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Physiological capillary regression is not dependent on reducing VEGF expression

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Abstract

Investigations into physiologically-controlled capillary regression report the provocative finding that microvessel regression occurs in the face of persistent elevation of skeletal muscle vascular endothelial growth factor-A (VEGF) expression. Thrombospondin-1 (TSP-1), a negative angiogenic regulator, is increasingly being observed to temporally correlate with capillary regression, suggesting that increased TSP-1 (and not reduction in VEGF *per se*) is needed to initiate, and likely regulate, capillary regression. Based on evidence being gleaned from physiologically-mediated regression of capillaries, it needs to be recognized that capillary regression (and perhaps capillary rarefaction with disease) is not simply the reversal of factors used to stimulate angiogenesis. Rather, the conceptual understanding that angiogenesis and capillary regression each have specific and unique requirements that are biologically constrained to opposite sides of the balance between positive and negative angioregulatory factors may shed light on why anti-VEGF therapies have not lived up to the promise in reversing angiogenesis and providing the cure that many had hoped toward fighting cancer. Emerging evidence from physiological controlled angiogenesis suggest that cases involving excessive or uncontrolled capillary expansion may be best treated by therapies designed to increase expression of negative angiogenic regulators, whereas those involving capillary rarefaction may benefit from inhibiting negative regulators (like TSP-1).

Keywords

thrombospondin-1 (TSP-1); angiogenesis; exercise training; detraining; hindlimb unloading

Introduction

Angiogenesis is critically dependent on expression of vascular endothelial growth factor-A (VEGF-A, henceforth referred to as VEGF) during growth and development. This is evident from observations that loss of the VEGF during embryogenesis results in early demise due to malformed nascent blood vessels in the developing cardiovascular system [25,33]. Although VEGF and its receptors are expressed in nearly all tissues throughout life, most

vascular beds are quiescent in adult life and physiologically-mediated angiogenesis is normally limited to only a few conditions, such as the female reproductive cycle [81], pregnancy [111], wound healing [7] and exercise-induced skeletal muscle angiogenesis [22]. The prevailing theory that mature blood vessels become independent of VEGF [96] is supported by evidence from studies involving muscle-targeted VEGF-deficient mice that show reductions in skeletal muscle capillarity only occur when VEGF-deficiency is introduced early in life. For example, when the muscle creatine kinase (MCK) promotor, whose expression begins during late embryogenesis, is used to drive *Cre recombinase* expression in VEGF/LoxP floxed mice, this results in a significant life-long reduction in skeletal muscle capillarity of up to 50% compared to aged-matched controls [79,99]. In contrast, when using a temporally-controlled VEGF deletion strategy that induces skeletal muscle VEGF gene deficiency in mice starting at 4 months of age, no significant alterations in basal skeletal muscle capillarity are observed [18]. However, regardless of the age when VEGF or its receptors are inhibited, subsequent angiogenesis is blocked in response to normal angiogenic eliciting stimuli, such as in response to exercise training [18,80], shear stress [102], muscle stretch overload [36], wound healing [7] and the normal female reproductive cycle [37,38]. Taken together, these data demonstrate that VEGF *is not* required to maintain the existing microvascular structure in postnatal life, but *is* an absolute requirement for physiologically-mediated angiogenesis at any stage of life.

The independence of mature blood vessels to VEGF does not however imply that VEGF is unneeded for vascular health. To the contrary, endothelial-cell targeted VEGF gene deletion (VEGF^{EC-KO}) in mice have established that significant impairment in the integrity of vascular networks occur in VEGF^{EC-KO} mice, resulting in aneurysms and hemorrhaging [61]. Indeed, VEGF is known to have a multifaceted role that includes regulating vascular permeability, and protection from apoptosis and neurodegeneration [94–96], and as such VEGF is still an important autocrine factor that is needed for the normal health and function of blood vessels. It is notable that capillary density remains unchanged in the organs/tissue of VEGF^{EC-KO} mice compared to control mice [61]. Thus, while VEGF is essential for many aspects of vascular and neuronal health, the evidence seems increasingly clear that mature blood vessels do not require VEGF to maintain already developed vascular networks, and at the same time demonstrate that loss of VEGF is not a trigger *per se* for capillary regression.

