Special Focus: Angiogenesis in the Central Nervous System

Mechanisms and targets for angiogenic therapy after stroke

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Abbreviations: CNS, central nervous system; HIF, hypoxia-inducible factor; VEGF, vascular endothelial growth factor; FGF, fibroblast growth factor; TGF-B, transforming growth factor-beta; PDGF, platelet-derived growth factor; G-CSF, granulocyte colony stimulating factor; BDNF, brain-derived growth factor; HGF, hepatocyte growth factor; P1GF, placenta growth factor; HB-EGF, heparin-binding epithelial growth factor; IGF, insulin-like growth factor; BBB, blood-brain barrier; FAK, focal adhesion kinase; Robo, roundabout proteins; MAPK, mitogen-activated protein kinase; VE-cadherin, vascular endothelial cadherin; N-cadherin, neural cadherin; E-cadherin, epithelial cadherin; MCAO, middle cerebral artery occlusion; PI3K, phospho inositide-3 kinase

Key words: angiogenic therapy, stroke, neuroprotection, neurogenesis, angiogenesis, neurovascular unit, cerebral ischemia, stroke recovery

Stroke remains a major health problem worldwide, and is the leading cause of serious long-term disability. Recent findings now suggest that strategies to enhance angiogenesis after focal cerebral ischemia may provide unique opportunities to improve clinical outcomes during stroke recovery. In this mini-review, we survey emerging mechanisms and potential targets for angiogenic therapies in brain after stroke. Multiple elements may be involved, including growth factors, adhesion molecules and progenitor cells. Furthermore, cross talk between angiogenesis and neurogenesis may also provide additional substrates for plasticity and remodeling in the recovering brain. A better understanding of the molecular interplay between all these complex pathways may lead to novel therapeutic avenues for tackling this difficult disease.

Introduction

Therapeutic options for clinical management in stroke remain quite limited. In the acute phase, thrombolytic reperfusion with recombinant tissue plasminogen activator is still only used in less than 5% of all ischemic stroke patients worldwide. During the chronic phase after stroke, standard treatments involving rehabilitation provide some support for recovering patients. However, many high-profile failures in a wide spectrum of pharmacologic neuroprotection trials have led to some pessimism in the field.1 In recent years, accumulating data suggest that damaged brain can be surprisingly plastic, and intriguing mechanisms of neurogenesis and angiogenesis might provide novel substrates for brain repair.2,3 Of course, this is not a new idea per se, and the reader is referred to many more detailed reviews that dissect mechanisms of neurogenesis and angiogenesis in stroke.4,6

In the brain, however, the responses and regulatory mechanisms that underlie angiogenesis might be more complex.7,8 Autopsy studies show that brain ischemia stimulates angiogenesis in part via stereotyped hypoxia-inducible factor (HIF) pathways.9 Although it seems imminent that a pro-angiogenic therapy in stroke could be beneficial by increasing blood flow, decreasing infarct size and by supporting reinvestment of vascular networks for faster neurological recovery—the clues for such a therapeutic orientation remain sporadic and indirect. Krupinski and his colleagues demonstrated that the number of new vessels in ischemic penumbral regions correlated with longer survival in ischemic stroke patients, suggesting that active angiogenesis might be beneficial for the ischemic brain.10 It is speculated that signals for angiogenesis should be activated in transient ischemic attacks and that such an upregulation could in part, explain why people with such attacks showed less severe brain damage than those without such episodes.11,12,13 The push for pro-angiogenic efforts in stroke recovery are based on the observed relationships between reduced cerebral blood flow, decreased vascular regeneration and reduced functional recovery in the brain. It is well known that stroke patients who later develop dementia show cerebral blood flow reduction in non-infarcted areas.14 Older patients tend to have decreased new vessel formation after stroke,15 and this may be correlated with worsened functional recovery,16,17 thus indirectly pointing to the role of endogenous angiogenesis in brain repair. It is believed that even if angiogenesis did not reduce the size of infarction, this may still bring

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better functional prognosis just by increasing the cerebral blood flow in adjacent brain.

