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REVIEW

Vascular endothelial growth factor-B: Impact on physiology and pathology

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ABSTRACT

Angiogenesis plays an important role in controlling tissue development and maintaining normal tissue function. Dysregulated angiogenesis is implicated in the pathogenesis of a variety of diseases, particularly diabetes, cancers, and neurodegenerative disorders. As the major regulator of angiogenesis, the vascular endothelial growth factor (VEGF) family is composed of a group of crucial members including VEGF-B. While the physiological roles of VEGF-B remain debatable, increasing evidence suggests that this protein is able to protect certain type of cells from apoptosis under pathological conditions. More importantly, recent studies reveal that VEGF-B is involved in lipid transport and energy metabolism, implicating this protein in obesity, diabetes and related metabolic complications. This article summarizes the current knowledge and understanding of VEGF-B in physiology and pathology, and shed light on the therapeutic potential of this crucial protein.

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Introduction

Angiogenesis is highly coordinated by a series of orchestrated events, and the interactions of VEGF family to its receptor have been well characterized. The VEGF family is major regulators of blood and lymphatic vessel development and growth,[1] comprising VEGF-A, -B, -C, -D, placenta growth factor (PlGF). In some literatures the VEGF-E (an Orf-virus-encoded protein) and VEGF-F (a variant isolated from snake venom)[2] were also included in this family. These grow factors are secreted as ~40-kDa dimeric glycoproteins and function in a paracrine fashion by signaling to three corresponding structurally homologous receptor tyrosine kinases expressed on endothelium cells (ECs) – vascular endothelial growth factor receptor (VEGFR)-1, −2 −3.[3] VEGF-A, VEGF-B and PlGF also bind to the neuropilin (NRP)-1 and −2.[4] Most biological angiogenic event occur through the VEGFR-2, whereas VEGFR-1 acts by synergistically augmenting VEGFR-2 signaling.[2] Their binding patterns are partially overlapping, and the feasible cross-talks may amplify the diversification of intracellular and intercellular interchange of communication. For example, the PlGF strengthened the activity of VEGF-A by displacing VEGF-A from VEGFR-1, facilitating its availability to the VEGFR-2.[5] Conversely, the transduced VEGF-B in RIP1-Tag2 islets possibly replaced VEGF-A and PlGF from VEGFR-1, and then diminishing pro-angiogenic effect.[6] In addition, the VEGFs are also engaged in the conversion from white adipose tissue (WAT) to brown adipose tissue (BAT),[7] leading to increased energy expenditure, and resulting in protection from diet induced obesity. The coexistence of angiogenic and browning effect may coordinate the organism to obtain a better adaptation to the external.

Discovered in 1996,[8] VEGF-B has approximate 47% and 37% amino acids sequence identical with VEGF-A and PlGF.[9] Owing to alternative splicing event, the VEGF-B gene generates two isoforms: VEGF-B_{167} and VEGF-B_{186}, 42/60 KDa homodimers, respectively.[9] Their N-terminal contains the receptor binding domain,[10] homologous with the regions in VEGF-A and PlGF, therefore sharing the common receptors. The diversity in their C-terminal properties affects their distribution in the body. VEGF-B_{167} has a heparin-binding domain, thus upon exudation it binds to cell surface heparin sulfate proteoglycans to anchor this isoform in extracellular matrix.[8] Unlike VEGF-B_{167}, VEGF-B_{186} does not connect the heparin, hence more diffusible.[11] The ratio of

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VEGF-B_{167}/VEGF-B_{186} varies significantly among species. The VEGF-B_{167} is prevalent in mouse, while the human tumor cell lines favor the VEGF-B_{186}.[12] Physiologically, the VEGF-B covers manifold organs and tissues, with the highest in the heart, skeletal muscle and lower levels in other tissues in adult mice.[13,14]

The *in vivo* role of VEGF-B remained elusive for decades. Due to its homologous structures, VEGF-B was initially recognized as an angiogenic factor. Subsequent studies, however, argued against the angiogenic activity of this molecule.[15-18] Physiologically, VEGF-B has little growth effects, as demonstrated in gain-of-function studies using transgenic[15] and adenoviral[16] expression of VEGF-B models and loss-of-function studies using VEGF-B null mice.[17,18] Under pathological conditions, this molecule can prevent cells from apoptosis and death. It showed both survival effect in laser injury-induced choroidal neovascularization or ischemia-originated retinal neovascularization models,[19] cardiac ischemia mouse,[20] and neuron-protective effect for the brain cortical neurons and retinal neurons[21] and motor neurons in the spinal cord.[22] The two effects may be relatively complemented, since the neural and vascular systems are inseparable and share the common molecular mechanisms for migration.[23] To underlie the survival effects, besides the anti-apoptotic effect *via* repressing the expression of pro-apoptotic BH3-only proteins and other apoptosis- and cell death-related proteins, including p53 and caspase family members,[21] VEGF-B might potentially enhance energy metabolism by regulating fatty acid (FAs) transport.[9,13] Surprisingly, at the high levels, VEGF-B acted as a growth-inhibiting molecule to forestall overgrowth and tumor growth.[6,9]