While the rationale and evidence for VEGF as an essential trigger to initiate angiogenesis has been well established; there is less, but growing, evidence that thrombospondin-1 (TSP-1) may be a similarly important factor for capillary regression and/or pathologically-mediated rarefaction. The current review brings together a diverse body of evidence that will specifically focus and highlight the evidence surrounding the respective importance of VEGF and TSP-1, and how these factors might interact and/or influence capillary regression. Although the focus is primarily on VEGF and TSP-1, due largely to the preponderance of evidence that is currently available, it should be recognized that this does not exclude the possibility that other angiogenic regulators could exert similar direct or indirect effects that may also significantly influence angioadaptation. The discussion is intended to identify evidence and events that might initiate capillary regression, and only

generally address the 'functional states' that might be involved. A more detailed handling of the stimuli and the highly choreographic sequence of events that is involved in the process of altering tissue capillarity can be found elsewhere [22,45,76].

Capillary regression correlates better to changes in TSP-1 than VEGF

Given the positive correlation between VEGF and microvessel density [4,45,51], along with evidence that VEGF inhibition strategies inhibits or impairs angiogenesis [62,63,66], it has generally been assumed that withdrawal of VEGF is also necessary for capillary regression. However, it is important to first emphasize, a positive correlation between VEGF and capillary expansion only provides evidence for the importance of VEGF towards stimulating angiogenesis and not regression *per se*. Second, the strategies involving VEGF inhibition used to study capillary regression are largely reported in the context of pathological responses, such as the aberrant regulation of angiogenesis seen with cancer [47], progression in renal disease [89], or in response to muscle ischemia [50,62,71,101]. In some cases, such as muscle ischemia, these approaches have also had only limited success [62], which is likely because high levels of VEGF appears to be only permissive, but not adequate, for angiogenesis - and also depends upon concomitant responses from its receptors [62,69]. Growing interest in the influence that impaired angiogenesis and/or disease-related capillary rarefaction have led investigators to study detraining and/or hindlimb suspension/unloading on skeletal muscle - to better understand the mechanisms underpinning capillary regression under normal physiological control - in order to understand the mechanisms being dysregulated (and leading to capillary rarefaction) in the context of disease.

Capillary regression with detraining

Recent studies involving training, followed by detraining, indicates that capillary regression *is not* dependent on reduction in VEGF expression [46,65,74]. For example, it has been shown that training-induced elevation in basal skeletal muscle VEGF levels persists even after 7 days of training cessation (i.e. detraining), and that, at this timeframe muscle capillarity had already reverted back to pre-training levels (despite the persistent elevation in muscle VEGF)[74]. This was a robust response seen in several muscles of the distal hindlimb (i.e. soleus, gastrocnemius, plantaris)(Table 1), each representing varying degrees of oxidative and glycolytic potential [74]. Consistent with this observation, two prior studies involving exercise training in rats, have also found detraining-induced capillary regression whilst basal muscle VEGF expression is elevated [46,65](Table 1). These studies provide the seemingly provocative observation that physiologically-mediated capillary regression *is not* dependent on the withdrawal of VEGF. Two of aforementioned studies, i.e. Huttemann *et al.* [46] and Olenich *et al.* [74], report the additional finding of elevated skeletal muscle expression of TSP-1, a potent negative angiogenic regulator. The temporal correlation of TSP-1 with detraining-induced capillary regression suggests that TSP-1, or perhaps more generally negative angiogenic regulators, may be the key determinant(s) in regulating capillary regression.

Capillary regression following hindlimb unloading

Rodent tail suspension resulting in hindlimb unloading (HU) of skeletal muscle is often used to mimic the loss of the gravity to study the long-term effects of spaceflight [72], which depending on HU duration can represent mild to severe skeletal muscle deconditioning. Importantly, however, HU does not involve a pathological etiology, but merely reduces the natural physical forces experienced by the unloaded muscles. Consistent to that observed with detraining studies in rodents, Roudier *et al.* [86] have reported that capillary regression occurring in the soleus muscle following 9-days HU was associated with reduction in the VEGF:TSP-1 ratio, due to increases TSP-1 protein expression and no change in VEGF protein (Table 1). In the plantaris muscle, HU did not alter TSP-1 protein expression, and no significant change in muscle capillarity was observed - in spite of a slight, but significant, elevation in VEGF [86]. Shown in Table 1, there are several studies that report on VEGF and TSP-1 responses with HU. One study reports no change in VEGF expression during HU-induced capillary regression, but significant reductions in the VEGF receptors, and in angiopoietin (Ang)-1 & Ang-2 signaling through its Tie-2 receptor [106]. Two other studies report decreased VEGF expression, with no significant change in TSP-1 protein expression, in conjunction with HU [52,53]. However, one of these studies report a 62-fold increase in the TSP-1 receptor CD-36 [53], suggesting that activation of TSP-1 signalling pathway was likely to occur even if TSP-1 itself was not elevated. The CD36 pathway is important in this context, because it is believed to be the primary receptor responsible for TSP-1 anti-angiogenic effects [91]. Taken together, HU studies that have assessed the TSP-1 pathway, appear to consistently find capillary regression when either TSP-1 or its receptors are elevated. In contrast, the expression level of VEGF is less predictable in association with capillary regression, showing a diversity of responses from elevated [86], unchanged [86,106] or decreased [52,53] VEGF in connection with HU (Table 1).