The post-stroke penumbra is extremely resilient and is a site of intense remodeling and active angiogenesis (Fig. 1). But in order to find an optimal window for application of angiogenic therapies, a rigorous timeline of angiogenic events needs to be drawn. It has been generally considered that proliferation of endothelial cells starts at several days after ischemic events.\(^{15,18}\) Studies using mice with middle cerebral artery occlusion demonstrated that endothelial cell proliferation might begin as early as 12–24 hours after ischemia and persist for up to several weeks thereafter.\(^{19,20}\) Studies using human brain samples also suggested that active angiogenesis takes place at three to four days after stroke\(^{10,11}\) and the number of vessels appeared to be correlated with the length of survival.\(^{31,15}\)

Beginning minutes after stroke in rodents, genes related to angiogenesis are upregulated, producing both message and translated proteins. Vascular endothelial growth factor (VEGF) signals appear in neurons for days after ischemic onset,\(^{21}\) and can be found in astrocytes for up to a few weeks.\(^{22}\) In humans, the angiogenic platelet-derived growth factor (PDGF) message can be detected around cystic infarction for weeks after stroke.\(^{23}\) Although brain cells manufacture and secrete angiogenic peptides after focal cerebral ischemia, the purpose of this angiogenic response remains speculative. Lyden and colleagues have proposed a ‘clean-up hypothesis’ whereby newborn vessels contribute to the clearing up of cellular debris.\(^{24,25}\) They demonstrated that microvessel density increased only in the ischemic margin adjacent to areas of pan-necrosis and was always associated with increased numbers of macrophages. Ischemic brain areas without macrophages displayed no vascular changes compared with normal animals. These data suggest that ischemia-induced microvessels are formed to facilitate macrophage infiltration and removal of necrotic brain.\(^{24}\) Several of the molecules implicated in this recovery response have multiple roles in stroke biology, often conflicting with information we have on their roles in effecting injury.

To exploit angiogenesis towards better stroke outcome, the focus ahead should be on evaluating mechanistic roles of specific molecules in defined windows of time. A critical mass of such information will have to be gathered before we can design pertinent angiogenic therapies customized to the heterogeneous evolution of stroke. Current thinking supports the notion that angiogenesis promotes neurogenesis,\(^{6}\) and that re-growth of vascular structure might provide the requisite molecular as well as anatomic support for recovering neural networks.\(^{26}\) It is generally assumed that angiogenesis is therapeutically advantageous, but it must be acknowledged that clinical evidence in stroke patients with a variety of disease baselines remain to be rigorously obtained.

The therapeutic ideal of optimized revascularization of the stroke penumbra and augmentation of post-stroke brain re-organization may be a tightrope walk. What are the best methods to induce angiogenesis—cytokines, peptides, antibodies, proteins or cells? Where and how should one target the angiogenic stimulus? When does one begin and stop treatments? How will the induced response interact with the endogenous response after stroke? What is the balance between beneficial neovascularization versus potentially dysfunctional angiogenesis? Even harder will be the process of evaluating patients who might be suitable for such therapies. Another layer of difficulty will be the prediction of differential responses in patients with various underlying vascular or metabolic diseases prior to specifying therapeutic regimens. For example, diabetic and/or hypertensive patients with inflammatory baselines may develop dysfunctional vasculature after stimulation. A pro-angiogenic strategy might be ineffective for patients with major artery occlusion in the brain as well. Promoting angiogenesis might be ideal for stroke subtypes with smaller microcirculatory lesions in the deeper layers of brain. Although the basic science of brain angiogenesis is moving forward very quickly, translating experimental findings into clinical treatment paradigms will be difficult.