Collectively, VEGF-B is more like a survival molecule rather than a growth factor.[24] Recently its participation in lipid transport and energy metabolism mediation was partially revealed, indicating its implication in lipids accumulation relevant metabolic diseases, e.g. the type 2 diabetes mellitus (T2DM). Here, we summarized recent advances on VEGF-B studies, with particular interest on its potential therapeutic application in diabetes therapy.

Diabetes, from lipid depots to targeting VEGF-B therapy

The prevalence of diabetes has been increasing during the past decades and, more importantly, diabetes is associated with a variety of severe complications, particularly cardiovascular events and renal dysfunction.[25] T2DM is characterized as insufficient insulin secretion from pancreatic β-cell, impaired insulin-stimulated glucose uptake into skeletal muscle and adipose tissue and defective insulin-dependent suppression of hepatic glucose output,[26] and these defects collectively result in hyperglycemia. Insulin resistance, a state that the metabolically active cells are less sensitive to the insulin dependent glucose handing, marks and exacerbates the T2DM. Insulin resistance stimulates β-cells to augment insulin production to lower blood glucose, and the compensate degree determines whether the individual develops diabetes or not.[27] Both genic mutations and environmental factors can lead to the T2DM, and the latter may contribute more, such as the obesity. Obesity, via deteriorating insulin resistance, places an depraved functional demand on the β-cell and accelerate β-cell failure.[25]

The classical remedy for T2DM is stepwise, starting from life style interventions such as caloric restriction via a very low-calorie diet (600 kcal/day)[28] or the exercise training;[29] afterwards the oral monotherapy, including incretion-based therapies such as glucagon-like peptide-1 and its stable analogs,[30] oral hypoglycemic agents (metformin and thiazolidinediones); further combination therapy, and ultimately insulin therapy.[31] In the following text, we first stated how the lipid dysregualtion contributes to insulin resistance development, then the targeted insulin resistance and VEGF-B for diabetes therapy.

Lipids metabolism, insulin resistance and T2DM

The relevance between lipid dysregulation and insulin resistance has been widely studied. Initially the Randle hypothesis explained that accumulative fatty acids (FAs) impaired pyruvate dehydrogenase and glycolysis.[32] Nevertheless, the diacylglycerol hypothesis[33] explicated the impaired insulin action and glucose disposal in chronic obesity states. Studies demonstrated the diacylglycerol and ceramides as pathogenic factors for insulin resistance.[34] The accumulation of them deteriorate insulin resistance by activating protein kinase C (PKC) family members[35] and impairing the Akt2 action,[36] separately. Mechanistically, PKC-θ phosphorylates insulin-receptor substrate (IRS-1) on ser 1101 to block IRS-1 tyrosine phosphorylation;[35] and ceramides impedes Akt2 activation via protein phosphatase 2A dephosphorylating,[36] further disrupting insulin signaling. Importantly, it is the intramyocellular diacylglycerols,[37] not the circulating lipids, that interrupt the insulin signaling and are responsible for insulin resistance progression. Besides, ectopic deposition of lipids in metabolically active organs can induce pathological activation of inflammation[38,39] and endoplasmic reticulum stress,[38,40] the ability of which to regulate insulin action may be reliant on their ability to alter the levels of key signaling intermediates.[38] It is highly likely that dysregulations of these pathways collectively contribute to insulin resistance.

Indeed, recent studies suggest that it is the lipids accumulation in liver and muscle, but not in

subcutaneous or visceral adiposity, accounts for insulin resistance.[41] The lipids in the islets are also concerned. Free fatty acids (FFAs) promote insulin production in the short term[42] yet repressing by interfering the co-localization of calcium channels and the secretory granules[43,44] in the long term. Obesity and high fatty diet (HFD) duplicated the effects of long-term incubation of islets with FFAs on the Ca^{2+} channel distribution and insulin output.[45] To figure out, the damaged β-cell function, rather than the declined number, may be the nature for the impaired insulin exudation.[25] Fig. 1 generalized the pathological progression from ectopic lipids deposition to insulin resistance and T2DM development. To conclude, the ectopic lipids aggradation is vital, and normalization of lipid storage might be able to attenuate insulin resistance in metabolically active organs such as liver and muscle.