There is very limited evidence available from spaceflight. But, two studies have reported increased circulating VEGF in astronauts during spaceflight [16,20], while one study involving mice flown in space finds increased circulating TSP-1 [40]. The effects of spaceflight on muscle capillarity also seem to be somewhat controversial [88], but there is evidence that long-term exposure to microgravity can reduce skeletal muscle vascular density [19]. While these data are purely circumstantial and cannot establish cause-and-effect relationship, they are consistent with the evidence from HU studies suggesting that increased TSP-1, and not reduced VEGF, likely mediates capillary regression associated gravitational unloading of skeletal muscle.

Capillary regression following spinal cord injury or denervation

Spinal cord injury (SCI) or muscle denervation is also known to impair vascular control and induce significant changes in skeletal muscle structure and function; which includes, but is not limited to, capillary regression [17,90]. Although clearly a pathological state, given the acute onset involving SCI and that skeletal muscle itself is not typically injured (at least not initially with the onset of SCI), the denervation of skeletal muscle could be viewed as an archetypal physiologically mediated feedback mechanism attempting to match blood supply to a muscle that is progressively becoming deconditioned due to loss or very low nerve activity to the muscle. In this context, it interesting to note that SCI studies report either no

change [10,60,103] or increased [17] VEGF expression, even though capillary regression is occurring (Table 1). Persistent elevation of VEGF has even been found following 25 months of denervation in the rat extensor digitorum longus muscle, despite a concurrent 9-fold decrease in muscle capillarity [17]. Here too, TSP-1 is found to increase as early as 12 hours following denervation, and persists even after 7 days, in otherwise healthy subjects with acute SCI [24,107]. In one study, TSP-1 mRNA was found to increase 58-fold in spinal cord microvascular endothelial cells [10]. Targeted deletion of either TSP-1, or its CD47 receptor, has also been shown to improve the acute epicenter vascularity in contused mice [73], implicating the importance of TSP-1 in reducing vascularity. However, the role of TSP-1 CD47 receptor is perhaps less clear in this context, as CD47 is found to primarily influence TSP-1 inhibitory effect on endogenous nitric oxide (NO) production [49]. But, it is interesting to note that neuronal NO synthase (nNOS) knockout mice have also recently been shown to have reduced VEGF mRNA expression in skeletal muscle [9], therefore it is tempting to speculate that TSP-1 inhibitory effect on NOS might also play a role in lower VEGF expression.

Capillary regression and angiogenesis during the ovarian cycle

The female ovarian cycle involves an active and regular cycle of capillary growth and regression, until menopause. This process involves a complex interaction of hormones, cytokines and growth factors that have been reviewed in depth by others [11,29,81], and the details of which are beyond the scope and focus of the current review. However, it is worth noting, consistent with aforementioned physiological conditions involving capillary regression, VEGF (and numerous other pro-angioregulatory factors, such as fibroblast growth factor-2, angiopoietins, and matrix metalloproteinases) have been found to be essential to stimulating angiogenesis during folliculogenesis and subsequent development of the corpus luteum (CL) [81]. Here too, upregulation of TSP-1 is thought to play a key role in follicular atresia by inhibiting angiogenesis [100]. Supporting this, use of a TSP-1 mimetic peptide in primates has been shown to suppress follicular angiogenesis and promotes follicular atresia [30]. Likewise, TSP-1 in human endometrium is found to be expressed in a cyclic manner that is linked with progesterone, and peak expression of TSP-1 occurs with periods associated with the lowest number of capillaries [48]. Thus, while angiogenesis that accompanies folliculogenesis, ovulation and luteal development requires VEGF, and the coordinated activity of multiple different angiogenic factors and cell types; the inhibition of angiogenesis (or regression of capillaries) found to occur during the menstrual cycle appears to correlate TSP-1 expression.