### Vascular Endothelial Growth Factor Therapy

Many growth factors may be involved in angiogenesis, but the prototypical mediator has been VEGF. In theory, VEGF-directed therapies can promote neurorestoration either directly as a...
neuroprotective agent or indirectly by inducing angiogenesis.\textsuperscript{27-29} VEGF is also known to promote neurogenesis, learning, memory and inhibition of apoptosis.\textsuperscript{30,31} Hence, VEGF monotherapy has garnered much attention as a formidable therapeutic avenue after stroke.\textsuperscript{32-34} One of the first studies of VEGF in ischemic stroke showed that mRNA and protein of VEGF\textsubscript{165} VEGF\textsubscript{189} and the receptor flk-1 were found to be upregulated in human post-mortem brain.\textsuperscript{35} In rodent models of stroke, an intra-cerebroventricular injection of VEGF via osmotic pump, starting 24 hours after onset of focal cerebral ischemia, the stimulation of angiogenesis coincided with a reduction of infarct volume.\textsuperscript{36} An increase in angiogenesis by the direct injection of VEGF via osmotic pump, starting 24 hours after onset of focal cerebral ischemia, the stimulation of angiogenesis coincided with a reduction of infarct volume.\textsuperscript{36} An increase in microvessel density three days after ischemia was greater in VEGF overexpressing transgenic mice overexpressing human VEGF\textsubscript{165} under a neuron-specific promoter was higher than wild-type mice before ischemia, and the increase in microvessel density three days after ischemia was greater than in wild-type, especially in the striatum. These data show that VEGF promotes revascularization after stroke. But how do the new vessels integrate into repair mechanisms? Some studies now suggest that under specific conditions, VEGF overexpression might in fact inhibit reperfusion after stroke, via hemodynamic steal. Newly formed vessels in adjacent non-middle cerebral artery territories may end up stealing blood supply from the actual ischemic areas.\textsuperscript{38} Recently, encapsulated cell grafts overexpressing VEGF were implanted into rat striatum before induction of focal cerebral ischemia resulting in brain edema.\textsuperscript{39} Angiogenesis was significantly increased around the area of the encapsulated graft after 24 h concomitant with a reduction in infarct size, but there was no increase in cerebral blood flow at 1, 7 and 14 days compared with control untreated animals. These data suggest that the link between increased vascularization, increased blood flow and recovery may not be inter-related or concomitant.

Unfortunately, the function of VEGF as a vascular permeability factor also means that an untitrated response may lead to blood-brain barrier (BBB) leakage, brain edema, vasodilatation and aberrant systemic hemodynamics.\textsuperscript{38,40-42} In addition, VEGF-induced angiogenic vessels are hemorrhagic, aggravating inflammatory responses in the recovering penumbra.\textsuperscript{43,44} The reader is referred to many other papers that have previously reviewed the benefits and pitfalls of VEGF monotherapy.\textsuperscript{45-47} Furthermore, VEGF appears to exert neuroprotective action at doses different than doses required for angiogenesis and angiogenic doses of VEGF may be directly neurotoxic (Fig. 2).\textsuperscript{48} Further studies are warranted to find the optimal balance between beneficial neuroprotection through angiogenesis versus notably deleterious effects of VEGF during stroke recovery.\textsuperscript{28,49,50}