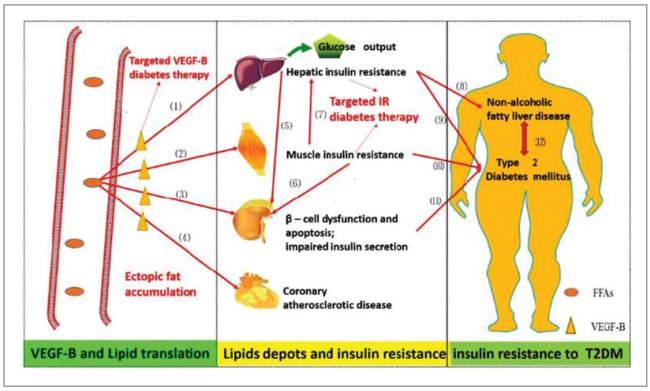

Figure 1. The progression from the ectopic lipids depots to the T2DM. Once the lipids overwhelm the capacity of adipose tissues, it shunts to the non-adipose tissues, leading to ectopic lipid deposition. (1), (2). The lipids deposition in the liver/muscle arouse the abnormal insulin behavior, resulting in muscle/hepatic insulin resistance. The insufficient insulin action gives rise to the glucose release from the liver and the lipids release from the adipose. Whereas, the glucose uptake is limited, relative to an increased lipids uptake by the tissue cells. (3). In the pancreatic islets, the lipid deposition would result in β-cell dysfunction and apoptosis,[6,46] and the weaken insulin production. (4). The lipids depots on the artery intima lead to the coronary atherosclerotic disease, and this can further develop into latter ischemic heart disease, eventually the heart failure (HF). (5), (6). The muscle/hepatic insulin resistance repress the glucose uptake. To let down the glucose level, the β-cell produces more insulin, which does not work for the already existing insulin resistance. The functional adaptation of the β-cell bring about a high rates of β-cell metabolism and risk of β-cell damage from mitochondrial and endoplasmic reticulum stress.[29] (7). Insulin resistance would impair storage of carbohydrate as glycogen in muscle, then carbohydrates are redirected to the liver and become substrates for hepatic de novo lipogenesis.[47] (8). The hepatic insulin resistance can deteriorate into the non-alcoholic fatty liver disease (NAFLD), even the more severe non-alcoholic steatohepatitis, or hepatocellular carcinoma (HCC). (9) – (11). These combinations together to cause the final T2DM. (12). The NAFLD and T2DM are regularly co-existing. The NAFLD imposes the risk for the diabetes and its complications, in turn, diabetes makes an individual more likely to have more severe NAFLD with the associative complications of cirrhosis and mortality.[48]

Treating diabetes by targeting insulin resistance

The peroxisome proliferator-activated receptors (PPARs) work as lipid sensors, modulating metabolic events by coordinately regulating the expression of genes linked to the energy homeostasis and insulin action, and therefore it can be regarded as a pharmacological target for management of metabolic disorders.[49] Thiazolidinediones (TZDs) can directly reduce peripheral systemic insulin resistance,[50] *via* the mighty activation of PPARγ, inducing the fat redirection from visceral to subcutaneous depots.[51] Given adipocytes own the highest PPARγ levels, these cells are the primary target for the glucose-lowering actions of TZDs.[52] Another insulin sensitizer-the apelin is also concerned with the magnified

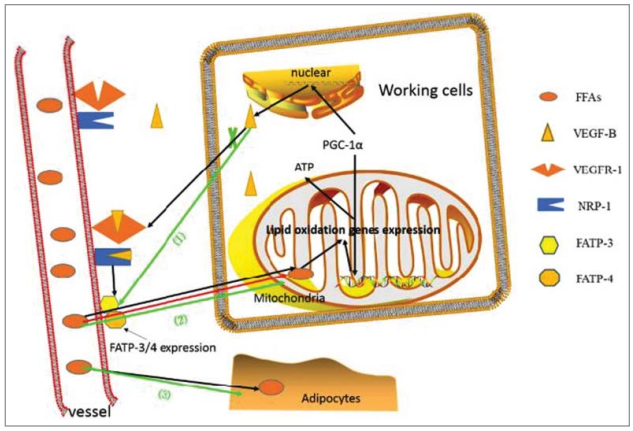

Figure 2. The involvement of the VEGF-B in the lipids translation, and the observed phenotype in the targeted VEGF-B treatment in various rodent animals. The black line stands for the pathways under physical conditions; the red for the pathological conditions; the green for the changes after the neutralizing VEGF-B strategies (the VEGF-$B^{-/-}$ model or VEGF-B antibody treatment). Under normal conditions, the PGC1-α regulates the coexpression of VEGF-B and the lipids oxidation associated genes, thus establishing a balance between the VEGF-B medicated lipids uptake and the energy demands of the metabolic cells, and the excessive lipids are stored in the adipocytes. When pathologic, the redundant lipids in the non-adipocyte tissues cause the abnormal insulin behavior, inducing the subsequent insulin resistance and the T2DM. Genetic or pharmacological inhibition of VEGF-B signaling leads to the (1). Decreased distribution of FATP3/4 on the ECs; (2). Less intracellular lipid droplets in working tissues; promoted insulin sensitivity and glucose uptake, ameliorated glucose tolerance; metabolic transformation from FAs to glucose oxidation; a lower risk for CVD; protected islet architecture and β-cell apoptosis; (3). Lipids redistribution to adipose tissues, leading to weight gain.