Balance Hypothesis for Physiological Angiogenesis and Regression

The concept that angiogenesis is stimulated by change in expression of pro- and anti-angiogenic stimulators was originally described by Judah Folkman in the context of an angiogenic switch to explain cancer/tumor mediated angiogenesis [27,28,44]. This concept implies that under normal conditions there is a dominant negative angiogenic phenotype preventing angiogenesis (i.e. in a binary 'off' position), and that in the context of cancer the 'off' signals are overridden by an abundance of tumor-derived pro-angiogenic factors that cause a shift in the balance of angiogenic regulators to an 'on' position and an angiogenic

phenotype for capillary growth [44]. The binary aspect for such a switch (i.e. 'on' versus 'off') might describe angiogenesis in the pathological state, but draws question in the context of physiologically-mediated angiogenesis for two reasons; 1) a greater abundance of negative angiogenic regulators compared to positive angiogenic factors (in 'off' position) could be expected to induce capillary regression [3], and not simply just prevent angiogenesis, and 2) a single acute bout of exercise increases expression of both stimulators and inhibitors of angiogenesis within skeletal muscle [31,78], suggesting a more dynamic control mechanism rather than a binary response. Thus, in the context of physiologically-regulated angiogenesis, the balancing scheme for angiogenic control has been viewed as a continual opposing balance between pro- and anti-angiogenic stimulators that neutralizes changes in microvascular bed [22,77](Figure 1), rather than a dominant negative 'off' state that switches 'on' in response stimuli eliciting angiogenesis.

Theoretically this implies that physiologically-mediated capillary expansion could occur by, 1) only increasing pro-angiogenic regulators, 2) only decreasing negative angiogenic regulators, or 3) some intermediate combination of changes in positive and negative factors (see Figure 1). Therefore studies have begun to examine and report VEGF:TSP-1 ratio in the context of capillaries changes in health [4,77,86] and disease [12,13,52]. On the surface this approach is very practical. In practice, however, it may actually prove experimentally problematic, at least when applied to protein expression levels of VEGF and TSP-1 in response to exercise. This is because the time frame for peak protein expression for exercise-induced VEGF and TSP-1 occurs at least 6 hours apart in skeletal muscle [75]. While this approach may prove less problematic for VEGF [14,31] and TSP-1 [78] mRNA responses, which both peak within 1–2 hours immediately post exercise, the concept of calculating a ratio between positive and negative angiogenic regulators may ultimately prove of limited value if there is an absolute requirement – or threshold – to initiate or trigger either capillary regression or angiogenesis. For example, as previously noted, there is now ample evidence that indicates without activation and/or upregulation of VEGF, angiogenesis is blocked (in response to either physiological or pathological conditions). If increasing VEGF is an absolute requirement for angiogenesis, it would follow that simply reducing expression of negative angiogenic factors would not by itself be able to elicit angiogenesis, unless there was concomitant change in VEGF (Figure 1). This is consistent with the greater muscle capillarity observed in TSP-1 KO compared to wild-type mice, where an associated increase in basal VEGF expression is also found in the muscle [64]. By the same token, the review of evidence presented in this manuscript, suggests that merely reducing VEGF is not sufficient to initiate or induce capillary regression. Rather, physiological regression of capillaries appears to occur primarily in association with elevation in a negative angiogenic regulator (e.g. TSP-1). Thus, changes in specific ratios of growth factors, such as between VEGF:TSP-1, may not always be consistent with morphological changes within the capillary bed (see Table 1), unless the constraints (i.e. trigger or threshold) of the angioregulatory system are first met. For angiogenesis the necessity of VEGF is well established, and for capillary regression it seems possible that a similar requirement might exist for TSP-1.

This does, however, raise some interesting, and as yet unanswered questions. Does the progress of regression induced by TSP-1 occur differently in the presence of normal or

elevated VEGF as compared to reduced levels of VEGF? Is TSP-1 an absolute requirement for capillary regression, or can other negative angiogenic regulators trigger this response too?. These, and many other, questions aimed at studying the role and mechanisms the negative angiogenic regulators play maintaining tissue capillarity throughout life are needed in future studies.