Given that VEGF is a potent angiogenic trigger, can we find ways to counteract the side effects and/or enhance the positive effects of the growth factor relevant to the demands of brain ischemia? Recent findings suggest that combinatorial therapy with other agents might be beneficial. The untoward side effects of VEGF were partially obviated by treatment with a combination of VEGF and angiopoietins.\textsuperscript{51} An alternate strategy might be to apply HIF prolyl hydroxylase inhibitors, which raise HIF-1 levels and increase expression of several hypoxia-response proteins, including VEGF, presumably in a regulated fashion that avoids vascular leakage.\textsuperscript{52} Molecules that counteract VEGF signaling in specific cellular pathways to eliminate its effects on permeability are being increasingly identified. Strategies that target Src family kinases, as downstream signaling mediators of VEGF-induced permeability were protective after myocardial infarction and stroke and prevented edema formation.\textsuperscript{38} In a mouse model of stroke, Src kinase inhibition reduced VEGF-mediated vascular permeability in the brain thereby reducing neuronal damage without influencing VEGF expression.\textsuperscript{53} The coupling of focal adhesion kinase (FAK) to integrin \(\alpha(v)\beta 5\), was found to be a critical event in the regulation of VEGF-mediated signaling and its biological responsiveness. Src kinase was found to regulate this coupling and recruit integrin alpha(v)beta5 into a FAK-containing signaling complex during growth factor-mediated biological responses.\textsuperscript{54} Several downstream effectors of VEGF have been tested as selective modulators of VEGF signaling.\textsuperscript{55} Inhibition of FAK or \(\alpha(v)\beta 5\) disrupted VEGF-mediated Ras and c-Raf activity on the chick chorio-allantoic membrane, suggesting that Ras-ERK pathway could represent another axis to alter vascular responses to VEGF-signaling in angiogenesis.\textsuperscript{56} The Roundabout (Robo) protein 4/Slit2 axis has been shown to selectively inhibit vascular endothelial growth factor (VEGF)-165-induced migration, tube formation and permeability in vitro and VEGF-165-stimulated vascular leak in vivo by blocking Src family kinase activation.\textsuperscript{57} Thus, targeting the Robo4-Slit2 signaling or in recovering vessels may open newer therapeutic options along with VEGF to minimize tissue injury and maximize its beneficial effects.\textsuperscript{58}

Figure 2. Exogenous VEGF can be directly neurotoxic. Histological analysis of untreated normal brain (A), normal brain treated by the low dose of VEGF\textsubscript{165} (B), normal brain treated by the intermediate dose of VEGF\textsubscript{165} (C), and normal brain treated by the high dose of VEGF\textsubscript{165} (D). The histology of untreated normal brain, normal brain treated by the low dose of VEGF\textsubscript{165}, and normal brain treated by the intermediate dose of VEGF\textsubscript{165} (C) is similar. In contrast, the adverse effects of the high dose of VEGF\textsubscript{165} on histology of the neurons and neuropil are conspicuous. Bar = 25 \(\mu\)m. Adapted from Manoonkitiwongsa et al. Vascular Pharmacology 2006; 44:316–25.
Beyond Growth Factors—Including the Neurovascular Plexus

Although there is inherent logic in promoting re-growth and recovery, the broader strategy of growth factor supplementation in stroke therapy has been challenging. Fibroblast growth factor-2 (FGF-2) mRNA and protein was reported to be upregulated in the brains, serum and cerebrospinal fluid of patients who died of acute ischemic stroke. The cellular localization of FGF-2 was found to be endothelial cells in these patients from days to months after stroke. These clinical findings were consistent with experimental models.

In rodent models of focal cerebral ischemia, FGF-2 administration correlated with reduced infarct volume. In addition, FGF-2 signaling was discovered to promote mitogenesis and differentiation in neural progenitor cells in vivo. Ren and Frankenstein have reviewed the downstream molecular aspects of FGF-2 signaling as well as its neuroprotective effects. Several growth factors such as transforming growth factor-β (TGFβ), granulocyte colony stimulating factor (G-CSF) and PDGF have also been found to be upregulated in brain ischemia and reports from in vivo studies remain optimistic. However, many human clinical trials in the USA and Europe were aborted because of high dose toxicity and inconclusive results. It is suggested that a combinational therapy of growth factors and/or their inhibitors may provide powerful therapeutic potential for enhancing stroke-induced neurogenesis and restoring the damaged tissue to function. Yet the translation of in vitro findings to in vivo studies and then finally to clinical initiatives will have to overcome the typical challenges of toxicity, untoward side-effects and questions in timing, dosage and patient selection.

