Regenerative Angiogenesis
Quality Over Quantity

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Angiogenesis can broadly be defined as the growth of new capillaries and blood vessels. A complex set of multiphasic signaling pathways regulate angiogenic blood vessel growth, and these pathways have been the target for both pro- and antiangiogenic therapies. Angiogenesis is a fundamental physiological process that is required for fetal development, wound healing, and tissue repair after ischemic damage. For these reasons, promoting proangiogenic pathways has therapeutic potential to combat diseases where tissue blood flow is compromised, such as peripheral artery disease, ischemic heart disease, or after ischemic stroke. Thus, quantitative methods to assess the degree of angiogenic growth are critical.

Typically, blood vessel density and the degree of angiogenic growth are quantified histologically by counting the number of capillaries observed in a defined cross section of tissue. When angiogenesis occurs, the ratio of capillaries per tissue surface area increases. Functional measures of angiogenesis include measuring perfusion to the tissue involved, and it is typically expected that these parameters (vascular density and perfusion) change in parallel. However, this is not always the case. In fact, injured tissue may demonstrate both an increase in capillary density and an increase in blood flow but still have underperfused areas. Such a discrepancy could be because of improper vasoregulation of new vessels, or creation of arteriovenous anastomoses that maintain overall tissue flow, but reduce perfusion to major segments of the tissue. Thus, it is important to consider both the quality of a neovascular network, which depends on its complex 4-dimensional architecture (3 physical dimensions and time), and how well the new network functions to deliver oxygen to the tissue compared with the native circulation (Figure).

In this issue of Circulation Research, Arpino et al provide novel functional/anatomic evidence that angiogenesis after ischemic injury does not necessarily result in a normally functioning network, despite a return of normal capillary density in the tissue. Using high-resolution structural imaging and red blood cell transit quantification, the authors showed proliferation of in vivo neocapillaries after a complete ischemic insult to the extensor digitorum longus muscle of C57BL/6 mice. They went on to show for the first time that the newly developed capillaries were not as effective in delivering oxygen to the tissues because immunohistological staining showed marked tissue hypoxia in regions of ample neovascularization. Further analysis revealed abnormal network lengths, branching patterns, and red blood cell capillary transit in the reperfused tissue. Also, compared with control animals, these newly developed vessels do not adequately regulate red blood cell flux to the muscle in response to a hypoxic stimulus, indicating, in the authors’ words, that the neovascularure is ineffective at delivering oxygen in accordance with local needs. This is an important finding that attests to the complexity of neovascularization of a posts ischemic and the need for a coordinated response by multiple signaling pathways. These data may help to explain the failure of some proangiogenic treatments for peripheral vascular disease and myocardial ischemia (reviewed by Giacca and Zacchigna) but also indicate the need to use functional assessments of tissue oxygenation to assess the efficacy of neovascularization.

A chronically dysfunctional neovascular capillary network is likely to have detrimental impact on the underlying ischemic skeletal muscle. The authors show that even under normoxic conditions, compared with control animals, the regenerated extensor digitorum longus muscle is hypoxic at 28 days post-occlusion of the femoral artery, presumably because of the dysfunctionality of the network which was regrown. What remains unexplored is how this chronic underperfusion translates to diminished muscle function. For example, it would be interesting to know both how the muscle architecture of the previously ischemic tissue compares to native muscle and whether the contractile properties of the revascularized muscle differ from native muscle. This could be tested by comparing contractile function and force generation of isolated regenerated muscle versus native muscle.

The degree of the ischemic insult in the study by Arpino et al was complete, as the right femoral artery of the test animals was ligated to eliminate blood flow to the limb. Regeneration of a completely obliterated vascular network might be too difficult to accomplish. Therefore, a model of subtotal injury may allow for more effective and functional angiogenesis and might be more relevant to clinical situations where chronic ischemia may promote some degree of collateral development. This raises another important consideration. In what way does the nature of the insult or
angiogenic stimulus (ischemia, hypoxia, physical, or chemical damage) direct the growth of functional versus dysfunctional angiogenic networks? Also, does angiogenesis which results from a natural stimulus like exercise\(^5,6\) yield a more functional network than therapeutically driven angiogenesis in an individual with cardiovascular disease? The methodological approach by Arpino et al\(^3\) creates an opportunity to assess the effectiveness of angiogenic networks stimulated by a variety of conditions to determine which result in a functional neovascular network that is capable of matching tissue perfusion with metabolic demand.

In the clinical setting, most pathologically relevant ischemic events (as mimicked in this study) occur in the presence of disease, not in otherwise healthy subjects. It will be important to reproduce these findings in a disease model to provide more relevance to the human condition. In support of this, studies have shown that angiogenesis in response to electric muscle stimulation is reduced in animals challenged with a high salt diet,\(^7\) as well as in hypertensive rodents.\(^8\)

Understanding the signaling pathways responsible for regenerative versus maladaptive angiogenesis may provide new insights for therapeutic angiogenesis. Understanding the timing of release of angiogenic factors\(^1,2\) may also provide clues to help avoid generation of a dysfunctional and insufficient neovascularure. Approximately 2000 people have received either protein-, cell-, or gene-based angiogenic therapies for the treatment of ischemic heart disease in the past 20 years,\(^9–11\) and although there have been some beneficial outcomes, the majority of these studies reported no beneficial effects in the patients. The reason for the failure of these studies is largely attributed to inefficient delivery of the angiogenic agent (typically vascular endothelial growth factor), but insufficient development of a functional angiogenic network may also be a key contributing factor. This speculation will require validation with better assessment of angiogenic function as described by Arpino et al.\(^3\)

References
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