Interaction between stimulators and inhibitors of angiogenesis

A potential explanation for the seemingly provocative observation of capillary regression whilst VEGF is elevated, may be that, in addition to TSP-1's antagonist effects on endothelial cell proliferation, migration and tube formation [93], TSP-1 can also interfere with VEGF binding to its receptors [15,39,41]. For example, TSP-1 via its CD-36 receptor can recruit Src homology 2 domain-containing protein tyrosine phosphatase (SHP-1) to suppress phosphorylation of VEGFR2 and inhibit its signalling [15]. Thus, when TSP-1 is elevated, the canonical VEGF signal pathways can be endogenously inhibited, regardless of whether VEGF levels are elevated or not. This notion is further bolstered by a recent report that exercise-induced increases in TSP-1 mRNA is blocked in endothelial cells following inhibition of its upstream transcription factor FoxO1/3a/4, resulting in faster skeletal muscle capillary adaptation in response to exercise training compared to wild-type mice [87,92]. The importance and action of TSP-1 are also highlighted by findings that systemic administration of a TSP-1 mimetic (i.e. ABT-510) can reduce skeletal muscle capillarity [3]. It should also be noted that impaired TSP-1 expression (as seen with either knockout mice or using antisense TSP-1 oligomers) also results in delayed wound healing (due to reduced macrophage response, prolonged persistence of inflammation and delayed scab loss)[1,21], highlighting again the importance of the TSP-1 in regulating the normal angiogenic response.

These data support the idea that VEGF's interaction with its receptors can be tempered or attenuated in the presence of TSP-1, and could, in part, explain why mature blood vessels become less dependent on VEGF during postnatal life [32,96]. Recent studies also suggest that murine double minute-2 (an E3 ubiquitin ligase that is an important negative regulator of the p53 tumor suppressor gene) can have an influence on both VEGF and TSP-1 expression, and therefore could influence muscle vascularity by its effects on the interaction between VEGF and TSP-1 [2,84,85]. Another possibility could involve upregulation of VEGF-165b, an alternatively spliced variant of VEGF-A₁₆₅ that exhibits anti-angiogenic properties [109]. VEGF-165b also competitively binds to the VEGF receptors and inhibits VEGF-R1 signalling. Thus far, however, evidence for VEGF-165b appears to be largely restricted pathological conditions and/or normal non-angiogenic tissue [8,105,109], perhaps contributing to the quiescence seen in most normal adult tissue. At present there appears to be only one study reporting on the VEGF-165b variant in the context of exercise, and no evidence was found to support its involvement with exercise training in humans [42]. Therefore the role, if any, for VEGF-165b as a mediator of involving physiological regression of capillaries remains questionable at the moment. Other regulators, such as endostatin and nucleolin [74], soluble Flt-1 [6], angiopoietins-1 & -2 and their Tie-2 receptors [43], and others, may also be important but will require future investigation to determine their role in mediating capillary regression.

The global picture that emerges is that physiological expansion vs. regression of blood vessels is likely to have different expression patterns for pro- and anti-angiogenic regulators and are not likely to depend, or be triggered, by the same factors (Figure 1). It is also possible, and likely, that these responses are different at different levels within the cardiovascular architecture (i.e. microvessels versus arterioles/venoules versus arteries/veins). Despite the apparent theoretical influence that either positive or negative factors can exert to alter angiogenicity, the mechanism(s) triggering or responsible for initiating microvessel expansion or regression appear to be biologically constrained and dependent on key angioregulatory peptides, much like a 'brake' and 'accelerator' on a car that requires separate input (force) to activate. In other words, it should no longer be assumed that the mechanism(s) involving capillary regression (and perhaps more importantly capillary rarefaction associated with chronic disease) are controlled by simply turning off the factors that stimulate angiogenesis.