It might be time to look beyond growth factors and their signal transduction pathways towards a more comprehensive view on the regulation of angiogenesis. A view that takes into account the entire neurovascular unit, cell-cell and cell-matrix interactions in the context of stroke. Actions of growth factors are specific. Local concentrations, timing of peak concentration and the consequences of growth factors signaling is largely determined by the overall state of the cell and its microenvironment. A single growth factor may mean different things to different cells in the neurovascular unit. For example, VEGF may lead to loosening of tight junctions in endothelial cells, trigger the autocrine production of other growth factors in astrocytes and pericytes, and stimulate pro-survival Akt signaling in neurons, all with vastly differing dose-response profiles. Since growth factors are such top-range global players in the orchestrated functions of the neurovascular unit, the idea of merely supplementing growth factors and cells as ‘factories’ of growth factors might be difficult to achieve clinically. Given redundancy at the level of growth factors and shared intracellular signaling machinery, the focus should be to look for targets that are involved in the sensitive control of the neurovascular environment in angiogenesis.

Cell Junction Molecules—Potential Targets?

In rethinking the considerations for a pro-angiogenic strategy for the recovering penumbra, the dangers of full-blown dysfunctional revascularization will be ghastly. This might be especially pertinent in stroke patients who are diabetic or have overall metabolic disease, wherein dysfunctional angiogenesis is a risk. How do our therapies interact with endogenous neurovascular mechanisms? Supplemental strategies in an already re-organizing vascular environment might be more pertinent than corrective measures. In this conservative view, the idea of augmenting existing angiogenesis without ‘triggering’ an over-robust response may be critical. For example, the delivery of survival signals to a proliferating endothelial cell may be sufficient; so it may persist just long enough to form stable tubules. Or perhaps one might use strategies to potentiate migratory mechanisms just enough to enhance tubule formation or to accelerate re-population of vascular structures with mural cells towards better maturation of the vascular architecture. Such subtle manipulations of the system might have greater potential to translate into clinically relevant therapies. In searching for such targets, we might have to consider smaller and specific molecules for every component process in angiogenesis and not larger stereotyped players like growth factors.

Cell junction molecules exist at the interface of multiple cellular decisions and play important roles in vascular permeability, quiescence, invasion and differentiation. As plasma membrane attachment sites for a variety of intracellular signaling molecules, they modulate inside-out and outside-in signaling in endothelial cells. The engagement of cell junctions control several component steps in the angiogenic cascade such as proliferation, migration, invasion, tubule formation and vascular maturation. Overall, cell adhesion molecules have emerged as formidable targets in a wide range of inflammatory diseases and neurodegenerative disorders owing to their distinct structure-function roles in normal and pathophysiological conditions. Anti-integrin therapy for tumor angiogenesis has gained ground in the recent times. Several antagonists targeted against these molecules have been found to have anti-angiogenic effects in vivo. Modulation of cell adhesion molecules and their signaling might be a useful strategy in stroke therapy either independently or because of their ability to alter responsiveness to growth factors—either potentiate growth factor signaling or attenuate its effects where necessary. A previous study showed that confluent endothelial cells with well-formed cell junctions respond poorly to the proliferative signals of VEGF and VE-cadherin. Also, the presence of VE-cadherin attenuates VEGF-induced VEGF receptor (VEGFR)-2 phosphorylation, p44/p42 MAP kinase phosphorylation, and cell proliferation. In cells overexpressing VE-cadherin, the half-life of VEGF-2 was increased revealing the existence of a novel layer of functional regulation of VEGFR-2 by VE-cadherin. These data suggest that cell junction molecule function could be altered as critical modifiers of exogenous growth factor therapy. Perhaps used in conjunction with VEGF, such applications might serve to sensitize or desensitize growth factor signaling in the recovering stroke penumbra.

