperfusion [43,44]. The effect has been interpreted as a stimulus to direct neovessel growth to the ischemic area, as VEGFs are mostly secreted from cardiomyocytes, and their receptors are mainly located on the endothelial cell surface. Physiological stimuli, such as exercise, also increase VEGF expression, and VEGFs are thought to play a role in physiological hypertrophy.

VEGFs are also known to influence the electromechanical properties of cardiomyocytes, where they regulate both expression and phosphorylation of connexins, proteins essential for propagation of action potentials in the myocardium [45,46]. VEGFs also alter ion channel expression in cardiomyocytes [47,48]. VEGF-B appears to have a unique effect profile, in regard to modulation of cardiomyocyte metabolism [49]. However, the effects seem to vary between animal models. VEGF-B over-expression has been shown to readjust cardiomyocyte metabolic pathways to favor glucose oxidation [50] and to increase cardiomyocyte fatty acid uptake [51], lipid accumulation and lipolysis and ceramide accumulation in cardiomyocytes [42]. As a result, the functional outcome in these experiments has varied from improved cardiac function and ischemia resistance to lipotoxicity.

1.1.5. Fibroblasts

Fibroblasts respond to various pathological stimuli and are central in scar formation after myocardial injury [52]. VEGFs induce fibroblast proliferation and activation leading to myofibroblast phenotype [53]. Myofibroblasts express VEGFR-1, -2, -3 and VEGF-A [34,54,55]. Over-expression of VEGFs has only minor effects on cardiac fibroblasts in normoxic heart, however, in the ischemic environment, VEGFR-1 ligands VEGF-B and VEGF-A have been shown to stimulate more fibroblast proliferation and myofibroblast differentiation, than for example, VEGF-D [9,12,56]. Fibroblasts can transdifferentiate to endothelial cells and even cardiomyocytes *in vitro* and *in vivo,* and VEGFs, among other endothelial factors, promote this process [57,58]. However, the biological significance and therapeutic potential of such phenomena remains unclear. Although scar formation is initially a repair mechanism, overactive fibroblast proliferation and collagen secretion may lead to stiffening of the myocardium and diminished cardiac function [59].

1.1.6. Stem cells

VEGFs can both recruit circulating and bone marrow derived stem cells to the heart, and stimulate proliferation of tissue resident stem cells [60,61]. Stem cells have been shown to differentiate into several cell types in the heart and to promote recovery after myocardial injury [62]. Stem cells appear to play dual role in cardiac repair processes. Several reports have shown that a small number of cells incorporate into the forming neovessels in the adult heart. However, due to apparent rarity of these events, the functional significance is likely to be limited [63], although different types of bone marrow and blood derived cells secrete cytokines and growth factors that are important for repair processes [64]. This mechanism appears to be important especially for flow-mediated collateral vessel growth. In addition, VEGFs promote embryonic stem cell differentiation into cardiomyocytes [65] and endothelial cells [66].

1.1.7. Neurons

Cardiac innervation regulates heart function under physiological conditions and is implicated in essentially all cardiac pathologies. Blood vessel and nerve development occur in parallel during embryogenesis, and these processes share common regulators. Peripheral nerves have been shown to express VEGFR-1, VEGFR-2 and Nrp-1. Hypoxia further increases the expression of VEGFR-1 and -2 in at least some neurons [67]. VEGFs can stimulate nerve growth and repair in several tissue environments including the cornea [68]. We have recently shown that VEGF-B stimulates sympathetic nerve growth in the heart. Nerve growth in the ischemic heart is initially a compensatory mechanism, aiming to salvage cardiac function and contractility. However, uncoordinated nerve growth, nerve-perfusion imbalance and hyperactive sympathetic activity, all increase the risk of arrhythmias and sudden cardiac death [69]. While nerve growth in other settings is a potential therapeutic target, in the ischemic heart, it is a potentially harmful side effect. Although the mechanism for VEGF-induced nerve growth is yet unclear, it is likely mediated via Nrp-1 and/or VEGFR-1, for example VEGF-D does not stimulate nerve growth in the heart (unpublished observation).

2. VEGFs in placenta

In addition to the heart, VEGF signaling has a crucial role in placental development. Decidual (i.e. mucous membrane of the uterus) expression of the VEGF-A attracts embryonic trophoblasts expressing VEGFR-1, and thus contributes to the crosstalk between maternal and embryonic/fetal tissues. Elevated plasma levels of circulating soluble VEGFR-1 (sVEGFR-1) during pregnancy are also associated with pregnancy complications. The role of VEGF in placental development and its association to placental dysfunction will now be reviewed. In addition, a novel therapy with VEGF for early onset fetal growth restriction will also be discussed.

2.1. Placental development

VEGF-A is upregulated in the menstrual cycle in the endometrium. In particular, the decidual expression of VEGF-A has been linked to the requirement of macrophages in the endometrium, where it is thought to promote the shift of the M1 to M2 phenotype. Macrophages are key players in the remodeling of the uterine wall during early pregnancy. The lack of available VEGF-A contributes to the macrophages being sustained as an M1 phenotype, which is linked to pregnancy complications [70].

Once the embryo has implanted into the endometrium, development of vascularity becomes essential for the growth of the fetus. Decidual expression of VEGF-A on the maternal side attracts embryonic trophoblasts expressing VEGFR-1 [71,72]. VEGF-A has also been shown to be expressed in villous mesenchymal cells on the fetal side, as well as in both endothelial and medial cells of the fetal villous blood vessels. Accordingly, VEGFR-2 has been located in villous trophoblasts and endothelial cells on the fetal side. On the other hand, placental expression of PlGF on the fetal side, a ligand to VEGFR-1, contributes to the crosstalk between maternal and embryonic/fetal tissue, as extravillous trophoblasts on the maternal side express VEGFR-1 throughout pregnancy [73]. Crosstalk between VEGF-A-expressing cells of the maternal origin and PlGF-expressing placental cells, leads to decidual spiral artery remodeling and invasion of extra villous trophoblasts from placenta to decidua. In turn, this enables blood flow from the maternal spiral arteries to enter the placenta and provide nutrients and oxygen for the developing fetus [74].

2.2. Placental dysfunction

Fetal growth restriction, or intrauterine growth restriction (FGR or IUGR, respectively), is diagnosed when the ultrasound imaging reveal the estimated fetus weight for the gestational age being below the 10^{th} percentile [75]. In FGR, retardation of the fetus growth is detected when subsequent ultrasound measurements are performed. SGA (small for gestational age) fetuses grow while pregnancy proceeds, although their weight is below the 10^{th} percentile. Several factors can contribute to the reduction of fetal growth, either alone or in combination. These include maternal, fetal, placental and genetic factors. Placental factors include, among others, abnormal maternal-placental vasculature. This abnormality can develop to placental dysfunction, which may lead to preeclampsia [76].

Elevated plasma levels of sVEGFR-1 during pregnancy are associated with complications, including FGR and preeclampsia. Placental cells expressing VEGFR-1, for yet unknown reasons, express the soluble form of VEGFR-1 and thus alter the maternal-placental vascularity [77]. Whether it is the local increase of the VEGF-A protein levels that triggers the excess expression of sVEGFR-1 or any other reason, the lack of available VEGF-A for spiral artery enlargement, diminishes the availability of nutrients and oxygen to the fetus, increases blood pressure and may disrupt the placental villi.

2.3. Novel treatment options

Currently there is no effective treatment to promote fetal growth in the uterus during FGR or preeclampsia. Maternal VEGF therapy has been suggested as a treatment strategy for early onset FGR where there is evidence of diminished utero-placental blood flow [78]. The aim is to transduce the maternal uterine artery cells to produce excess VEGF-A, which is secreted to the spiral arteries. This could then trigger the VEGFR-1 expressing trophoblasts to remodel the spiral arteries and/or, more directly, trigger VEGFR-2 expressing endothelial cells to proliferate and express nitric oxide to induce vasodilatation. So far, this method has been tested in guinea pig and sheep models, with both VEGF-A and VEGF-$D^{\Delta N \Delta C}$ via adenoviral vector delivery. In both animal models, an increase of fetal growth, as well as utero-placental and umbilical blood perfusion, have been observed [79,80]. In addition, safety studies with an *in vitro* human placenta perfusion model, and an ethical evaluation were recently published [81,82]. Accordingly, a pregnant rabbit reproductive toxicology model has been established and toxicology studies have already been performed (unpublished data). The EU FP-7 funded EVERREST project is a clinical phase I/IIa trial using this approach. The proposed treatment window is between gestation weeks 22 and 28 [82].

3. VEGFs in hemangiomas and arteriovenous malformations

Finally, as well as the heart and placenta, VEGFs and their receptors play significant roles in the pathogenesis of various vascular anomalies, such as hemangiomas and arteriovenous malformations (AVMs). In both, genetic mutations in VEGFR or genes affecting the VEGF signaling pathway have been found. Propranolol, the first-line treatment of complicated infantile hemangiomas, has been shown to reduce VEGFR expression and secretion of VEGF-A. Besides endothelial cells, multiple other cell types are involved in the pathogenesis of these diseases. In hemangiomas, mainly stromal cells and stem cells express VEGF-A. In AVMs, VEGF-A expression locates to the endothelium, subendothelial layer and brain parenchyma. The involvement of various cell types in VEGF signaling and pathogenesis of hemangiomas and AVMs are described below.