phosphorylation of Akt and glucose uptake in skeletal muscle.[53]

Hurdles remain since most TZDs exert the risk of cardiovascular morbidities, and rosiglitazone has been withdrawn from the market.[54] To solve this issue, the selective peroxisome proliferator-activated receptor estrogen receptor modulators[55] might provide a more tolerable therapy for T2DM, without the cardiomegaly adverse effect or fewer. Additionally, the dual agonists of PPAR-α/γ or even PPAR-$\alpha/\gamma/\delta$ pan agonists[56] showed promising results in the simultaneous treatment of diabetic hyperglycemia and dyslipidemia.

VEGF-B and lipids transportation

The VEGF-B is critical in coordinating ECs-mediated long-chain fatty acids (LCFA) uptake with the energy demand of the surrounding tissue via its co-expression with the mitochondrial gene cluster,[13] consisting primarily of genes coding for proteins within the oxidative phosphorylation machinery.[57] This may be under the transcriptional regulation of estrogen-related receptor α and co-regulator peroxisome proliferator-activated receptor gamma coactivator-1 alpha (PGC-1α).[58] VEGF-B released by the tissue cells promotes the distribution of fatty acids transport proteins (FATP)3 and FATP4 on the ECs, *via* its binding to VEGFR-1 and NRP-1, further facilitating the lipids transport into the tissue cells. The receptor knockout studies abolished the growing expression of FATP3/4[13] while the co-expression of two FATPs led to the highest uptake of LCFA, suggesting a synergistic effect. Both isoforms of VEGF-B promoted the expression of FATP3/4 in several lipid-metabolizing peripheral tissues at transcriptional and translational levels, with the soluble form-VEGF-B_{186} being more efficient.[13] VEGF-B_{167}, with a better tissue specificity, might be more likely to fulfill the tissue-specific demand of FA uptake to cooperate with the oxidative capacity of specific tissue.[59] In summary, the VEGF-B creates a metabolic cross-talk between the ECs and the tissue cells, hence guaranteeing the energy accommodation and simultaneously tackling intracellular lipids accumulation and lipotoxicity.[60] However, the passively lipids transportation in obesity states can progressed into the insulin resistance and the subsequent T2DM.

Targeted VEGF-B therapy for T2DM

Due to its important roles in mediating lipid transport and metabolism, VEGF-B has been proposed as a novel therapeutic molecule for T2DM remedy via existing methods including gene deletion, gene slicing and the neutralizing monoclonal antibodies (mAbs). Both the construction of genetically engineered VEGF-$B^{-/-}$ model[13,61] and VEGF-B neutralizing bodies treatment[13] ameliorated the diabetic phenotype, although the obesity phenotype remained. Many insights have been provided, despite in the animal models, providing the likelihood of developing the therapy tactics for the patients. In the VEGF-$B^{-/-}$ models,[13] the first phenotypic alteration observed is the declined levels of FATP3/4, resulting in the degressive lipids uptake, thereupon smaller and less abundant intracellular lipid droplets in heart, muscle, liver and BAT. Then, the accompanied promoted insulin sensitivity, glucose transporter-4 distribution on the membrane, glucose uptake; decreased plasma glucose and ameliorating glucose tolerance were observed, accordingly maintaining the euglycemia. These led to a compensatory amplification in carbohydrate utilized for energy production, indicating a metabolic transformation from FAs oxidation to glucose burning. Then, the unconsumed FAs were transferred to WAT, causing a growing body weight. Maybe the FAs uptake into the WATs is a process utilizing molecules other than VEGF-B?[59] Nonetheless, various VEGF-B deficient mouse strains exhibited inconsistent phenotypes in baseline conditions,[46] and the phenotypes described above are the comprehensive analysis of several studies.[13,61] For the impact of VEGF-B deletion in HFD mice and in diabetic db/db mice, a gene dosage effect in males was observed since the VEGF-$B^{+/-}$ mice showed slightly elevated blood glucose levels compared with VEGF-$B^{-/-}$ mice.[61] Also, the VEGF-$B^{-/-}$ mice showed lower plasma triglycerides (TGs) and LDL/VLDL-bound cholesterol ratio, and higher levels of HDL-bound cholesterol, suggesting a lower risk for cardiovascular diseases (CVD).[61] Moreover, therapeutic inhibition of VEGF-B preserved islet functionality and insulin production by protecting islet architecture and guarding against β-cell apoptosis, possibly via the blunted lipotoxicity.[61] Antibody-mediated pharmacological controlling of VEGF-B phenocopied most of outcomes aforementioned. The neutralizing VEGF-B antibody (2H10) blocked the VEGF-B to binding its receptors, bringing about a long-lasting neutralization effect.[2]