As independent candidates for angiogenic revitalization of the penumbra, the prospect of selectively targeting specific molecules on specific cells of the neurovascular unit seems attractive. Like many other molecules, cell junction molecules might have biphasic roles in mechanisms of stroke injury and repair. The ability to utilize their distinct molecular configurations in each phase might in fact make for better vascular supportive and neuroprotective strategies. The selective targeting of angiogenic tumor vasculature by function-blocking antibodies and small molecule antagonism has been effective in inhibiting tumor growth without affecting vascular permeability. With small molecule antagonism and peptidomimetics, it is now plausible to target specific epitopes and domains in these molecules.
Mechanisms and targets for angiogenic therapy after stroke

87 Cadherin antagonists, targeted to specific ectodomains of the cadherin molecule have been tested in models of tumor angiogenesis and retinal neovascularization with promising results. The invasion promoting activities of N-cadherin signaling has been studied in tumor angiogenesis as well. N-, E-cadherin antagonists tested in human trials for anti-angiogenic therapies for aggressive cancers like ovarian and endometrial cancers have been reported to act with relatively low cytotoxicity and side-effects.

Additionally, the use of soluble adhesion molecules to deliver survival signals, alter growth factor responsiveness and facilitate pertinent cell-cell communication within the remodeling penumbra needs to be evaluated. Recently, soluble N-cadherin fragments were found to stimulate migration of endothelial cells through the FGF-receptor and modulate fibroblast growth factor receptor dependent neurite outgrowth.

During vasculogenesis, N-cadherin mediates adhesion, recognition and signaling between pericytes and endothelial cells and is required for normal vascular morphogenesis. N-cadherin deficiency impairs pericyte recruitment in embryonic stem cell-derived angiogenesis pointing to the pivotal role played by cell junction molecules in signaling pathways crucial to angiogenesis and neurogenesis. The significant diversity in expression of cell junction molecules, the expression of tissue specific isoforms and their spatio-temporal functions in the CNS can be exploited to better vascular morphogenesis and neurogenerative outcomes.

The last decade has seen accelerated drug discovery efforts, aimed at manipulating the function of a variety of cell adhesion molecules through monoclonal antibodies, peptides, peptidomimetics and non-peptide small molecules for diagnostic and therapeutics. Such toolkits for cell junctional molecules may provide a way to discover attractive targets for either head-on pro-angiogenic strategies or for combinatorial therapy with growth factors.

Angiogenesis, Neurogenesis and Cell therapy

Strategies to induce angiogenesis are part of larger neurorestorative strategies aimed to extend the therapeutic window for stroke recovery, in order to increase the diversity in therapeutic options for a variety of patients. The ordained goal in the post-acute phase of ischemia is to enrich the intrinsic potential in the brain for

Figure 3. A peptide antagonist to VE-cadherin inhibits retinal angiogenesis without disrupting endothelial cell junctions. (A) VE-cadherin antagonist blocks retinal angiogenesis in a mouse model of oxygen-induced retinopathy in a dose-dependent manner. (B) The vascular endothelial cadherin (VE-cadherin) antagonist does not disrupt existing endothelial cell junctions. Representative images of confluent bovine retinal endothelial cell monolayers stained for VE-cadherin in untreated (I), control peptide-treated (II), and antagonist-treated (III) cells. The antagonist had no effect on the structural integrity of the monolayer as demonstrated by continuous VE-cadherin staining along the cell borders. Bars indicate 10 μm. (D) The function of the cell junctions was assessed by measuring the permeability of the monolayer using fluorescein isothiocyanate-dextran. The permeability of the antagonist-treated cells was not significantly different from untreated cells or cells treated with control peptide. Adapted from Navaratna et al. Archives of Ophthalmology 2008; 126:1082–8.
Mechanisms and targets for angiogenic therapy after stroke