3.1. General features of benign vascular tumors: infantile hemangioma

Infantile hemangioma (IH) is a benign, high-flow vascular tumor with an incidence of 4–10% in all neonates and 4.5% in mature neonates (female:male ratio 4:1, Caucasian prevalence) [83,84]. The majority of the lesions locate in the head and neck region and are found in skin, subcutaneous fat or mucous membranes. Predisposing factors

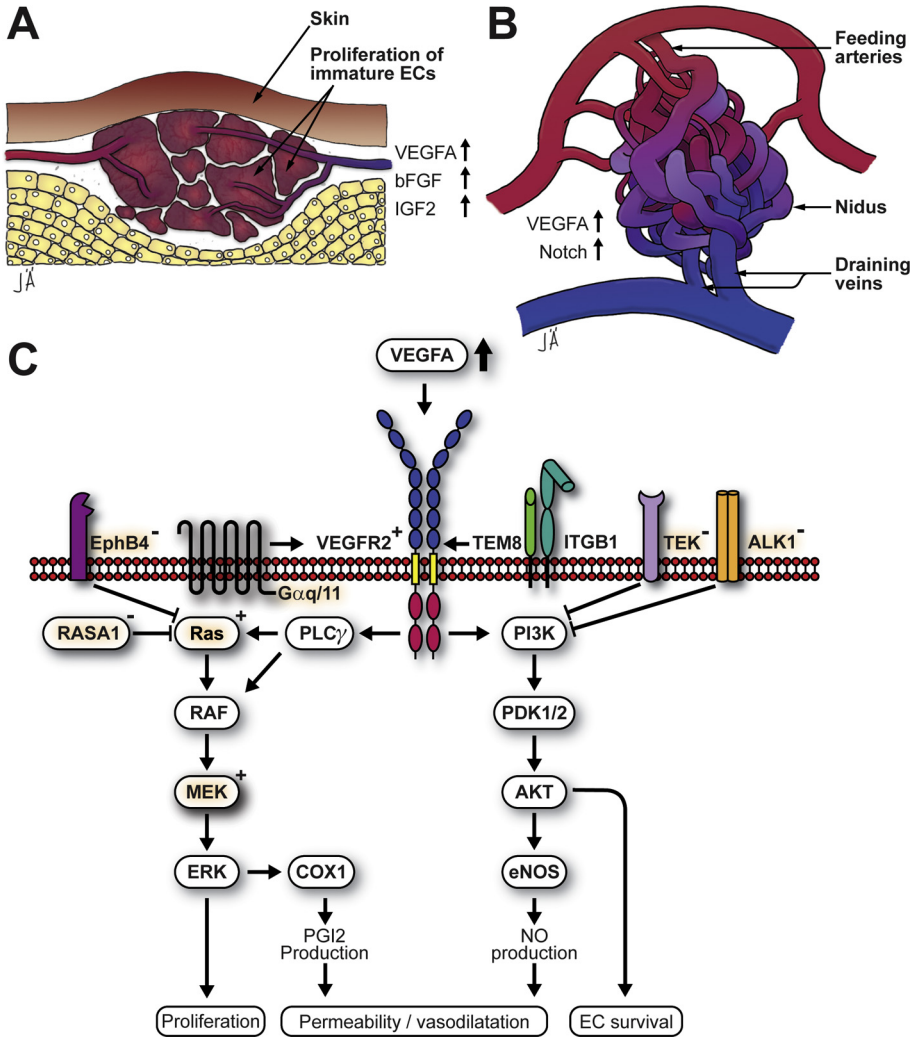

Fig 2. Involvement of VEGF-A and its downstream signaling in high-flow vascular anomalies. (A, B) Schematic illustrations showing the general structure and pathological vasculature of proliferating phase IH (A) and AVM (B) as well as key factors found to be upregulated in the lesion. (C) Genetic mutations in VEGFR-2, TEM8 and Gαq/11 are associated with vascular tumors and promote elevated VEGFR-2 activity and/or decreased VEGFR1 expression. Genetic mutations in EphB4, RASA1, KRAS, MAP2K1, TEK and ALK1 are associated with susceptibility to AVMs and promote prolonged/constitutive MEK/ERK or PI3K/AKT signaling. Signaling factors with significant incidence of mutations are highlighted. +, mutation promoting constitutive activity; -, inactivating mutation.

include low birth weight, family history of IH, multiple-gestation pregnancy, placental anomalies and intrauterine complications [83,85,86]. Lesions manifest during the first weeks of the postnatal period. In proliferative phase, immature endothelial cells divide rapidly, mast cells emerge and the basement membrane thickens (Fig. 2A). After reaching a plateau, the lesion starts to involute, as endothelial cells undergo apoptosis. As involution progresses, the amount of arterial vascular channels decrease and the remaining vessels enlarge, containing mature endothelial cells and pericytes. In the final regression phase, blood vessels are replaced with a fibrous adipocyte-rich tissue [83,85,87,88].

Besides IH, rare forms of hemangiomas are fully grown before birth. Fetal or congenital hemangiomas are divided in three forms: rapidly involuting (RICH), non-involuting (NICH) and partially involuting (PICH). Other rare benign vascular tumors include tufted angioma, spindle cell hemangioma, epithelioid hemangioma and pyogenic granuloma (ISSVA classification; http://www.issva.org/UserFiles/file/ISSVA-Classification-2018.pdf). Previous studies link a perturbation of the VEGF signaling pathway to most of these vascular tumors [89–93].

3.1.1. Genetic mutations and the role of VEGFR signaling in hemangiomas

The majority of IHs are sporadic. For familial IHs, two mechanisms of inheritance have been proposed, autosomal dominant and maternal transmission [84]. Although multiple studies suggest genetic contribution, the genetic background of familial IHs is not well-known [94]. Based on genetic mapping, 3 families were shown to have linkage to 5q31-33 region, from which VEGFR-3, fibroblast growth factor receptor 4 and platelet-derived growth factor receptor B have been linked to angiogenesis [95]. Very rare genetic mutations have also been detected in a small number of patients e.g. in VEGFR-2, VEGFR-3 and Angiopoietin-1 receptor TEK [96–98] (Table 1). In the context of VEGFR signaling, Jinnin *et al.* (2008) showed that in hemangioma-derived endothelial cells, VEGFR-2/TEM8/β1 integrin signaling complex suppresses expression of VEGFR-1 and NFAT-regulated genes, which in turn, leads to hyperactivation of VEGFR-2 signaling and a more proliferative endothelial cell phenotype [98]. Furthermore, a C482R mutation in VEGFR-2, found in a few IH patients [98], may induce ligand-independent activation of VEGFR-2 pathway [99]. In addition to these, mutations in genes regulating VEGFR-2 phosphorylation, such as G protein-coupled receptor alpha subunits GNA11 and GNAQ, have been found in some vascular anomalies [100–102] (Table 1). Thus, dysfunction of VEGFR-1 and VEGFR-2 signaling appears to be a common denominator in many of the benign vascular tumors (Fig. 2C).

Since IHs usually regress, reversible pathophysiological events besides genetic mutations, may act as a triggering mechanism for lesion formation and induce constitutive activation of VEGFR-2. A current hypothesis suggests that hypoxia upregulates VEGF-A expression which further induces CD133-positive hemangioma stem cells to proliferate and differentiate to immature endothelial cells [103]. Besides VEGF-A, basic fibroblast growth factor, insulin-like growth factor 2 and matrix metalloproteinases are upregulated in proliferative IH, but not in involuting IH [104,105]. Increased VEGF-A plasma levels are detected in patients having proliferative IH [106,107]. Interestingly, although expression of insulin-like growth factor 2 is detected in the endothelium

Table 1
Mutations and genetic variants associated with susceptibility to high-flow vascular anomalies.

Disease	Gene	Mutation(s)	Mutation/patient cohort	Reference
Infantile hemangioma	VEGFR-2	C482R	n = 2/9	[98]
		P1147S	n = 1/15	[96]
	VEGFR-3	P954S	n = 1/15	[96]
	TEM8	A326T	n = 1/9	[98]
	DUSP5	S147P	n = 1/3	[97]
	TEK	Y897C, R915C	n = 2/23	[186]
Pyogenic granuloma	TEK	R915C	n = 1/23	[186]
	BRAF	V600E, G464E	n = 4/25	[101]
	KRAS	G13R	n = 1/25	[101]
	HRAS	E49K, Q61R, G13S	n = 4/4	[187]
Pyogenic granuloma associated with port-wine stain	BRAF	V600E	n = 8/10	[101]
	NRAS	Q61R	n = 1/10	[101]
	GNAQ	R183Q	n = 9/10	[101]
Epithelioid hemangioma	TEK	Y897C	n = 1/23	[186]
	WWTR1-CAMTA1	translocation in t(1;3)(p36.3;q25)	n = 13/13 (FISH), confirmed WWTR1-CAMTA1 fusion n = 3/13	[188]
NICH	GNAQ	E209P, E209L	n = 5/10	[102]
	GNA11	E209L	n = 2/10	[102]
RICH	GNAQ	E209L, E209H	n = 3/5	[102]
	GNA11	E209L	n = 2/5	[102]
Tufted angioma	GNA14	Q204L	n = 1	[100]
Spindle cell hemangioma	IDH1	R132C	n = 18/28	[189]
	IDH2	R172T, R172M	n = 2/28	[189]
Sporadic extracranial AVM	MAP2K1	Q56P, Q58_E62del, K57N, F53L + D67Y	n = 16/25	[190]
	TEK	L914F, S917I, R918H, Y897C, R915C, R918C	n = 10/106	[186]
	BMPR2	del in exon 6 + exon 7	n = 1	[191]
Sporadic intracranial AVM	KRAS	G12D, G12V, Q61H	n = 45/72	[192]
	VEGF-A	IVS5-892T > C, 3596A > G, −634C > G −8339A > T in intronic and regulatory regions	IVS5-892TT/TC, 3596AA/AG, -634CG/GG and -8339AA genotypes more frequent in AVM than control group	[193]
	ANGPTL4	Synonymous variant 9511G > A	Minor allele more frequent in AVM than control group	[194]
	ALK1	Intronic variant IVS3-35A > G	Major allele more frequent in AVM than control group	[195,196]
	ENG	synonymous variant 207G > A	GG genotype more frequent in AVM than control group; not significant alone but in combination with ALK1 IVS3-35A allele	[195]
	TGFB2	Promoter variant −875A > G	AG genotype more frequent in AVM than control group	[197]
	IL6	Promoter variant −174G > C	GG genotype more frequent in AVM than control group	[198]
	IL1β	Promoter variants −511C > T, −31T > C	-511TT and -31CC genotypes more frequent in AVM than control group	[199]
	IL1α	−889 C > T in 5' regulatory region	Minor allele more frequent in AVM than control group	[200]
	MMP3	Promoter variant −707A > G	Minor allele more frequent in AVM than control group	[201]
CM-AVM	RASA1	Several, cause reduced expression or loss of function	n = 6/17 families n = 68/100	[202] [203]
	EPHB4	Several, cause reduced expression or loss of function	n = 54/365	[204]
Hereditary hemorrhagic telangiectasia	ENG ALK1	Several, cause reduced expression or loss of function	85% of all HHT patients carry mutations either in ENG or ALK1	[205,206]
	SMAD4	Several, cause reduced expression	n = 3/30 n = 2/109	[207] [208]
	BMP9	P85L, R68, R333W	n = 3/191	[209]
	BMPR2	Q433X	n = 1	[210]

and interstitium of IH, VEGF-A is mainly expressed in stromal cells and not in the endothelium. Instead, hemangioma-derived stem cells express and secrete VEGF-A [88]. Accordingly, hemangioma-derived pericytes upregulate VEGF-A and have reduced levels of angiopoietin-1 that may lead to vessel instability [108]. These data thus support the role of stem cells in initiating lesion formation and emphasizes the complexity of cellular crosstalk between pericytes, endothelial cells and stem cells in IH life cycle. As mast cells are known to release VEGF-A [109] and they are most prominent in the proliferative phase of IH [88], this cell type may play a role in lesion formation and VEGFR-2 signaling.