Despite these progresses, cautions should be taken when further translating to humans. Many T2DM patients also suffer from myocardial ischemia, coronary artery disease and diabetic neuropathy, thus would potentially benefit from VEGF-B coronary arteriogenic, neuron-protective and neurogenic effects.[46] Since the VEGF-B levels did not differ between T2DM patients and normal controls,[62] abrogating these effects may be detrimental. The tumor angiogenesis inhibiting

effects also deserve cogitation.[46] Maybe the tissue specific deletion or the shRNA and the targeting delivery of anti-VEGF-B-body represent the possible curative orientation. A pinpointed understanding of the tissue/cell-specific expression pattern of VEGF-B and VEGFR-1 is necessary, and reporter-gene expressing models may help.[63]

Robciuc[64] *et al.* implied the promise of VEGF-B transgenic or VEGF-B protein delivery to ameliorate insulin sensitivity (to improve insulin sensitivity or to ameliorate insulin resistance), diminishes obesity, and alleviate metabolic syndrome, by displacing VEGF-A from VEGFR-1 to activate VEGFR-2 and increasing adipose tissue vascularity, thereupon providing a therapeutic tactics for counteracting obesity. Similar findings were reported in another report.[59] The contradiction aroused that how to settle the discrepancies between the two studies.[13,64] We compared, in order to make them seem harmonious. (i) The insulin resistance in the two studies was derived from the insufficient vascularization and consecutive hypoxia and the ectopic fat accumulation, thus the VEGF-B linked downstream angiogenic event and the suppression of the VEGF-B medicated lipids trafficking could help, respectively. (ii) The VEGF-B gain-of-function may be applicable to the early period when the body owns the functional islets and normal insulin levels, mainly act on the adipose tissue; however, the VEGF-B loss-of-therapy is probably suitable for the terminal stages, targeting for the non-adipose tissue. (iii) Were the amelioration of the gain-of-therapy can be enlarged to the muscular tissues, such as muscle and liver, its suitability could be enlarged. If so, the possible side effect of the VEGF-B loss-of-function in the diabetes patient might be evaded and conversely can exert both anti-diabetic and neuron-protecting, cancer-inhibiting effects. (iv) Were restricted, along the deterioration of insulin resistance, the therapeutic regimen develops from the single gain-of-function to the combination of the gain/loss-of-function. Therefore, to suppress the VEGF-B in the muscular tissue and to enhance the role of VEGF-B in the adipose tissue simultaneously and to avoid the potential side-effect, a tissue-specific delivery of VEGF-B is required, which may be achieved by the newly designed tPep-VEGF-B targeting the adipose tissue.[65]

Cardiovascular diseases, to achieve the tissue specific effect

Mickle studies have implicated the role of VEGF-B in cardiac development, indicating its potential therapeutic application in treating heart diseases. The heart owns the highest mRNA level of VEGF-B[13,14] and the developing heart (pre- and postnatal) exhibited the primary VEGF related factor expression.[12,66] During embryonic (E12.5-17.5) and early postnatal (P3) development, the most prominent cardiac VEGF-B expression changed from right to left ventricular wall,[67,68] however the right ventricular wall after postnatal cardiac remodeling (P18) and in the adult, implying the coordination of its expression adaptation with the changes in cardiac energy requirements at various development stages.[63,69] Gene knockout studies showed that VEGF-B does participate in coronary vasculature development and normal physiological responses to ischemia and vascular occlusion.[18] And, the HF patients showed the declined VEGF-B,[70] both in ischemic and dilated cardiomyopathy. Mice lacking VEGF-B displayed mild cardiac phenotypes, such as the slightly smaller heart and dysfunctional coronary vasculature in Bellomo VEGF-B$^{-/-}$ mice[67] and a prolonged PQ interval in Aase VEGF-B$^{-/-}$ mice.[71] Though minor phenotype differs, these inferred a protective role of VEGF-B in the normal or ischemic heart. Additional, individuals with diabetes are prone to suffer from CVD. Given the apparent role in the cardiac development and amelioration in the metabolic symptoms, VEGF-B therapy may show its appliance value in the HF patients.