Clinical insights

These findings are also likely to be relevant in the context of disease, particularly since elevation of TSP-1 has been observed and associated with numerous conditions involving capillary rarefaction and found to confer relative protection from cancer [23,35,49,82]. For example, capillary architecture is altered due to an imbalance between VEGF and TSP-1 in the muscle of ovariectomized rats [97]. Increased TSP-1 and capillary rarefaction in the kidney has been observed in chronic renal disease [54,98]. At the same time, the renal angiogenic response to hypoxia and ischemic reperfusion injury is impaired with elevated TSP-1 [68,83]. Elevated TSP-1 expression and capillary rarefaction has also been observed with type-1 [58] and type-2 diabetes [34,59], and the responsiveness of muscle VEGF to an angiogenic stimulus (i.e. acute exercise) is impaired with diabetes [57]. Our laboratory has made a similar observation in obese Zucker rats (OZR; a model for metabolic syndrome and type-2 diabetes), where TSP-1 expression can be elevated up to 3-fold in OZR whole muscle extracts, but VEGF protein expression is unchanged in OZR compared to similar-aged healthy lean controls (IM Olfert & JC Frisbee unpublished data). It is also interesting to note, using laser capture microdissection in streptozocin induced type-1 diabetic mice, TSP-1 mRNA has been found to be elevated (nearly 3-fold) in capillaries within muscle, but not in the muscle fibers themselves - whereas VEGF mRNA was unchanged in either tissue [57].

While the role of the TSP-1 in chronic disease has been, and continues to be, extensively studied (see reviews [23,49]), the conceptual realization that capillary rarefaction does not primarily depend on the withdrawal or lowering of pro-angiogenic mitogens offers critical insight and greater clarity toward the signalling pathways that may be best to target. Equally important, these data suggest which pathways are also not likely the best targets, and as such could help explain why anti-VEGF therapies have been disappointingly unsuccessful in reducing microvascular density in the majority of cancers [5,70,104,110]

In retrospect, it seems that much of the current understanding of the angiogenic process comes from studies in situations where angiogenesis is dysregulated, such as cancer or other chronic disease states [27,44], rather than studies involving physiologically-mediated

angiogenesis. If the dysregulation of angiogenesis that defines pathology does not behave or respond as that observed for vascular beds under physiological control, it must be recognized this approach will not likely provide the best strategies to attack mal-angioadaptation. Indeed, it seems essential that we understand the mechanisms regulating microvessel quiescence and adaptation while under physiological control if we hope to develop the best therapies and strategies aimed at reversing the abnormal microvessel changes in chronic disease. In this context, studies being performed in early life (i.e. during or around the perinatal period associated with growth and development) may also fall short in helping to understand abnormal capillary changes with chronic disease in adult life, since most vessels in normal tissue seem to lose dependence on VEGF after development. Nonetheless, it is clear that VEGF is an absolute requirement to initiate angiogenesis at any age, therefore clinical inhibition of VEGF may be most useful in cases when tumors can be detected or treated early in pathogenesis, and therefore able to prevent or minimize the initial development or expansion of the abnormal microvascular bed. Whereas, the usefulness of anti-VEGF or anti-VEGF receptor therapies appears to be more limited, and less effective, once tumors have already established a microvascular supply [26,55,56,108]. Unfortunately early detection is not currently a reality for the majority of neoplastic malignancies, and therefore - perhaps not surprising - that anti-VEGF therapies have not lived up to the promise so many had hoped for. The evidence from physiological regulation of capillary regression suggest that therapies targeting increases in TSP-1 (or other negative angiogenic factors) will likely be the most helpful to reducing microvessel density where excessive angiogenesis is occurring, and that decreasing negative angiogenic mediators will likely hold the greatest promise for stopping or preventing capillary rarefaction.

Summary

Based on evidence being gleaned from physiologically regulated capillary regression, it is increasingly clear that capillary regression (and likely disease-related capillary rarefaction) is not merely the reverse of angiogenesis, but rather each process (microvessel expansion vs. regression) has a distinct expression pattern that involves a complex interaction involving shared but unique angio-regulatory programming. This represents a complex, but exquisite, control process that ensures a microvascular bed is appropriately and optimally matched to supply the metabolic demands of the organ/tissues it feeds.

Perspective

This article is based on lecture delivered at the 10th World Congress for Microcirculation in Kyoto, Japan, 2015. The article highlights lessons learned from studying physiological regulation of angiogenesis and capillary regression and provides potential insights that may be important to understanding and developing the best treatment strategies in combating pathological conditions involving maladaptation within the microvascular bed.

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Abbreviations

CL	Corpus luteum
FoxO1	Forkhead box protein O1
HU	Hindlimb unloading
MCK	Muscle creatinine kinase
OZR	Obese Zucker rat
TSP-1	Thrombospondin-1
VEGF-A or VEGF	Vascular endothelial growth factor

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Balance Hypothesis

Figure 1.