3.1.2. Management, treatment and emerging therapeutic concepts of infantile hemangioma

IHs can be deforming, ulcerated or obstructive, thus needing therapeutic intervention (10-12% of all IHs). Although VEGF-A seems to play a role in the pathogenesis of many benign vascular tumors, inhibition of VEGF-A signaling pathway e.g. by sunitinib, bevacizumab, suratinib has been mostly tested in separate case studies or in small patient cohorts of adult hepatic hemangiomas with mixed results [110]. Since 2008, propranolol, a beta-adrenergic receptor antagonist, has been a first-line treatment for complicated IHs, with a response rate of 96–98% [83,85]. After 6 months of propranolol treatment, 60% of the IH lesions are completely or near-completely regressed [111–113]. At a cellular level, propranolol has been suggested to target hemangioma stem cells, endothelial cells and pericytes. A recent study showed that propranolol targets beta adrenergic receptor β2AR, reduces cAMP levels and increases MAPK activity leading to reduction of hemangioma stem cell proliferation and induction of apoptosis [114]. Propranolol treatment has also been suggested to decrease VEGFR-1 and VEGFR-2 expression levels [115]. As VEGF-A and HIF-1α are upregulated by cAMP and βAR1/βAR2 ligand noradrenaline [116,117], a reduction of cAMP levels by propranolol could lead to sustained inhibition of VEGF-A signaling and thus regression of the lesion. In accordance, VEGF-A levels in plasma are reduced in propranolol-treated patients [118,119]. However, no significant reduction of VEGF-A protein expression was detected in propranolol-treated IH lesions [120]. As a recent study showed that β1AR receptor blocker atenolol had an equivalent efficacy

as propranolol for treatment of IHs, it appears that both βAR1 and βAR2 are important in IH pathogenesis [121].

Recurrence of lesions after propranolol treatment (10–15% of cases) occurs particularly in segmental or deep IHs [83]. Delayed referral to treatment affects success rate, as propranolol is the most effective in the early phase of the IH life cycle [122]. As propranolol crosses the blood-brain barrier, hydrophilic B blockers e.g. atenolol, nadolol, acebutolol have been suggested as an alternative for the treatment of IH, to reduce central nervous system related side effects, such as sleep and memory disturbances [83]. Besides transient gross motor delay in IH patients treated with propranolol [123], no developmental risks or growth impairment were reported in a recent follow-up study [124]. Lately, propranolol-loaded nanoparticles [125] and nanoparticles with CD133-aptamers [126] were used to reduce proliferation of hemangioma-derived stem cells *in vitro*. Thus, a nanoparticle-aided approach with local delivery could be an interesting option to reduce adverse effects of propranolol. In addition, topical beta blockers, e.g. timolol maleate 0.5% gel, have already been tested in the clinics for the treatment of superficial IHs alone or in combination with propranolol [127,128].

3.2. General features of arteriovenous malformations

AVMs are benign, congenital high-flow vascular anomalies. Their typical characteristics are an aggregation of dilated arteries and veins and the lack of a capillary bed (Fig. 2B). This results in abnormal arteriovenous shunting which promotes higher physiological blood flow and increased pressure towards the wall of the draining vein. AVMs belong to rare diseases with an estimated incidence in the general population of 0.001–0.02%. They could appear in any tissue and organ in the body. Most common locations are central nervous system, followed by head and neck regions, limbs, trunk and viscera [129–133]. Some autosomal inherited genetic diseases, like hereditary hemorrhagic telangiectasia and capillary malformation-arteriovenous malformation are associated with higher incidence of AVMs [134–139]. No other predisposing factors are currently known. In accordance to their congenital nature, lesions are present at birth. Some of them remain in a quiescence state. However, ca. 82% of extracranial AVMs progress towards an expansive and destructive phase during adolescence/early adulthood [129–131]. Expansive life cycle is a common feature also for brain AVMs [132,133,140] which further elevates venous hypertension, ischemic symptoms and changes in the vessel wall structure [129–131]. The etiology behind AVMs is not well-known. Current knowledge suggests that defects in vascular development or genetic mutations in key angiogenic/vasculogenic pathways, in conjunction with second-hit mutation, injury/trauma, infection or inflammation may function as triggering factors and initiate focal formation of lesions [131,133,141].

3.2.1. Genetic mutations and the role of VEGF-A in arteriovenous malformations

The majority of AVMs are sporadic. Familial syndromes associated with AVMs are caused by inactivating mutations in transforming growth factor (TGF) -β pathway genes or RASA1/EPHB4 (Table 1) [134–139]. TGFβ signaling is involved in vessel maturation by recruitment of mural cells; additionally, it participates in VEGFR2 signaling by regulating PI3K/AKT activity and VEGF-A expression (Fig. 2C) [142–145]. Thus, defects in TGFβ pathway have devastating effects on vessel development and structure. Interestingly, EPHB4 and RASA1 both act as negative regulators of Ras/MEK/ERK pathway [137,139]. Recent studies have also detected somatic activating mutations in the Ras family member KRAS, and the Ras downstream mediator MAP2K1, in > 60% of sporadic AVMs [146,147]. Together, these findings suggest that activation of Ras/MEK/ERK pathway is a central disease mechanism for AVMs [137,139,146,147]. TEK mutations with a potential ability to disturb vessel maturation and formation of proper endothelial junctions have also been found in a few patients [148].

Higher expression levels of VEGF-A is detected from AVMs, compared to control tissue [131,149–152]. In brain AVMs, VEGF-A is expressed in endothelial cells, the subendothelial layer and brain parenchyma next to pathological vessels [149–151,153]. Involvement of endothelial cells in VEGF-A production has also been confirmed in cell cultures derived from AVMs [154,155]. Interestingly, a polymorphism in the intronic and 5' regulatory regions of the VEGF-A gene with a potential ability to regulate gene expression has been associated with susceptibility to brain AVMs [156]. As tissue ischemia and venous hypertension themselves are capable of potentiating VEGF-A expression [157–160], it is still debatable whether VEGF-A upregulation is a cause of the disease or a compensatory mechanism promoted by venous hypertension/ischemic symptoms. Nevertheless, increased VEGF-A may promote neovascularization and growth of the lesion, matrix remodeling, vascular leakage and recruitment of inflammatory cells [131,157,161], and thus participate in the disease progression. VEGF-A expression has been detected especially in rapidly growing or recurring AVMs [140,158,162]. In accordance, acceleration of the growth of vascular lesions was achieved by VEGF-A overexpression in the ALK1 knockout mouse model [144].

High expression of Notch1, Notch4 and their target genes, Hes and Hey2, is also a common feature in brain AVMs. Notch signaling is activated by e.g. VEGF-A, Ras/MEK/ERK pathway and elevated blood flow and it regulates sprouting angiogenesis, tip cell/stalk cell selection, arterial-venous specification and expansion of the vessels [163–165]. In preclinical animal models, introduction of constitutive Notch4 activity promotes formation of AVMs [166–168]. Notch receptors and their polymorphisms have also been associated with intracranial hemorrhage caused by AVM [146,167,169–171]. AVM-associated polymorphism in inflammatory genes and MMP3 could further participate in lesion formation and progression [172–175].

3.2.2. Management, treatment and emerging concepts in therapeutics

Clinical presentation of AVMs depends on location and size of the lesion as well as its relation with other organs/structures. Although early stage lesions may be symptomatic, more serious symptoms and morbidity manifest in expansive and destructive phases of the growth [129–131]. The symptoms associated with AVMs include pain, tissue edema, structural and functional defects, significant risk of vessel wall rupture, bleeding and ulceration, as well as headache, seizures, neurological defects and a risk of hemorrhagic stroke. Noteworthy, brain AVMs are a leading cause of hemorrhagic stroke especially in young adults and children. In the most severe cases, extensive AVMs may lead to a high-output heart failure [130–133].

Current treatment options for AVMs include sclerotherapy, endovascular embolization, stereotactic radiotherapy and microsurgical resection. Choice of the treatment method depends on the location, size, and shape as well as angioarchitectural structure of the lesion. Although efficient, all invasive treatments are associated with a risk of treatment-related side effects and morbidity, as well as a risk of incomplete AVM resection leading to recurrence after the treatment [130–133,176,177]. Thus, non-invasive medical treatment with a potential ability to normalize the pathological vasculature of the lesion, and/or prevent recurrence, would be of importance. Bevacizumab, thalidomide and propranolol have been utilized to decrease epistaxis and improve hemoglobin concentrations in hereditary hemorrhagic telangiectasia [178,179]. Among these factors, the anti-angiogenic and anti-inflammatory agent thalidomide, showed the best efficacy; however, teratogenic effects and side effects need to be taken into consideration [179]. Despite clear association of VEGF-A to hereditary hemorrhagic telangiectasia, bevacizumab did not have any significant effects on epistaxis [178,179]. Partial improvement in clinical outcome was achieved, with patients suffering from high output cardiac failure and/or severe hemorrhages [180,181]. Recent findings on genetic studies and mouse models suggest the potentiality of MEK and PI3K inhibition for the treatment of sporadic AVMs [143,144,146,147]. Importantly, chemical inhibitors targeting MEK (UO126) or PI3K (Buparlisib,

Taselisib) are already in clinical use for the treatment of cancer and are well tolerated [182,183]. In addition, normalization of Notch signaling was shown to decrease arteriovenous shunting and blood vessel size in preclinical animal models [184,185] and could become a potential target for further drug development.

