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Therapeutic angiogenesis as a novel therapeutic modality for peripheral arterial disease

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ABSTRACT

Neovascularization or angiogenesis is the process by which new blood vessels develop from a pre-existing vascular network. It is a complex, multi-step process that requires different cell types and many genes with correct spatial and temporal orchestration. Tissue ischemia resulting from the constriction and obstruction of blood vessels leads to an illness that may affect many organs including the heart, brain, kidney, and legs. In contrast to coronary and cerebral artery disease, peripheral arterial disease (PAD) remains an under-appreciated condition that is rarely diagnosed and even less frequently treated despite being serious and extremely prevalent. Since mortality in PAD patients exceeds that in patients with myocardial infarction and stroke, under-treatment of PAD could translate to a major unmet clinical problem. In recent years, much progress has been made in the field of therapeutic angiogenesis. Based on better knowledge of the mechanisms associated with vascular growth, pro-angiogenic therapy aims at generating new blood vessels and increasingblood flow to ischemic tissues using mainly angiogenic growth factors. So far, this new approach has met some clinical success and is expected to provide a new treatment option for those patients who are unsuited to conventional revascularization therapies. However, since there are a great number of angiogenic factors, improvements in the strategy must be actively sought to have better therapeutic outcome. Those approaches would require the introduction of new form of *in vitro* assays and *in vivo* imaging systems for assessing angiogenesis. In addition, individualized angiogenesis-based therapies must be found for a genuine cure of ischemia and prevention of organ failure. Here, issues related to pro-angiogenesis and its future directions are discussed. Especially, transcription factor- based angiogenic gene therapy as an alternative to combination therapy is covered in detail.

Key words : angiogenesis, vessel formation, ischemia, peripheral arterial disease

Introduction

Angiogenesis is essential in certain physiological states as well as for normal development. Imbalance of angiogenesis has been incriminated in a number of pathologic states (15). Tissue ischemia resulting from the constriction or obstruction of blood vessels leads to an illness that may affect many organs, including the heart, brain, kidney, and legs. In recent years, considerable progress has been made in the field of therapeutic angiogenesis, where angiogenic growth factors are administered to ischemic sites to generate new blood vessels. Therapeutic angiogenesis has been clinically applied to severe ischemic limb as well as ischemic heart disease as a new therapy (13,14,16).

Usually, invasive therapies, i.e. intravascular treatment and revascularization, are the first choice treatments for severe ischemic limb. However, there are many cases to which invasive therapy is unapplicable and the case which does not show

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definitive indications. Such patients are at a high risk of having lower limb amputation after all, requiring new therapies. In this respect, therapeutic induction of *in vivo* angiogenesis is gaining more attention as a novel form of therapy to cure those "no-option patients" who are unsuited to conventional therapies. In the present paper, examples of novel gene therapy strategies that can be clinically applied to peripheral arterial disease (PAD) are discussed.

PAD is vastly under-appreciated

PAD broadly encompasses the vascular diseases that are primarily caused by atherosclerosis and thrombo-embolic pathophysiological processes which alter the normal structure and function of the aorta, its visceral arterial branches, and the arteries of the lower extremities (8). The major cause of lower extremity PAD (hereafater, simply PAD) is atherosclerosis. Risk factors for atherosclerosis such as cigarette smoking, diabetes, dyslipidemia, hypertension, and hyperhomocysteinemia increase the chance of developing PAD. Early symptoms of PAD include transient pain in the leg upon walking (intermittent claudication) caused by ischemia. Up to 25% of these ischemic patients will progress to develop cirtical limb ischemia (CLI) frequently requiring amputation that is associated with high mortality rates (5,8).

The incidence of PAD is rising rapidly these days and, despite advances in clinical management, many problems remain unsolved. Current treatment options for PAD are limited to symptomatic control and are plagued with side-effects and limited efficacy. As of yet, there is no therapy to help disease regression or cure PAD. Historically, treatment of PAD has been dominated by interventional procedures and drugs that were designed to provide palliative relief. Recently, however, a great deal of attention is focused on new approaches for medical manipulation of vascular growth. The development of new therapeutic approaches based on recombinant growth proteins and gene- and cell-based therapies promise to provide a set of powerful options that will have a significant impact on the practice of cardiovascular medicine. Better knowledge of the molecular mechanisms and consequences will open the way for development of new treatment strategy aimed at facilitating the reperfusion of ischemic tissues.