An intraventricular injection of human hepatocyte growth factor (HGF) gene was both angiogenic and neuroprotective and resulted in reduced brain ischemic insult.102 PlGF, a ligand for VEGFR and neuropilin-1 is thought to simultaneously regulate angiogenesis and neurogenesis after stroke.103 Functional recovery following MCAO in a rat model of stroke was elevated coincident with enhanced neurogenesis and angiogenesis after an adenovirus-mediated gene transfer of heparin-binding EGF-like growth factor (HB-EGF).104 Although, it is generally accepted that angiogenesis and neuronal health are coupled in normal brain physiology, there are conflicting views on thinking of angiogenesis as a back-door approach to neuroprotection and neurogenesis. VEGF was demonstrated to exert neuroprotective action significantly before its classical pro-angiogenic functions, possibly involving the PI3K/Akt pathway.105-108 These data suggest that at the molecular level, angiogenesis might not be coupled to neurogenesis after stroke—even though VEGF is implicated in both processes. The rediscovery of many angiogenic agents such as VEGF, FGF and BDNF as neuroprotective factors at different concentrations with different temporal characteristics,109,110 supports the idea that endothelial-neuron survival might be synergistic. But simultaneously, it questions the notion that these two processes might not be evoked simultaneously in therapy with the use of growth factors and cells secreting these growth factors.

Several cell-based approaches to stimulating angiogenesis have been attempted as part of neurorestorative therapies in stroke.111 Recruitment of bone-marrow cells induced revascularization after ischemic injury and augmented blood vessel stabilization in a murine model of MCAO.112 Systemic administration of CD34+ human cord blood-derived cells also induced neovascularization and facilitated neurogenesis113 through secretion of VEGF, FGF-2 and IGF-1.114,115 However, pharmacological and cell-based approaches to induce rapid angiogenesis run the danger of leading to dysfunctional tissue architecture and exacerbating neuronal damage. How these promising experimental leads can be tested long-term in patients remains to be carefully assessed.

The idea of substituting cells after ischemic injury in order to create a cellular microenvironment geared towards functional recovery is appealing. However, it is not clear if such efforts to accelerate angiogenesis will translate to enhancing endogenous neurogenesis and improved functional outcomes. Given the fact that untoward side-effects of full-blown growth factor supplementation in ischemic stroke models remains unsolved, the idea of transplanting whole cells capable of secreting multiple growth factors altering many cellular pathways in multiple ways in the neurovascular environment should be approached with caution. Before these can evolve into clinically viable strategies, a better understanding of molecular interplay between cells in the neurovascular unit has to be accomplished. It might also be time to rethink neuroprotection with the neurovascular unit in mind. Neuroprotection could be facilitated by the delivery of survival signals that mimic modes of paracrine coupling within the neurovascular unit. For example, the delivery of soluble decoy ligands that trigger desired survival responses by mimicking cell-cell interactions in the neurovascular unit. Ultimately, strategies that enable sensitive augmentation of penumbral remodeling are far likely to succeed clinically over larger moves to trigger or induce angiogenesis—given the inundating task of customizing stroke therapy to age, gender, individual physiology and metabolic baselines. The trick might be to think globally but tinker locally: to target smaller molecules with specific functions for greater control.

Conclusions

Neurovascular remodeling is a key component of recovery after stroke. It is increasingly recognized that without vascular support, we cannot have neuronal plasticity. Over the past few years, complex mechanisms are beginning to be dissected that underlie these endogenous responses in damaged brain parenchyma. As we understand more of these pathways, we may design better therapies to augment angiogenesis for promoting recovery. VEGF remains the prototypical growth factor involved. But understanding its interactions with other growth factors and cell adhesion molecules will be critical to yield vascular recovery without BBB leakage. In the final analysis, it likely that a combinatorial platform with growth factors, adhesion molecule modifiers and cellular therapies might provide the optimal approach for promoting angiogenesis in brain (Fig. 4).

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