4. Summary

VEGFs and their receptors are important for the maintenance of normal endothelial function of blood vessels. VEGF therapy, aiming to improve cardiac function, has largely concentrated on myocardial angiogenesis and improving blood flow. VEGFs are also important mediators of vascular growth in placental abnormalities, hemangiomas and AVMs. Since VEGFs affect various cell types via paracrine signaling, a broad spectrum evaluation of their influence is needed to understand both VEGF-induced therapeutic effects and the role of VEGFs in pathological states. Techniques, such as massive parallel sequence-based gene expression analysis, high-resolution *in vivo* imaging of transgenic/knock-out animal models and co-culture assays of multiple cell types, will improve our understanding of VEGF-induced cellular effects leading to significant biological outcomes.

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References

[1] A. Namiki, E. Brogi, M. Kearney, et al., Hypoxia induces vascular endothelial growth factor in cultured human endothelial cells, J. Biol. Chem. 270 (52) (1995) 31189–31195.
[2] Y. Liu, S.R. Cox, T. Morita, S. Kourembanas, Hypoxia regulates vascular endothelial growth factor gene expression in endothelial cells. Identification of a 5' enhancer, Circ. Res. 77 (3) (1995) 638–643.
[3] E. Brogi, G. Schatteman, T. Wu, et al., Hypoxia-induced paracrine regulation of vascular endothelial growth factor receptor expression, J. Clin. Invest. 97 (2) (1996) 469–476.
[4] T.T. Rissanen, J.E. Markkanen, M. Gruchala, et al., VEGF-D is the strongest angiogenic and lymphangiogenic effector among VEGFs delivered into skeletal muscle via adenoviruses, Circ. Res. 92 (10) (2003) 1098–1106.
[5] J. Rutanen, T.T. Rissanen, J.E. Markkanen, et al., Adenoviral catheter-mediated intramyocardial gene transfer using the mature form of vascular endothelial growth factor-D induces transmural angiogenesis in porcine heart, Circulation 109 (8) (2004) 1029–1035.
[6] J.E. Lahteenvuo, M.T. Lahteenvuo, A. Kivela, et al., Vascular endothelial growth factor-B induces myocardium-specific angiogenesis and arteriogenesis via vascular endothelial growth factor receptor-1- and neuropilin receptor-1-dependent mechanisms, Circulation 119 (6) (2009) 845–856.
[7] A. Hoeben, B. Landuyt, M.S. Highley, H. Wildiers, A.T. Van Oosterom, E.A. De Bruijn, Vascular endothelial growth factor and angiogenesis, Pharmacol. Rev. 56 (4) (2004) 549–580.
[8] J. Woolard, H.S. Bevan, S.J. Harper, D.O. Bates, Molecular diversity of VEGF-A as a regulator of its biological activity, Microcirculation 16 (7) (2009) 572–592.
[9] J. Rutanen, T.T. Rissanen, J.E. Markkanen, et al., Adenoviral catheter-mediated intramyocardial gene transfer using the mature form of vascular endothelial growth factor-D induces transmural angiogenesis in porcine heart, Circulation 109 (8) (2004) 1029–1035.
[10] P. Vempati, A.S. Popel, Gabhann F. Mac, Extracellular regulation of VEGF: isoforms, proteolysis, and vascular patterning, Cytokine Growth Factor Rev. 25 (1) (2014) 1–19.
[11] T. Zhao, W. Zhao, Y. Chen, R.A. Ahokas, Y. Sun, Vascular endothelial growth factor (VEGF)-A: role on cardiac angiogenesis following myocardial infarction, Microvasc. Res. 80 (2) (2010) 188–194.
[12] J.E. Lahteenvuo, M.T. Lahteenvuo, A. Kivela, et al., Vascular endothelial growth factor-B induces myocardium-specific angiogenesis and arteriogenesis via vascular endothelial growth factor receptor-1- and neuropilin receptor-1-dependent mechanisms, Circulation 119 (6) (2009) 845–856.
[13] D.O. Bates, S.J. Harper, Regulation of vascular permeability by vascular endothelial growth factors, Vasc. Pharmacol. 39 (4-5) (2002) 225–237.
[14] J.P. Laakkonen, J.P. Lappalainen, T.L. Theelen, et al., Differential regulation of angiogenic cellular processes and claudin-5 by histamine and VEGF via PI3K-signaling, transcription factor SNAI2 and interleukin-8, Angiogenesis 20 (1) (2017) 109–124.
[15] S.M. Weis, D.A. Cheresh, Pathophysiological consequences of VEGF-induced vascular permeability, Nature 437 (7058) (2005) 497–504.
[16] L.H. Huang, K.J. Lavine, G.J. Randolph, Cardiac lymphatic vessels, transport, and healing of the infarcted heart, JACC Basic Transl. Sci. 2 (4) (2017) 477–483.
[17] T. Vuorio, A. Tirronen, S. Yla-Herttuala, Cardiac lymphatics – a new avenue for therapeutics? Trends Endocrinol. Metab. 28 (4) (2017) 285–296.
[18] A. Dashkevich, C. Hagl, F. Beyersdorf, A.I. Nykanen, K.B. Lemstrom, VEGF pathways in the lymphatics of healthy and diseased heart, Microcirculation 23 (1) (2016) 5–14.
[19] L. Klotz, S. Norman, J.M. Vieira, et al., Cardiac lymphatics are heterogeneous in origin and respond to injury, Nature 522 (7554) (2015) 62–67.
[20] O. Henri, C. Pouehe, M. Houssari, et al., Selective stimulation of cardiac lymphangiogenesis reduces myocardial edema and fibrosis leading to improved cardiac function following myocardial infarction, Circulation 133 (15) (2016) 1484–1497 discussion 1497.
[21] A. Aspelund, S. Antila, S.T. Proulx, et al., A dural lymphatic vascular system that drains brain interstitial fluid and macromolecules, J. Exp. Med. 212 (7) (2015) 991–999.
[22] A. Louveau, I. Smirnov, T.J. Keyes, et al., Structural and functional features of central nervous system lymphatic vessels, Nature 523 (7560) (2015) 337–341.
[23] T. Vuorio, H. Nurmi, K. Moulton, et al., Lymphatic vessel insufficiency in hypercholesterolemic mice alters lipoprotein levels and promotes atherogenesis, Arterioscler. Thromb. Vasc. Biol. 34 (6) (2014) 1162–1170.
[24] B.R. Thomson, S. Heinen, M. Jeansson, et al., A lymphatic defect causes ocular hypertension and glaucoma in mice, J. Clin. Invest. 124 (10) (2014) 4320–4324.
[25] A. Chanakira, R. Dutta, R. Charboneau, R. Barke, S.M. Santilli, S. Roy, Hypoxia differentially regulates arterial and venous smooth muscle cell proliferation via PDGFR-beta and VEGFR-2 expression, Am. J. Physiol. Heart Circ. Physiol. 302 (5) (2012) H1173–H1184.
[26] C.L. Grosskreutz, B. Anand-Apte, C. Duplaa, et al., Vascular endothelial growth factor-induced migration of vascular smooth muscle cells in vitro, Microvasc. Res. 58 (2) (1999) 128–136.
[27] A. Ishida, J. Murray, Y. Saito, et al., Expression of vascular endothelial growth factor receptors in smooth muscle cells, J. Cell. Physiol. 188 (3) (2001) 359–368.
[28] C. Betsholtz, P. Lindblom, H. Gerhardt, Role of pericytes in vascular morphogenesis, EXS 94 (94) (2005) 115–125.
[29] E. Fagiani, G. Christofori, Angiopoietins in angiogenesis, Cancer Lett. 328 (1) (2013) 18–26.
[30] S. Lorquet, S. Berndt, S. Blacher, et al., Soluble forms of VEGF receptor-1 and -2 promote vascular maturation via mural cell recruitment, FASEB J. 24 (10) (2010) 3782–3795.
[31] A. Parenti, L. Brogelli, S. Filippi, S. Donnini, F. Ledda, Effect of hypoxia and endothelial loss on vascular smooth muscle cell responsiveness to VEGF-A: role of flt-1/VEGF-receptor-1, Cardiovasc. Res. 55 (1) (2002) 201–212.
[32] M.O. Hiltunen, M. Laitinen, M.P. Turunen, et al., Intravascular adenovirus-mediated VEGF-C gene transfer reduces neointima formation in balloon-denuded rabbit aorta, Circulation 102 (18) (2000) 2262–2268.
[33] M. Bruczko, M. Wolanska, A. Malkowski, K. Sobolewski, R. Kowalewski, Evaluation of vascular endothelial growth factor and its receptors in human neointima, Pathobiology 83 (1) (2016) 47–52.
[34] Q. Zhao, K. Egashira, K. Hiasa, et al., Essential role of vascular endothelial growth factor and flt-1 signals in neointimal formation after periadventitial injury, Arterioscler. Thromb. Vasc. Biol. 24 (12) (2004) 2284–2289.
[35] R. Hutter, F.E. Carrick, C. Valdiviezo, et al., Vascular endothelial growth factor regulates reendothelialization and neointima formation in a mouse model of arterial injury, Circulation 110 (16) (2004) 2430–2435.
[36] R. Khurana, S. Shafi, J. Martin, I. Zachary, Vascular endothelial growth factor gene transfer inhibits neointimal macrophage accumulation in hypercholesterolemic rabbits, Arterioscler. Thromb. Vasc. Biol. 24 (6) (2004) 1074–1080.
[37] S.E. Heinonen, A.M. Kivela, J. Huusko, et al., The effects of VEGF-A on atherosclerosis, lipoprotein profile, and lipoprotein lipase in hyperlipidaemic mouse models, Cardiovasc. Res. 99 (4) (2013) 716–723.
[38] X.G. Chen, Y.X. Lv, D. Zhao, et al., Vascular endothelial growth factor-C protects heart from ischemia/reperfusion injury by inhibiting cardiomyocyte apoptosis, Mol. Cell. Biochem. 413 (1-2) (2016) 9–23.
[39] L. Zentilin, U. Puligadda, V. Lionetti, et al., Cardiomyocyte VEGFR-1 activation by VEGF-B induces compensatory hypertrophy and preserves cardiac function after myocardial infarction, FASEB J. 24 (5) (2010) 1467–1478.
[40] A.G. Marneros, Effects of chronically increased VEGF-A on the aging heart, FASEB J. 32 (3) (2018) 1550–1565.
[41] L. Lottonen-Raikaslehto, R. Rissanen, E. Gurzeler, et al., Left ventricular remodeling leads to heart failure in mice with cardiac-specific overexpression of VEGF-B167: echocardiography and magnetic resonance imaging study, Physiol. Rep. 5 (6) (2017), https://doi.org/10.14814/phy2.13096.
[42] T. Karpanen, M. Bry, H.M. Ollila, et al., Overexpression of vascular endothelial growth factor-B in mouse heart alters cardiac lipid metabolism and induces myocardial hypertrophy, Circ. Res. 103 (9) (2008) 1018–1026.
[43] T. Zhao, W. Zhao, Y. Chen, L. Liu, R.A. Ahokas, Y. Sun, Differential expression of vascular endothelial growth factor isoforms and receptor subtypes in the infarcted heart, Int. J. Cardiol. 167 (6) (2013) 2638–2645.
[44] Y. Wang, A. Gabrielsen, P.R. Lawler, et al., Myocardial gene expression of angiogenic factors in human chronic ischemic myocardium: influence of acute ischemia/cardioplegia and reperfusion, Microcirculation 13 (3) (2006) 187–197.
[45] R.C. Pimentel, K.A. Yamada, A.G. Kleber, J.E. Saffitz, Autocrine regulation of myocyte Cx43 expression by VEGF, Circ. Res. 90 (6) (2002) 671–677.
[46] R.K. Iyer, D. Odedra, L.L. Chiu, G. Vunjak-Novakovic, M. Radisic, Vascular endothelial growth factor secretion by nonmyocytes modulates connexin-43 levels in