Therapeutic angiogenesis is a novel strategy for the treatment of PAD

Angiogenesis is the process by which new blood vessels develop from a pre-existing vascular network. It is central to many physiological and pathological phenomena and plays a critical role in the response to ischemia associated with PAD. The current treatments for PAD are limited to percutaneous transluminal angioplasty (PTA) or surgical revascularization (1). Unfortunately, many patients with PAD are poor candidates for either procedure. Therefore, for such 'no-option' patients, therapeutic angiogenesis is a novel promising tool to treat PAD.

In recent years, much progress has been made in the field of therapeutic angiogenesis. Using mainly angiogenic growth factors, pro-angiogenic therapy aims at generating new blood vessels around the clogged area and increasing blood flow to ischemic tissues (**Fig 1**). There are many factors within different categories that are known to have angiogenic potentials (**Table 1**). Although single angiogenic growth factor like VEGF or FGF-2 may be successful in induc-

Table 1. Angiogenic Factors with Therapeutic Potential.

Growth Factors	Chemokines	Transcription Factors	Other Substances
VEGF family	MCP-1	HIF-1α/VP16	Tissue kallikrein
FGF family		Egr-1*	PAR-activators
Angiopoietins		Prox-1	Thrombin
HGF, PDGF-BB		Ets-1	Del-1, Cyr61
IGF-1, IGF-2		Foxo	PR39
NGF, GM-CSF			eNOS, iNOS
			Secreted frizzled-related protein

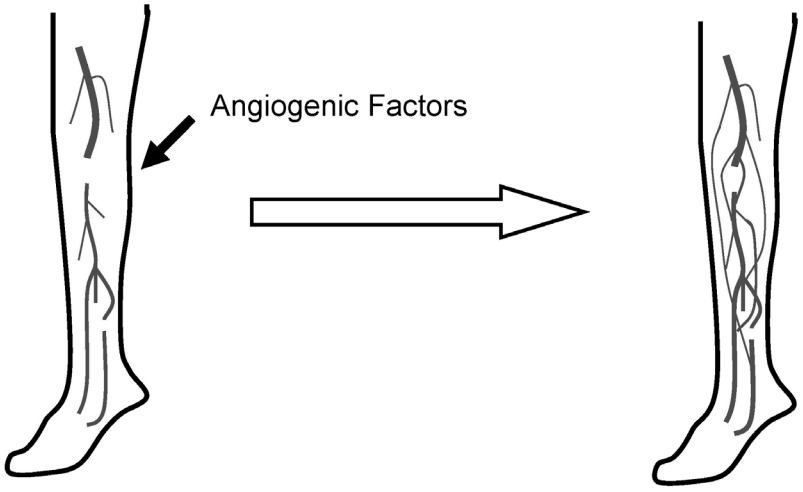

Fig 1. Schematic drawing of therapeutic angiogenesis for PAD. Local injection of angiogenic factors can induce formation of new blood vessels around the clogged arteries. Since the clinical outcome of PAD can be altered by the collateral vessels (thin lines) that form around the clogged region, therapeutic angiogenesis can alleviate or even cure the symptoms of PAD by promoting formation of collaterals. Angiogenic factor can be either chemicals, peptides, genes or even stem cells like endothelial progenitor cell.

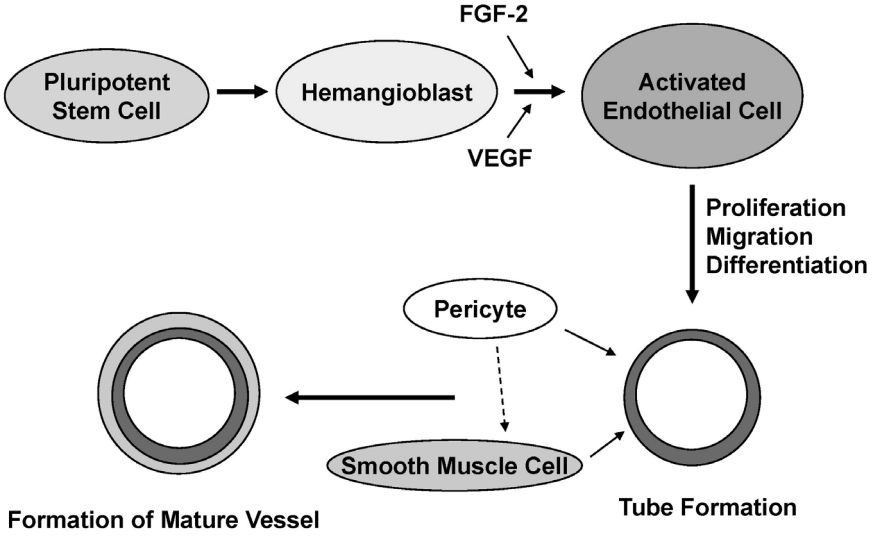

Fig 2. Developmental process and multifactorial regulation of blood vessel formation. There are temporally and spatially regulated expressions of many angiogenic factors in a variety of cell types. Pericytes and vascular smooth muscle cells surround the endothelial cell layer and forms mature vessels. VEGF: vascular endothelial growth factor, FGF-2: fibroblast growth factor-2.