Although several tactics are applied in the ischemic cardiomyopathy, such as acute coronary care, reperfusion of occluded coronary vessels and improvements in pharmacologic therapy, the mortality is still substantially ascending.[72] Genetic therapeutic vascular growth to induce the angiogenesis and arteriogenesis event may be an succedaneous approach for those with myocardial or peripheral ischemia who are unsuitable to conventional revascularization options.[73] Table 1 simplified several

Table 1. Summary of VEGF-B overexpression studies

isoforms	animal	Vectors	observed activity	Ref
VEGF-B_{167}	Rat	Ad	angiogenic	74
VEGF-B	Rat	TG	angiogenic/myocardial hypertrophy	75
VEGF-B	Rat	TG/AAV	angiogenic/metabolism/myocardial hypertrophy	70
VEGF-B	Mice	TG	metabolism/myocardial hypertrophy	15
VEGF-B_{167}	Rat	AAV	antiapoptotic	76
VEGF-B_{186}	Pig/Rabbit	Ad	angiogenic/metabolism/antiapoptotic	77

Abbreviations: Ad, adenoviral; TG, transgenic; AAV: adeno-associated virus.

VEGF-B gene transfer studies, providing us the insight of its protective role in myocardial ischemia and HF models.

Although VEGF-B is dispensable for maintaining normal cardiac capability under unstressed conditions,[17,78] mounting evidence suggested distinct but complementary roles for VEGF-B in the maintenance of cardiac contractility and coronary perfusion.[70] Opposited to the pathological cardiac hypertrophy, the VEGF-B overexpression derived adaptive hypertrophy did not deteriorate into HF,[70] and this maybe also a paracrine event: the VEGFR-1 produced by the cardiomyocytes[79] however distributed on the ECs, establishing the ECs-Cardiomyocyte cross-talks. As for the metabolic altering, the strengthened MAPK and weakened AMPK signaling modulate cardiomyocytes to favor glucose oxidation and macromolecular biosynthesis.[70] The enhanced glucose utilization could avoid the ischemic myocardium and limit the myocardial ischemia/reperfusion injury, by augmenting the energy production in the energy-depleted myocardium.[80,81] Coincidentally, such a metabolic shift was also occurred on the VEGF-B$^{-/-}$ mice,[13] and may be favoring the glucose oxidation is profitable for cardiac ability. The vascular growth also linked to the ECs metabolism.[82] Potentially, the exogenous VEGF-B expression can touch off the endogenous expression of co-expressed mitochondrial genes, given endogenous VEGF-B levels are highest in the heart.[8] The antiapoptotic impact was accounted to the antiapoptotic gene expression profile in cardiomyocytes and the metabolic change. To summarize, the effect of VEGF-B overexpression reflected in three aspects, cardiac growth promoting effect therefore displayed a compensatory hypertrophy; angiogenic effect and its related metabolic effects to modulate metabolism of ECs[82] and cardiomyocytes;[70] the protection of cardiomyocytes; from apoptosis; the manipulation of cardiac stem cell to protect against short- and long-term ischemia-reperfusion injury.[83] The combination of these yielded a prolonged beneficial influence on the heart, making the VEGF-B a promising candidate for the treatment of myocardial ischemia.

There are a few points worthy further investigations. First, a complementary relevancy existed between these effects: the cardiac hypertrophy could be the relevant consequence of the others. Vascularization is crucial for adaptive hypertrophy, as enlarged heart tissue must match with the concordant expansion of the coronary vasculature to maintain an adequate supply of oxygen and nutrients for the heart.[15,84] The VEGF-B mediated lipids accumulation in the heart might also contributed, since inherited and acquired cardiomyopathies have the marked cardiac intracellular lipid accumulation.[85]

Second, despite some similar phenotype changes were observed in two studies, discrepancy existed, even completely contradictory results. For example, in the two studies,[70,77] the common myocardial-specific angiogenesis and arteriogenes activity were observed. Whereas, in rabbits and the pigs,[77] the aggrandizement of FATP4 expression and lipids and glycogen accumulation in the myocardium were observed; while there is no difference in cardiac or skeletal muscle FAs influx between the VEGF-B transgenic, gene-deleted and wild type rats.[70] It is important to note that FAs and TGs levels were reduced in the transgenic rat. The reason might be the FATP4 was actually a fatty Acyl-CoA synthase,[86] which meant the FATP4 directs the FAs to synthetic pathways rather than oxidation.