cardiac organoids, Tissue Eng. Part A 18 (17-18) (2012) 1771–1783.
[47] N. Naumenko, J. Huusko, T. Tuomainen, et al., Vascular endothelial growth factor-B induces a distinct electrophysiological phenotype in mouse heart, Front. Physiol. 8 (2017) 373.
[48] W. Xing, C. Gao, D. Qi, et al., Enhanced effect of VEGF165 on L-type calcium currents in guinea-pig cardiac ventricular myocytes, Biomed. Pharmacother. 85 (2017) 697–703.
[49] M.I. Zafar, J. Zheng, W. Kong, et al., The role of vascular endothelial growth factor-B in metabolic homoeostasis: current evidence, Biosci. Rep. 37 (4) (2017), https://doi.org/10.1042/BSR20171089 (Print 2017 Aug 31).
[50] R. Kivela, M. Bry, M.R. Robciuc, et al., VEGF-B-induced vascular growth leads to metabolic reprogramming and ischemia resistance in the heart, EMBO Mol. Med. 6 (3) (2014) 307–321.
[51] C.E. Hagberg, A. Falkevall, X. Wang, et al., Vascular endothelial growth factor B controls endothelial fatty acid uptake, Nature 464 (7290) (2010) 917–921.
[52] S.D. Prabhu, N.G. Frangogiannis, The biological basis for cardiac repair after myocardial infarction: from inflammation to fibrosis, Circ. Res. 119 (1) (2016) 91–112.
[53] T. Zhao, W. Zhao, W. Meng, et al., Vascular endothelial growth factor-D mediates fibrogenic response in myofibroblasts, Mol. Cell. Biochem. 413 (1-2) (2016) 127–135.
[54] V. Chintalgattu, D.M. Nair, L.C. Katwa, Cardiac myofibroblasts: a novel source of vascular endothelial growth factor (VEGF) and its receptors flt-1 and KDR, J. Mol. Cell. Cardiol. 35 (3) (2003) 277–286.
[55] T. Zhao, W. Zhao, W. Meng, C. Liu, Y. Chen, Y. Sun, Vascular endothelial growth factor-C: its unrevealed role in fibrogenesis, Am. J. Physiol. Heart Circ. Physiol. 306 (6) (2014) H789–H796.
[56] T.T. Rissanen, J.E. Markkanen, M. Gruchala, et al., VEGF-D is the strongest angiogenic and lymphangiogenic effector among VEGFs delivered into skeletal muscle via adenoviruses, Circ. Res. 92 (10) (2003) 1098–1106.
[57] H. Yamakawa, N. Muraoka, K. Miyamoto, et al., Fibroblast growth factors and vascular endothelial growth factor promote cardiac reprogramming under defined conditions, Stem Cell Rep. 5 (6) (2015) 1128–1142.
[58] L. Qian, Y. Huang, C.I. Spencer, et al., In vivo reprogramming of murine cardiac fibroblasts into induced cardiomyocytes, Nature 485 (7400) (2012) 593–598.
[59] K.M. Herum, J. Choppe, A. Kumar, A.J. Engler, A.D. McCulloch, Mechanical regulation of cardiac fibroblast profibrotic phenotypes, Mol. Biol. Cell 28 (14) (2017) 1871–1882.
[60] S. Taghavi, J.C. George, Homing of stem cells to ischemic myocardium, Am. J. Transl. Res. 5 (4) (2013) 404–411.
[61] U. Eriksson, K. Alitalo, VEGF receptor 1 stimulates stem-cell recruitment and new hope for angiogenesis therapies, Nat. Med. 8 (8) (2002) 775–777.
[62] Y. Xie, Y. Fan, Q. Xu, Vascular regeneration by stem/progenitor cells, Arterioscler. Thromb. Vasc. Biol. 36 (5) (2016) e33–e40.
[63] W.H. Zimmermann, T. Eschenhagen, Questioning the relevance of circulating cardiac progenitor cells in cardiac regeneration, Cardiovasc. Res. 68 (3) (2005) 344–346.
[64] T. Kinnaird, E. Stabile, M.S. Burnett, S.E. Epstein, Bone-marrow-derived cells for enhancing collateral development: mechanisms, animal data, and initial clinical experiences, Circ. Res. 95 (4) (2004) 354–363.
[65] Y. Chen, I. Amende, T.G. Hampton, et al., Vascular endothelial growth factor promotes cardiomyocyte differentiation of embryonic stem cells, Am. J. Physiol. Heart Circ. Physiol. 291 (4) (2006) H1653–H1658.
[66] A.A. Blancas, N.E. Lauer, K.E. McCloskey, Endothelial differentiation of embryonic stem cells, Curr. Protoc. Stem Cell Biol. (2008) (Chapter 1:Unit 1F.5.
[67] A. Wick, W. Wick, J. Waltenberger, M. Weller, J. Dichgans, J.B. Schulz, Neuroprotection by hypoxic preconditioning requires sequential activation of vascular endothelial growth factor receptor and akt, J. Neurosci. 22 (15) (2002) 6401–6407.
[68] V.H. Guaiquil, Z. Pan, N. Karagianni, S. Fukuoka, G. Alegre, M.I. Rosenblatt, VEGF-B selectively regenerates injured peripheral neurons and restores sensory and trophic functions, Proc. Natl. Acad. Sci. U. S. A. 111 (48) (2014) 17272–17277.
[69] P.S. Chen, L.S. Chen, J.M. Cao, B. Sharifi, H.S. Karagueuzian, M.C. Fishbein, Sympathetic nerve sprouting, electrical remodeling and the mechanisms of sudden cardiac death, Cardiovasc. Res. 50 (2) (2001) 409–416.
[70] K.C. Wheeler, M.K. Jena, B.S. Pradhan, et al., VEGF may contribute to macrophage recruitment and M2 polarization in the decidua, PLoS One 13 (1) (2018) e0191040.
[71] D.S. Charnock-Jones, A.M. Sharkey, C.A. Boocock, et al., Vascular endothelial growth factor receptor localization and activation in human trophoblast and choriocarcinoma cells, Biol. Reprod. 51 (3) (1994) 524–530.
[72] J.C. Cooper, A.M. Sharkey, J. McLaren, D.S. Charnock-Jones, S.K. Smith, Localization of vascular endothelial growth factor and its receptor, flt, in human placenta and decidua by immunohistochemistry, J. Reprod. Fertil. 105 (2) (1995) 205–213.
[73] K. Kumazaki, M. Nakayama, N. Suehara, Y. Wada, Expression of vascular endothelial growth factor, placental growth factor, and their receptors flt-1 and KDR in human placenta under pathologic conditions, Hum. Pathol. 33 (11) (2002) 1069–1077.
[74] S. De Falco, The discovery of placenta growth factor and its biological activity, Exp. Mol. Med. 44 (1) (2012) 1–9.
[75] J.A. Copel, M.O. Bahtiyar, A practical approach to fetal growth restriction, Obstet. Gynecol. 123 (5) (2014) 1057–1069.
[76] D. Sharma, S. Shastri, P. Sharma, Intrauterine growth restriction: antenatal and postnatal aspects, Clin. Med. Insights Pediatr. 10 (2016) 67–83.