ing angiogenesis, combination of multiple growth factors is increasingly sought these days to augment the therapeutic response. This trend is proper since blood vessel formation is a complex and multi-step process that requires the actions of many different factors (**Fig 2**). Indeed, to form healthy blood vessels, there has to be temporally and spatially regulated expressions of many angiogenic factors in a variety of cell types including endothelial cells and vascular smooth

muscle cells (4,6,12). Supporting this concept, a lot of recent studies and clinical trials on therapeutic angiogenesis began to question the therapeutic efficacy of single factor approach. Therefore, to meet the growing need for functionally significant tissue perfusion in the ischemic tissues, a novel strategy that can provide concerted actions of multiple factors are required. One way to achieve such a goal is to use transcription factor, for example, hypoxia-inducible factor-1α (HIF-1α) that functions as a master regulator of oxygen homeostasis and orchestrate the expression of multiple target genes and thus can induce significant level of angiogenesis in the ischemic region.

Alone or in modified forms including combination with transactivation domain of VP16, several transcription factors have been studied for therapeutic angiogenesis purposes (**Table 1**). Although HIF-1α is a major transcription factor that mediates cellular responses under hypoxic conditions, there are other transcription factors like early growth response factor-1 (Egr-1) that provides another axis of hypoxic response within the cell. Egr-1 is an immediate early gene that is rapidly induced in response to a variety of stimuli including hypoxia (7) and has a number of early downstream target genes that are related to angiogenesis. Consistent with this, it was recently shown that Egr-1 gene delivery can enhance new blood vessel formation (10). When a constitutively active form of Egr-1, Egr-1*, was delivered via adenoviral vector to the ischemic hindlimb of mouse, significant increase in local blood flow was achieved. The tissue histology data also indicated significantly increased arteriogenesis in the Egr-1*-injected group.

Imaging of angiogenesis

Imaging of angiogenesis would be valuable in risk stratification of patients with PAD. The progress in noninvasive imaging strategies to assess angiogenesis has been made possible with the availability of many technological advances, which include dedicated hybrid SPECT-CT and PET-CT systems and agents targeted at molecular markers of the angiogenic process, involving both receptor-probe interactions and reporter gene technology (2,11). These novel targeted approaches for imaging angiogenesis will complement standard imaging of physiological parameters and will play a crucial role for evaluation of therapeutic interventions to promote angiogenesis.

The ability to quantitatively image expression of angiogenic growth factor and its receptors in a non-invasive manner can aid in many areas including new drug development and validation, lesion detection, patient stratification, treatment monitoring, and dose optimization. It can also help cardiologists decide when or whether to start angiogenic treatment targeting specific angiogenic factor and its receptors (3). Much research effort will be needed in the future to improve the *in vivo* stability, targeting efficacy, and pharmacokinetics of the imaging probes. With the development of new tracers having better targeting efficacy and desirable pharmacokinetics, clinical translation will be made easier.

Conclusion

Despite current advances in surgery and interventional therapy, lower limb amputation is still required in a large percentage of patients with PAD. To address this, a great deal of attention is focused on new approaches for medical treatment of PAD. Among different approaches, therapeutic angiogenesis can be a promising new treatment for PAD. By combating the insufficiency of, or insensitivity to angiogenic factors in the setting of atherosclerotic-induced arterial occlusion, it aims at promoting compensatory angiogenesis in hypoxic tissues and in cases of poor wound healing.

However, significant problems still exist in translating experimental findings into beneficial clinical approaches. Studies of ischemic skeletal muscles disclosed evidence of endogenous angiogenesis and adaptive skeletal muscle metabolic changes in response to hypoxia [9]. Since genetic and environmental factors could also account for the great heterogeneity in the expression of the angiogenic genes, development of preclinical assays that are equivalent in terms of efficacy or relevance to human disease may be crucial for the successful development of novel angiogenic therapies. The use of proper animal models to assay angiogenesis is also essential to the search for therapeutic agents that stimulate angiogenesis in

the clinical setting. Future improvements in the strategy would require the introduction of *in vitro* assays and *in vivo* imaging systems for assessing human angiogenesis. When these efforts are combined together, development of individualized angiogenesis-based therapies for a genuine cure of ischemia and prevention of organ failure would be achieved in not-so-distant future. Therapeutic angiogenesis should at least find its place as an adjunct therapy for current surgical revascularization techniques.

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