Third, the diverse mechanisms may lead in the same myocardial hypertrophy phenotype. For example, the transgenic mice developed an invalid cardiac hypertrophy due to an enlarged size of the cardiomyocytes but lacked an arteriogenic phenotype, failed to compromise heart ability;[15] however, the cardiac hypertrophy observed in the transgenic or AAV-VEGF-B overexpression rats could be attributed to the coronary tree expanding and reprogram of the cardiomyocyte energy substrate utilization from FAs oxidation towards glucose oxidation.[70] In turn, the alike manifold lipid and glycogen accumulation in the myocardium caused the metabolic changes,[77] cardiac hypertrophy,[70] mitochondrial lipotoxicity and the consecutive mouse death,[15] respectively in three distinct studies.

Furthermore, just noticing the angiogenic role of VEGF-B *in vivo*, an apparent disagreement exists ranging from no angiogenic ability at all in several tissues after adenovirus gene delivery;[87] ability to potentiate rather than bringing about angiogenesis when transduced into the endothelial barrier;[88] a restricted revascularization in the ischemic myocardium.[18,77] Several factors had been proposed to participate, such as the diversity of genetic background, VEGF-B isoforms or means for VEGF-B overproduction (recombinant proteins, naked plasmid DNA or adenoviral vectors).[89] In this respect, adenoviruses and AAV vectors administration represented the most efficient vectors to transfer genes into the adult myocardium and ensured a prolonged effect.[90] The AAV vectors had acquired increasing popularity due to its ability to transduce postmitotic cells, such as cardiomyocytes, at high efficiency and to drive lasting periods of gene expression with noticeable inflammation.[91] Other vector systems, such as the first generation adenoviruses, could activate innate immune responses.[92]

Regarding the VEGF-B as an endogenous protective and repair-promoting cardiac proteins, its overexpression

can be efficacious to prevent cardiac damage and enhance tissue repair.[93] The other members, VEGF-A[94] and PlGF[95] also showed protective ability. Howbeit, the risk of adverse effects, consisting of bleeding, leakage, hypotension, malignancy, had limited clinically systemic administration of VEGF-A for the revascularization of ischemic tissues;[96] and the Ad-PlGF induced myocardial angiogenesis and cardiac hypertrophy was abolished by the nitric oxide synthase inhibitor L-arginine Methyl Ester (L-NAME).[97] The L-NAME did not cancel VEGF-B activity, emphasizing the uniqueness of VEGF-B. To explain these different effects, Ad-VEGF-B_{186} and Ad-PlGF might signal through different receptor binding sites and/or structural variants, or alternatively, acted by recruiting distinct co-receptors to the signaling complex,[13] thereby inducing various downstream events,[77] which was verified since VEGF-B did not rescue development in PlGF deficiency mice.[95] In comparison, the VEGF-B has marked superiority over its family members. First, VEGF-B showed high selectivity to stimulate angiogenesis, especially in the ischemic myocardium,[18] being expected to stimulate angiogenic without causing adverse effects. The upregulation of VEGF-B levels in the ischemic heart but not in the ischemic muscle might partly account for its specificity. Another possible mechanism might be the distinct ECs differentiation in the isolated tissue and organs.[98] Then, VEGF-B provided a more balanced vascularization, embracing microvessel maturation, arteriogenesis besides mere angiogenesis. Nevertheless, the rAAV-VEGF-A barely led to vessel formation but failed to enhance collateralization and perfusion, unless platelet-derived growth factor-B was co-transfected.[99] Moreover, high amounts of VEGF-B were well tolerated, predicating the much wider therapeutic window than VEGF-A or VEGF-C.[70] This uniqueness of VEGF-B warrant further cogitation of the therapeutic potential of VEGF-B for promoting functional recovery of myocardial ischemia.

Neurodegenerative disorders, potent protection without angiogenic by- effect

VEGF-B also manifested its safeguarding role in the neurodegenerative disorders. Indeed, the up-regulated transcriptional activation of VEGF-B in response to midbrain neurodegenerative challenges was observed in Parkinson's Disease (PD), and VEGF-B produced by astrocytes and motor neurons exerted a neuroprotective affect.[22,100] Preclinical studies in PD[101] and amyotrophic lateral sclerosis model[22] had shown promising results, despite the lack of clinical studies. VEGF-B treatment rescued brain neurons from apoptosis in stroke mouse[21] and protected cultured primary motor neurons against degeneration,[22] with little retinal neovascularization effect. The co-expression of VEGF-B with FAs oxidation relative mitochondrial genes was observed in rat midbrain, suggesting the mitochondria as the target site.[102] Moreover, VEGF-B possessed the considerably robust survival ability,[101,102] as a single VEGF-B_{186} protein treatment at a dose of 3 mg per rat partially protected dopaminergic fibers in the striatum and rescued the dopaminergic neurons in the caudal sub-region of the substantianigra.[101]