[77] H. Suzuki, C. Hirashima, S. Nagayama, et al., Increased serum levels of sFlt-1/PlGF ratio in preeclamptic women with onset at /=32weeks, Pregnancy Hypertens 12 (2018) 96–103.
[78] A.L. David, Maternal uterine artery VEGF gene therapy for treatment of intrauterine growth restriction, Placenta 59 (Suppl. 1) (2017) S44–S50.
[79] V. Mehta, K.N. Abi-Nader, P. Shangaris, et al., Local over-expression of VEGF-DDeltaNDeltaC in the uterine arteries of pregnant sheep results in long-term changes in uterine artery contractility and angiogenesis, PLoS One 9 (6) (2014) e100021.
[80] A.M. Swanson, C.A. Rossi, K. Ofir, et al., Maternal therapy with ad.VEGF-A165 increases fetal weight at term in a guinea-pig model of fetal growth restriction, Hum. Gene Ther. 27 (12) (2016) 997–1007.
[81] M. Sheppard, R.N. Spencer, R. Ashcroft, A.L. David, EVERREST Consortium, Ethics and social acceptability of a proposed clinical trial using maternal gene therapy to treat severe early-onset fetal growth restriction, Ultrasound Obstet. Gynecol. 47 (4) (2016) 484–491.
[82] M. Desforges, A. Rogue, N. Pearson, et al., In vitro human placental studies to support adenovirus-mediated VEGF-D(DeltaNDeltaC) maternal gene therapy for the treatment of severe early-onset fetal growth restriction, Hum. Gene Ther. Clin. Dev. 29 (1) (2018) 10–23.
[83] C. Leaute-Labreze, J.I. Harper, P.H. Hoeger, Infantile haemangioma, Lancet 390 (10089) (2017) 85–94.
[84] E. Castren, P. Salminen, M. Vikkula, A. Pitkaranta, T. Klockars, Inheritance patterns of infantile hemangioma, Pediatrics 138 (5) (2016), https://doi.org/10.1542/peds.2016-1623 (Epub 2016 Oct 12).
[85] G.G. Behr, C. Johnson, Vascular anomalies: hemangiomas and beyond–part 1, fast-flow lesions, AJR Am. J. Roentgenol. 200 (2) (2013) 414–422.
[86] K.G. Chiller, D. Passaro, I.J. Frieden, Hemangiomas of infancy: clinical characteristics, morphologic subtypes, and their relationship to race, ethnicity, and sex, Arch. Dermatol. 138 (12) (2002) 1567–1576.
[87] A.N. Haggstrom, B.A. Drolet, E. Baselga, et al., Prospective study of infantile hemangiomas: demographic, prenatal, and perinatal characteristics, J. Pediatr. 150 (3) (2007) 291–294.
[88] J.B. Mulliken, P.E. Burrows, S.J. Fishman, Mulliken's and Young's Vascular Anomalies, (2013).
[89] R. Bragado, E. Bello, L. Requena, et al., Increased expression of vascular endothelial growth factor in pyogenic granulomas, Acta Derm. Venereol. 79 (6) (1999) 422–425.
[90] G. Dyduch, K. Okon, W. Mierzynski, Benign vascular proliferations–an immunohistochemical and comparative study, Pol. J. Pathol. 55 (2) (2004) 59–64.
[91] M. Sadeghpour, R.J. Antaya, R. Lazova, C.J. Ko, Dilated lymphatic vessels in tufted angioma: a potential source of diagnostic confusion, Am. J. Dermatopathol. 34 (4) (2012) 400–403.
[92] A. Picard, E. Boscolo, Z.A. Khan, et al., IGF-2 and FLT-1/VEGF-R1 mRNA levels reveal distinctions and similarities between congenital and common infantile hemangioma, Pediatr. Res. 63 (3) (2008) 263–267.
[93] M. Miettinen, M.S. Rikala, J. Rys, J. Lasota, Z.F. Wang, Vascular endothelial growth factor receptor 2 as a marker for malignant vascular tumors and mesothelioma: an immunohistochemical study of 262 vascular endothelial and 1640 nonvascular tumors, Am. J. Surg. Pathol. 36 (4) (2012) 629–639.
[94] J.F. Grimmer, M.S. Williams, R. Pimentel, et al., Familial clustering of hemangiomas, Arch. Otolaryngol. Head Neck Surg. 137 (8) (2011) 757–760.
[95] J.W. Walter, F. Blei, J.L. Anderson, S.J. Orlow, M.C. Speer, D.A. Marchuk, Genetic mapping of a novel familial form of infantile hemangioma, Am. J. Med. Genet. 82 (1) (1999) 77–83.
[96] J.W. Walter, P.E. North, M. Waner, et al., Somatic mutation of vascular endothelial growth factor receptors in juvenile hemangioma, Genes Chromosom. Cancer 33 (3) (2002) 295–303.
[97] K. Pramanik, C.Z. Chun, M.K. Garnaas, et al., Dusp-5 and snrk-1 coordinately function during vascular development and disease, Blood 113 (5) (2009) 1184–1191.
[98] M. Jinnin, D. Medici, L. Park, et al., Suppressed NFAT-dependent VEGFR1 expression and constitutive VEGFR2 signaling in infantile hemangioma, Nat. Med. 14 (11) (2008) 1236–1246.
[99] S. Sarabipour, K. Ballmer-Hofer, K. Hristova, VEGFR-2 conformational switch in response to ligand binding, elife 5 (2016) e13876.
[100] Y.H. Lim, A. Bacchiocchi, J. Qiu, et al., GNA14 somatic mutation causes congenital and sporadic vascular tumors by MAPK activation, Am. J. Hum. Genet. 99 (2) (2016) 443–450.
[101] L. Groesser, E. Peterhof, M. Evert, M. Landthaler, M. Berneburg, C. Hafner, BRAF and RAS mutations in sporadic and secondary pyogenic granuloma, J. Invest. Dermatol. 136 (2) (2016) 481–486.
[102] U.M. Ayturk, J.A. Couto, S. Hann, et al., Somatic activating mutations in GNAQ and GNA11 are associated with congenital hemangioma, Am. J. Hum. Genet. 98 (4) (2016) 789–795.
[103] M.E. Kleinman, M.R. Greives, S.S. Churgin, et al., Hypoxia-induced mediators of stem/progenitor cell trafficking are increased in children with hemangioma, Arterioscler. Thromb. Vasc. Biol. 27 (12) (2007) 2664–2670.
[104] K. Takahashi, J.B. Mulliken, H.P. Kozakewich, R.A. Rogers, J. Folkman, R.A. Ezekowitz, Cellular markers that distinguish the phases of hemangioma during infancy and childhood, J. Clin. Invest. 93 (6) (1994) 2357–2364.
[105] J. Chang, D. Most, S. Bresnick, et al., Proliferative hemangiomas: analysis of cytokine gene expression and angiogenesis, Plast. Reconstr. Surg. 103 (1) (1999) 1–9 (discussion 10).
[106] L. Zhang, X. Lin, W. Wang, et al., Circulating level of vascular endothelial growth factor in differentiating hemangioma from vascular malformation patients, Plast.

Reconstr. Surg. 116 (1) (2005) 200–204.
[107] P. Przewratil, A. Sitkiewicz, E. Andrzejewska, Local serum levels of vascular endothelial growth factor in infantile hemangioma: intriguing mechanism of endothelial growth, Cytokine 49 (2) (2010) 141–147.
[108] E. Boscolo, J.B. Mulliken, J. Bischoff, Pericytes from infantile hemangioma display proangiogenic properties and dysregulated angiopoietin-1, Arterioscler. Thromb. Vasc. Biol. 33 (3) (2013) 501–509.
[109] J. Boesiger, M. Tsai, M. Maurer, et al., Mast cells can secrete vascular permeability factor/ vascular endothelial cell growth factor and exhibit enhanced release after immunoglobulin E-dependent upregulation of fc epsilon receptor I expression, J. Exp. Med. 188 (6) (1998) 1135–1145.
[110] M. Lee, J.Y. Choi, J.S. Lim, M.S. Park, M.J. Kim, H. Kim, Lack of anti-tumor activity by anti-VEGF treatments in hepatic hemangiomas, Angiogenesis 19 (2) (2016) 147–153.
[111] C. Leaute-Labreze, P. Hoeger, J. Mazereeuw-Hautier, et al., A randomized, controlled trial of oral propranolol in infantile hemangioma, N. Engl. J. Med. 372 (8) (2015) 735–746.
[112] P.H. Hoeger, J.I. Harper, E. Baselga, et al., Treatment of infantile haemangiomas: recommendations of a european expert group, Eur. J. Pediatr. 174 (7) (2015) 855–865.
[113] E. Wedgeworth, M. Glover, A.D. Irvine, et al., Propranolol in the treatment of infantile haemangiomas: lessons from the european propranolol in the treatment of complicated haemangiomas (PITCH) taskforce survey, Br. J. Dermatol. 174 (3) (2016) 594–601.
[114] N.C. Munabi, R.W. England, A.K. Edwards, et al., Propranolol targets hemangioma stem cells via cAMP and mitogen-activated protein kinase regulation, Stem Cells Transl. Med. 5 (1) (2016) 45–55.
[115] H. Chim, B.S. Armijo, E. Miller, C. Gliniak, M.A. Serret, A.K. Gosain, Propranolol induces regression of hemangioma cells through HIF-1alpha-mediated inhibition of VEGF-A, Ann. Surg. 256 (1) (2012) 146–156.
[116] J.M. Fredriksson, J.M. Lindquist, G.E. Bronnikov, J. Nedergaard, Norepinephrine induces vascular endothelial growth factor gene expression in brown adipocytes through a beta -adrenoreceptor/cAMP/protein kinase A pathway involving src but independently of Erk1/2, J. Biol. Chem. 275 (18) (2000) 13802–13811.
[117] S. Greenberger, J. Bischoff, Pathogenesis of infantile haemangioma, Br. J. Dermatol. 169 (1) (2013) 12–19.
[118] W.L. Yuan, Z.L. Jin, J.J. Wei, Z.Y. Liu, L. Xue, X.K. Wang, Propranolol given orally for proliferating infantile haemangiomas: analysis of efficacy and serological changes in vascular endothelial growth factor and endothelial nitric oxide synthase in 35 patients, Br. J. Oral Maxillofac. Surg. 51 (7) (2013) 656–661.
[119] X.D. Chen, G. Ma, J.L. Huang, et al., Serum-level changes of vascular endothelial growth factor in children with infantile hemangioma after oral propranolol therapy, Pediatr. Dermatol. 30 (5) (2013) 549–553.
[120] P. Przewratil, J. Kobos, A. Wnek, et al., Serum and tissue profile of VEGF and its receptors VGFR1/R2 in children with infantile hemangiomas on systemic propranolol treatment, Immunol. Lett. 175 (2016) 44–49.
[121] C.B. Bayart, J.E. Tamburro, A.T. Vidimos, L. Wang, A.B. Golden, Atenolol versus propranolol for treatment of infantile hemangiomas during the proliferative phase: a retrospective noninferiority study, Pediatr. Dermatol. 34 (4) (2017) 413–421.
[122] G. Onnis, I. Dreyfus, J. Mazereeuw-Hautier, Factors associated with delayed referral for infantile hemangioma necessitating propranolol, J. Eur. Acad. Dermatol. Venereol. (2018).
[123] C. Mahon, G. Heron, D. Perkins, A. Drage, O. Wargon, Oral propranolol for infantile haemangioma may be associated with transient gross motor delay, Br. J. Dermatol. (2018).
[124] A.V. Moyakine, J.M. Kerstjens, S. Spillekom-van Koulil, C.J. van der Vleuten, Propranolol treatment of infantile hemangioma (IH) is not associated with developmental risk or growth impairment at age 4 years, J. Am. Acad. Dermatol. 75 (1) (2016) 59–63.e1.
[125] X. Guo, X. Zhu, D. Liu, Y. Gong, J. Sun, C. Dong, Continuous delivery of propranolol from liposomes-in-microspheres significantly inhibits infantile hemangioma growth, Int. J. Nanomedicine 12 (2017) 6923–6936.
[126] X. Guo, X. Zhu, J. Gao, D. Liu, C. Dong, X. Jin, PLGA nanoparticles with CD133 aptamers for targeted delivery and sustained release of propranolol to hemangioma, Nanomedicine (London) 12 (21) (2017) 2611–2624.
[127] H.M. Marey, H.F. Elmazar, S.S. Mandour, H.A. Khairy, Combined oral and topical beta blockers for the treatment of early proliferative superficial periocular infantile capillary hemangioma, J. Pediatr. Ophthalmol. Strabismus 55 (1) (2018) 37–42.
[128] H.W. Wu, C. Liu, X. Wang, et al., Topical application of 0.5% timolol maleate hydrogel for the treatment of superficial infantile hemangioma, Front. Oncol. 7 (2017) 137.
[129] M.P. Kohout, M. Hansen, J.J. Pribaz, J.B. Mulliken, Arteriovenous malformations of the head and neck: natural history and management, Plast. Reconstr. Surg. 102 (1998) 643–654.
[130] A.S. Liu, J.B. Mulliken, D. Zurakowski, S.J. Fishman, A.K. Greene, Extracranial arteriovenous malformations: natural progression and recurrence after treatment, Plast. Reconstr. Surg. 125 (2010) 1185–1194.
[131] A.K. Greene, D.B. Orbach, Management of arteriovenous malformations, in: A.K. Greene, C.A. Perlyn (Eds.), Vascular Anomalies, Elsevier Inc, Philadelphia, 2011, pp. 94–106.
[132] A. Laakso, J. Hernesniemi, Arteriovenous malformations: epidemiology and clinical presentation, Neurosurg. Clin. N. Am. 23 (2012) 1–6.
[133] M.T. Lawton, W.C. Rutledge, H. Kim, et al., Brain arteriovenous malformations, Nat. Rev. Dis. Primers. 1 (2015) 15008.
[134] K.A. McAllister, K.M. Grogg, D.W. Johnson, et al., Endoglin, a TGF-beta binding protein of endothelial cells, is the gene for hereditary haemorrhagic telangiectasia type 1, Nat. Genet. 8 (1994) 345–351.
[135] D.W. Johnson, J.N. Berg, M.A. Baldwin, et al., Mutations in the activin receptor-like kinase 1 gene in hereditary haemorrhagic telangiectasia type 2, Nat. Genet. 13 (1996) 189–195.
[136] C.J. Gallione, J.A. Richards, T.G.W. Letteboer, et al., SMAD4 mutations found in unselected HHT patients, J. Med. Genet. 43 (2006) 793–797.
[137] N. Revencu, L.M. Boon, A. Mendola, et al., RASA1 mutations and associated phenotypes in 68 families with capillary malformation-arteriovenous malformation, Hum. Mutat. 34 (2013) 1632–1641.
[138] W.L. Wooderchak-Donahue, J. McDonald, B. O'Fallon, et al., BMP9 mutations cause a vascular-anomaly syndrome with phenotypic overlap with hereditary hemorrhagic telangiectasia, Am. J. Hum. Genet. 93 (2013) 530–537.
[139] M. Amyere, N. Revencu, R. Helaers, et al., Germline loss-of-function mutations in EPHB4 cause a second form of capillary malformation-arteriovenous malformation (CM-AVM2) deregulating RAS-MAPK signaling, Circulation 136 (2017) 1037–1048.
[140] D. Kashiwazaki, R. Kobayashi, K. Houkin, S. Kuroda, Increased expression of vascular endothelial growth factor and its receptor in enlarging brain arteriovenous malformations–a case report, Br. J. Neurosurg. 28 (2014) 119–121.
[141] H. Kim, H. Su, S. Weinsheimer, L. Pawlikowska, W.L. Young, Brain arteriovenous malformation pathogenesis: a response-to-injury paradigm, Acta Neurochir. Suppl. 111 (2011) 83–92.
[142] H. Sadick, F. Riedel, R. Naim, et al., Patients with hereditary hemorrhagic telangiectasia have increased plasma levels of vascular endothelial growth factor and transforming growth factor-beta1 as well as high ALK1 tissue expression, Haematologia 90 (2005) 818–828.
[143] R. Ola, A. Dubrac, J. Han, et al., PI3 kinase inhibition improves vascular malformations in mouse models of hereditary haemorrhagic telangiectasia, Nat.Commun. 7 (2016) 13650.
[144] Y. Jin, L. Muhl, M. Burmakin, et al., Endoglin prevents vascular malformation by regulating flow-induced cell migration and specification through VEGFR2 signalling, Nat. Cell Biol. 19 (2017) 639–652.
[145] S.I. Cunha, P.U. Magnusson, E. Dejana, M.G. Lampugnani, Deregulated TGF-β/BMP signaling in vascular malformations, Circ. Res. 121 (2017) 981–999.
[146] S.I. Nikolaev, S. Vetiska, X. Bonilla, et al., Somatic activating KRAS mutations in arteriovenous malformations of the brain, N. Engl. J. Med. 378 (2018) 250–261.
[147] J.A. Couto, A.Y. Huang, D.J. Konczyk, et al., Somatic MAP2K1 mutations are associated with extracranial arteriovenous malformation, Am. J. Hum. Genet. 100 (3) (2017) 546–554.
[148] C. Ye, L. Pan, Y. Huang, et al., Somatic mutations in exon 17 of the TEK gene in vascular tumors and vascular malformations, J. Vasc. Surg. 54 (2011) 1760–1768.
[149] D. Rothbart, I.A. Awad, J. Lee, J. Kim, R. Harbaugh, G.R. Criscuolo, Expression of angiogenic factors and structural proteins in central nervous system vascular malformations, Neurosurgery 38 (1996) 915–924.
[150] E. Hatva, J. Jääskeläinen, H. Hirvonen, K. Alitalo, M. Haltia, Tie endothelial cell-specific receptor tyrosine kinase is upregulated in the vasculature of arteriovenous malformations, J. Neuropathol. Exp. Neurol. 55 (1996) 1124–1133.
[151] T. Kiliç, M.N. Pamir, S. Küllü, F. Eren, M.M. Ozek, P.M. Black, Expression of structural proteins and angiogenic factors in cerebrovascular anomalies, Neurosurgery 46 (2000) 1179–1191.
[152] T. Koizumi, T. Shiraishi, N. Hagihara, K. Tabuchi, T. Hayashi, T. Kawano, Expression of vascular endothelial growth factors and their receptors in and around intracranial arteriovenous malformations, Neurosurgery 50 (2002) 117–124.
[153] Y. Nakamura, Y. Sugita, S. Nakashima, et al., Alternatively activated macrophages play an important role in vascular remodeling and hemorrhaging in patients with brain arteriovenous malformation, J. Stroke Cerebrovasc. Dis. 25 (2016) 600–609.
[154] M.N. Jabbour, J.B. Elder, C.G. Samuelson, et al., Aberrant angiogenic characteristics of human brain arteriovenous malformation endothelial cells, Neurosurgery 64 (2009) 139–146.
[155] M. Xu, H. Xu, Z. Qin, J. Zhang, X. Yang, F. Xu, Increased expression of angiogenic factors in cultured human brain arteriovenous malformation endothelial cells, Cell Biochem. Biophys. 70 (2014) 443–447.
[156] H. Chen, Y. Gu, W. Wu, et al., Polymorphisms of the vascular endothelial growth factor A gene and susceptibility to sporadic brain arteriovenous malformation in a chinese population, J. Clin. Neurosci. 18 (4) (2011) 549–553.
[157] N. Ferrara, Vascular endothelial growth factor: basic science and clinical progress, Endocr. Rev. 25 (4) (2004) 581–611.
[158] U. Sure, N. Butz, A.M. Siegel, H.D. Mennel, S. Bien, H. Bertalanffy, Treatment-induced neoangiogenesis in cerebral arteriovenous malformations, Clin. Neurol. Neursurg. 103 (2001) 29–32.
[159] T. Kojima, S. Miyachi, Y. Sahara, et al., The relationship between venous hypertension and expression of vascular endothelial growth factor: hemodynamic and immunohistochemical examinations in a rat venous hypertension model, Surg. Neurol. 68 (2007) 277–284.
[160] E. Sho, M. Komatsu, M. Sho, et al., High flow drives vascular endothelial cell proliferation during flow-induced arterial remodeling associated with the expression of vascular endothelial growth factor, Exp. Mol. Pathol. 75 (2003) 1–11.
[161] A.B. Persson, I.R. Buschmann, Vascular growth in health and disease, Front. Mol. Neurosci. 4 (2011) 14.
[162] T.J. Buell, D. Ding, R.M. Starke, R. Webster Crowley, K.C. Liu, Embolization-induced angiogenesis in cerebral arteriovenous malformations, J. Clin. Neurosci. 21 (11) (2014) 1866–1871.
[163] M. Hellström, L.K. Phng, J.J. Hofmann, et al., Dll4 signalling through Notch1 regulates formation of tip cells during angiogenesis, Nature 445 (2007) 776–780.