The neurobiological activity of VEGF-A consists of neuroprotection, neurogenesis, and angiogenesis.[103] Whereas, VEGF-B lacks the undesired adverse angiogenic vitality,[100] consequently it could be regarded as a trophic divisor to reduce effects of neurodegeneration. And, the VEGF-B had no visible neurorestoration effect.[102] Given the tempting neuroprotective activity combined with negligible angiogenic/permeability activity,[21,22] strategies such as adding exogenous VEGF-B or up-regulating the endogenous VEGF-B levels to strengthen this natural protective response may have the potential to be a disease modifying therapy for PD.[100]

Cancer, dual effects on cancer metastasis and growth

The correlation of VEGF-B with cancer remains unclear. Considering the cancer tissues had the higher VEGF-B level,[104] The VEGF-B might promote the cancer progression, especially in advanced cancers. However, the VEGF-B also retarded tumor growth in the RIP1-Tag2 mouse of pancreatic neuroendocrine tumorigenesis,[6] and the reduced blood perfusion in VEGF-B-T241 tumors might explain, at least in part, the anti-tumor growth effect.[105] The antigrowth effect might also be accounted for the approximate 15% heavier weight displayed in the VEGF-$B^{-/-}$ mice.[6,13] The VEGFR-1 may explained the antigrowth and antiangiogenic effect, since it is served as the decoy receptor of VEGF-A.[106] The VEGFR-1 had a negative role in developmental vascularization, and VEGFR-$1^{-/-}$ embryos died early due to VEGF-A dependent vessels overgrowth and disorganization.[107] Moreover, the VEGF-B advanced tumor invasiveness both in HCC patients[108] and mouse tumors,[105] via remodeling of the tumor microvasculature, leading to leaky vascular networks that are highly permissive for invasion.[105] So, VEGF-B might have paradoxical roles in cancer initiation and further progression, inhibiting growth and promoting metastasis, which insinuated the uncoupling of the metastasis and primary tumor growth.[105]

Overall, the tumor retains the higher VEGF-B levels, associated with high rates of distant failure and poor overall survival,[109] and it was responsible to expect that lowering the VEGF-B may show anti-cancer effect.

Actually, the recent reported metformin made it.[110] To let down it, several methods have been put forward. The gene deletion therapy can provide the prolonged effect though the tissue specific deletion barriers and the existing physical function of VEGF-B astricted its application. Similar therapeutic outcomes can be achieved by administrating monoclonal antibodies. Given its potential role for in vivo function characterization and the identification for the new therapeutic strategies, more therapeutic antibodies against human VEGF-B, and small molecule tyrosine kinase inhibitors are deserved to be raised.

Concluding remarks and future prospects

VEGF-B is inert under physiological conditions while showing a potent and safe therapeutic potential in treating metabolic dysregulations, correlative with its widely distribution and multifaceted features. These seeming contradictory features enable the VEGF-B the valuable therapeutic significance in clinical at an attractive safety profile, even the feasibility of developing into the drugs.

The studies of recombinant VEGF-B protein had been limited, due to the burdens in the purification and the lacking of VEGFR-1 mediated responses that might form the basis of a simple cell-based assay system.[14] The exist for VEGF-B purification are largely based on affinity chromatography.[65,111] Comparing with the molecular pharmacological interventions, gene therapy may provide a long-lasting therapeutic effect.[72] In terms of the mAbs, more studies are warranted to interpret the intrinsic molecular basis and to design new molecules with optimized pharmacokinetics/pharmacodynamics, given a series of variables such as potency, half-life, binding stability, bioavailability, and dosing regimen of the existing VEGF-A blockers reflected in clinical efficacy[112] and the complex relationship between clinical studies.[113] And, considering the unsuspected feed-back loops and cross-talk between diversified signaling pathways, the efficacy of conventional molecule has been less than expected, there by the design of mAbs that targeting multiple pathways, especially the intracrine (intracellular and autocrine) signaling pathways, perhaps be an optional orientation, such as epidermal growth factor receptor-VEGF(R) pathway cross-talk in the cancer angiogenesis.[114]

Nevertheless, it is just the multiple features that also limit its application, such as the side effect of the VEGF-B in diabetes. Among these models, diabetes represents the most promising, however others are limited to the rodent studies. More studies are under consideration to uncover the more precise role of VEGF-B under physiological conditions and its possible application in treating diseases. How does the context-dependent varied features switch occur? How it achieved the cardiac specific angiogenesis effect? Also, were the VEGF-B gain-of-therapy enlarged its ability to the muscular tissues, how to actualize the cell/tissue specific delivery of the VEGF-B or its antibodies to avert the possible by-effect, and the development of the long-acting analogs or some other VEGF mimetics are also worth exploring. The clarify of the cross-talk of the family members, if necessary be specific to the tissue, is also a huge task in the long time.

Disclosure of potential conflicts of interest

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