[164] F. Lanner, K.L. Lee, G.C. Ortega, et al., Hypoxia-induced arterial differentiation requires adrenomedullin and notch signaling, Stem Cells Dev. 22 (2013) 1360–1369.
[165] B. Ubezio, R.A. Blanco, I. Geudens, et al., Synchronization of endothelial Dll4-notch dynamics switch blood vessels from branching to expansion, elife 5 (2016) e12167.
[166] P.A. Murphy, T.N. Kim, L. Huang, et al., Constitutively active Notch4 receptor elicits brain arteriovenous malformations through enlargement of capillary-like vessels, Proc. Natl. Acad. Sci. U. S. A. 111 (2014) 18007–18012.
[167] P.A. Murphy, M.T. Lam, X. Wu, et al., Endothelial Notch4 signaling induces hallmarks of brain arteriovenous malformations in mice, Proc. Natl. Acad. Sci. U. S. A. 105 (2008) 10901–10906.
[168] T.R. Carlson, Y. Yan, X. Wu, et al., Endothelial expression of constitutively active Notch4 elicits reversible arteriovenous malformations in adult mice, Proc. Natl. Acad. Sci. U. S. A. 102 (28) (2005) 9884–9889.
[169] Q. ZhuGe, M. Zhong, W. Zheng, et al., Notch-1 signalling is activated in brain arteriovenous malformations in humans, Brain 132 (2009) 3231–3241.
[170] S. Li, R. Wang, Y. Wang, et al., Receptors of the Notch signaling pathway are associated with hemorrhage of brain arteriovenous malformations, Mol. Med. Rep. 9 (2014) 2233–2238.
[171] D. Delev, A. Pavlova, A. Grote, et al., NOTCH4 gene polymorphisms as potential risk factors for brain arteriovenous malformation development and hemorrhagic presentation, J. Neurosurg. 126 (5) (2017) 1552–1559.
[172] M. Fontanella, E. Rubino, E. Crobeddu, et al., Brain arteriovenous malformations are associated with interleukin-1 cluster gene polymorphisms, Neurosurgery 70 (1) (2012) 12–17.
[173] H. Kim, P.G. Hysi, L. Pawlikowska, et al., Common variants in interleukin-1-beta gene are associated with intracranial hemorrhage and susceptibility to brain arteriovenous malformation, Cerebrovasc. Dis. 27 (2009) 176–182.
[174] Y. Zhao, P. Li, W. Fan, et al., The rs522616 polymorphism in the matrix metalloproteinase-3 (MMP-3) gene is associated with sporadic brain arteriovenous malformation in a Chinese population, J. Clin. Neurosci. 17 (2010) 1568–1572.
[175] H. Kim, P.G. Hysi, L. Pawlikowska, et al., Population stratification in a case-control study of brain arteriovenous malformation in latinos, Neuroepidemiology 31 (2008) 224–228.
[176] B. Kim, K. Kim, P. Jeon, et al., Long-term results of ethanol sclerotherapy with or without adjunctive surgery for head and neck arteriovenous malformations, Neuroradiology 57 (2015) 377–386.
[177] J. Pekkola, K. Lappalainen, P. Vuola, T. Klockars, P. Salminen, A. Pitkäranta, Head and neck arteriovenous malformations: results of ethanol sclerotherapy, AJNR Am. J. Neuroradiol. 34 (2013) 198–204.
[178] P. Stokes, J. Rimmer, Intranasal bevacizumab in the treatment of HHT – related epistaxis: a systematic review, Rhinology (2017).
[179] A.A. Halderman, M.W. Ryan, C. Clark, et al., Medical treatment of epistaxis in hereditary hemorrhagic telangiectasia: an evidence-based review, Int. Forum Allergy Rhinol. (2018).
[180] A. Guilhem, A.E. Fargeton, A.C. Simon, et al., Intra-venous bevacizumab in hereditary hemorrhagic telangiectasia (HHT): a retrospective study of 46 patients, PLoS One 12 (2017) e0188943.
[181] S. Dupuis-Girod, I. Ginon, J.C. Saurin, et al., Bevacizumab in patients with hereditary hemorrhagic telangiectasia and severe hepatic vascular malformations and high cardiac output, JAMA 307 (9) (2012) 948–955.
[182] C.J. Caunt, M.J. Sale, P.D. Smith, S.J. Cook, MEK1 and MEK2 inhibitors and cancer therapy: the long and winding road, Nat. Rev. Cancer 15 (2015) 577–592.
[183] F. Janku, Phosphoinositide 3-kinase (PI3K) pathway inhibitors in solid tumors: from laboratory to patients, Cancer Treat. Rev. 59 (2017) 93–101.
[184] P.A. Murphy, T.N. Kim, G. Lu, A.W. Bollen, C.B. Schaffer, R.A. Wang, Notch4 normalization reduces blood vessel size in arteriovenous malformations, Sci. Transl. Med. 4 (2012) 117ra8.
[185] Y. Yao, J. Yao, M. Radparvar, et al., Reducing jagged 1 and 2 levels prevents cerebral arteriovenous malformations in matrix gla protein, Proc. Natl. Acad. Sci. U. S. A. 110 (2013) 19071–19076.
[186] C. Ye, L. Pan, Y. Huang, et al., Somatic mutations in exon 17 of the TEK gene in vascular tumors and vascular malformations, J. Vasc. Surg. 54 (6) (2011) 1760–1768.
[187] Y.H. Lim, S.R. Douglas, C.J. Ko, et al., Somatic activating RAS mutations cause vascular tumors including pyogenic granuloma, J. Invest. Dermatol. 135 (6) (2015) 1698–1700.
[188] C. Errani, L. Zhang, Y.S. Sung, et al., A novel WWTR1-CAMTA1 gene fusion is a consistent abnormality in epithelioid hemangioendothelioma of different anatomic sites, Genes Chromosom. Cancer 50 (8) (2011) 644–653.
[189] K.C. Kurek, T.C. Pansuriya, M.A. van Ruler, et al., R132C IDH1 mutations are found in spindle cell hemangiomas and not in other vascular tumors or malformations, Am. J. Pathol. 182 (5) (2013) 1494–1500.
[190] J.A. Couto, A.Y. Huang, D.J. Konczyk, et al., Somatic MAP2K1 mutations are associated with extracranial arteriovenous malformation, Am. J. Hum. Genet. 100 (3) (2017) 546–554.
[191] T. Handa, Y. Okano, N. Nakanishi, T. Morisaki, H. Morisaki, M. Mishima, BMPR2 gene mutation in pulmonary arteriovenous malformation and pulmonary hypertension: a case report, Respir. Investig. 52 (3) (2014) 195–198.
[192] S.I. Nikolaev, S. Vetiska, X. Bonilla, et al., Somatic activating KRAS mutations in arteriovenous malformations of the brain, N. Engl. J. Med. 378 (3) (2018) 250–261.
[193] H. Chen, Y. Gu, W. Wu, et al., Polymorphisms of the vascular endothelial growth factor A gene and susceptibility to sporadic brain arteriovenous malformation in a Chinese population, J. Clin. Neurosci. 18 (4) (2011) 549–553.
[194] S.A. Erkinova, E.A. Sokolova, K.Y. Orlov, et al., Angiopoietin-like proteins 4 (ANGPTL4) gene polymorphisms and risk of brain arteriovenous malformation, J. Stroke Cerebrovasc. Dis. 27 (4) (2018) 908–913.
[195] L. Pawlikowska, M.N. Tran, A.S. Achrol, et al., Polymorphisms in transforming growth factor-beta-related genes ALK1 and ENG are associated with sporadic brain arteriovenous malformations, Stroke 36 (10) (2005) 2278–2280.
[196] M. Simon, D. Franke, M. Ludwig, et al., Association of a polymorphism of the ACVRL1 gene with sporadic arteriovenous malformations of the central nervous system, J. Neurosurg. 104 (6) (2006) 945–949.
[197] N. Jiang, X. Li, T. Qi, S. Guo, F. Liang, Z. Huang, Susceptible gene single nucleotide polymorphism and hemorrhage risk in patients with brain arteriovenous malformation, J. Clin. Neurosci. 18 (9) (2011) 1279–1281.
[198] H. Kim, P.G. Hysi, L. Pawlikowska, et al., Population stratification in a case-control study of brain arteriovenous malformation in latinos, Neuroepidemiology 31 (4) (2008) 224–228.
[199] H. Kim, P.G. Hysi, L. Pawlikowska, et al., Common variants in interleukin-1-beta gene are associated with intracranial hemorrhage and susceptibility to brain arteriovenous malformation, Cerebrovasc. Dis. 27 (2) (2009) 176–182.
[200] M. Fontanella, E. Rubino, E. Crobeddu, et al., Brain arteriovenous malformations are associated with interleukin-1 cluster gene polymorphisms, Neurosurgery 70 (1) (2012) 12–17.
[201] Y. Zhao, P. Li, W. Fan, et al., The rs522616 polymorphism in the matrix metalloproteinase-3 (MMP-3) gene is associated with sporadic brain arteriovenous malformation in a Chinese population, J. Clin. Neurosci. 17 (12) (2010) 1568–1572.
[202] I. Eerola, L.M. Boon, J.B. Mulliken, et al., Capillary malformation-arteriovenous malformation, a new clinical and genetic disorder caused by RASA1 mutations, Am. J. Hum. Genet. 73 (6) (2003) 1240–1249.
[203] N. Revencu, L.M. Boon, A. Mendola, et al., RASA1 mutations and associated phenotypes in 68 families with capillary malformation-arteriovenous malformation, Hum. Mutat. 34 (12) (2013) 1632–1641.
[204] M. Amyere, N. Revencu, R. Helaers, et al., Germline loss-of-function mutations in EPHB4 cause a second form of capillary malformation-arteriovenous malformation (CM-AVM2) deregulating RAS-MAPK signaling, Circulation 136 (11) (2017) 1037–1048.
[205] S.I. Cunha, P.U. Magnusson, E. Dejana, M.G. Lampugnani, Deregulated TGF-beta/BMP signaling in vascular malformations, Circ. Res. 121 (8) (2017) 981–999.
[206] L. Ruiz-Llorente, E. Gallardo-Vara, E. Rossi, D.M. Smadja, L.M. Botella, C. Bernabeu, Endoglin and alk1 as therapeutic targets for hereditary hemorrhagic telangiectasia, Expert Opin. Ther. Targets 21 (10) (2017) 933–947.
[207] C.J. Gallione, J.A. Richards, T.G. Letteboer, et al., SMAD4 mutations found in unselected HHT patients, J. Med. Genet. 43 (10) (2006) 793–797.
[208] T. Nishida, M.E. Faughnan, T. Krings, et al., Brain arteriovenous malformations associated with hereditary hemorrhagic telangiectasia: gene-phenotype correlations, Am. J. Med. Genet. A 158A (11) (2012) 2829–2834.
[209] W.L. Wooderchak-Donahue, J. McDonald, B. O'Fallon, et al., BMP9 mutations cause a vascular-anomaly syndrome with phenotypic overlap with hereditary hemorrhagic telangiectasia, Am. J. Hum. Genet. 93 (3) (2013) 530–537.
[210] C.M. Rigelsky, C. Jennings, R. Lehtonen, O.A. Minai, C. Eng, M.A. Aldred, BMPR2 mutation in a patient with pulmonary arterial hypertension and suspected hereditary hemorrhagic telangiectasia, Am. J. Med. Genet. A 146A (19) (2008) 2551–2556.