Schematic diagram depicting the balance hypothesis for positive (blue boxes) and negative (red triangles) angiogenic regulators in response to physiologically-mediated angiogenesis and capillary regression. *Top two panels* represent the theoretical extremes by which altering expression of angioregulatory factors, by either (i.e. increasing) positive or (i.e. decreasing) negative factors, tips the balance of factors to stimulate angiogenesis. Physiologically controlled angiogenesis likely uses a compromise somewhere between these two extremes (denoted as three dots between the panels), however it is important to note that

existing evidence does not support the idea that angiogenesis can be stimulated in the absence of increasing VEGF (see text for details). Simply decreasing negative angiogenic factors is not sufficient by itself to stimulate angiogenesis under physiological conditions. In contrast, the *bottom two panels* represent the theoretical extremes by which altering expression of angioregulatory factors, by either (i.e. decreasing) positive or (i.e. increasing) negative factors, can tip the balance of factors to stimulate capillary regression. Similar to angiogenesis, physiologically controlled capillary regression likely uses a compromise somewhere between these two extremes. But, in this case, emerging evidence suggest capillary regression *is not* dependent on changes in positive angiogenic regulators (such as VEGF), but rather that negative angiogenic regulators (such as TSP-1) are essential and will determine whether or not capillary regression occurs. VEGF and TSP-1 are used as the principal examples of positive and negative angiogenic regulators based on the preponderance of the evidence highlighting the importance of these factors, but it is likely angiopoietin (Ang)-1 and Ang-2, and other factors, are also important and exert either direct or indirect roles on regulating angioadaptation in health and disease.

Table 1
Basal skeletal muscle protein expression for VEGF and TSP-1 in response capillary regression

Condition	Species	Tissue	Tissue Capillarity	VEGF expression	TSP-1 expression	Relative VEGF:TSP-1	Reference
Detraining (7 days)	Male/Female C57Bl/6 mice	Gastrocnemius m.	↓ 28% [†]	n.s.	↑ 114%	Low (0.65)	[74]
		Plantaris m.	↓ 19% [†]	↑ 26%	↑ 172%	Low (0.77)	
		Soleus m.	↓ 11% [†]	↑ 64%	↑ 212%	Low (0.63)	
Detraining (14 days)	Male Sprague-Dawley rats	Plantaris m.	↓ ~15% [†]	↑ 270%	not reported	?	[65]
		Soleus m.	↓ ~28% [†]	n.s.	not reported	?	
Detraining (30 days)	Male C57Bl/6 mice	Quadriceps m.	↓ ~45% [†]	↑ 36%	n.s.	High (1.36)	[46]
Hindlimb unloading (9 days)	Male Sprague-Dawley rats	Heart	↓ ~29% [†]	n.s.	not reported	?	[67]
	Female Wistar Rats	Plantaris m.	n.s.	↑ 57%	n.s.	High (1.57)	[86]
		Soleus m.	↓ 23%	n.s.	↑ 185%	Low (0.35)	
Hindlimb unloading (7 days)	Male Wistar Rats	Soleus m.	↓ 11%	↓ ~38%	n.s.	Low (0.62)	[52]
Hindlimb unloading (10 days)	Female CD1 mice	Gastrocnemius m.	↓ 19.5%	n.s.	not reported	?	[106]
Hindlimb unloading (14 days)	Male Wistar Rats	Soleus m.	↓ ~24%	↓ ~70%	n.s.	Low (0.30)	[53]
					TSP-1 receptor ↑ ~60-fold (CD36)		
Unilateral Sciatic Nerve Denervation (25-months)	Male Wt/HickCar rats	Extensor digitorum longus m.	↓ 94%	↑ §	not reported	?	[17]
Spinal cord injury > 4 years	Male Humans	Vastus Lateralis m.	not measured ↓ 33% artery dia. [‡]	↓ 1.9-fold	↑ 1.6-fold	Low (0.33)	[60]
					↑ 2.4-fold	Low (0.26)	
T9/10 Spinal cord injury	Female C57Bl/6 mice	spinal cord microvascular endothelial cells	↓ 55% ^{††}	n.s.	↑ 58-fold	Low (0.02)	[10]
T10 spinal cord injury	Wild-type Female C57Bl/6 mice	Spinal cord	↓ ~56% ^{‡‡}	not reported	not reported	?	[73]
	TSP-1 KO Female C57Bl/6 mice		↓ ~47% ^{‡‡}	not reported	not applicable (TSP-1 KO mice)	?	
	CD-47 KO Female C57Bl/6 mice		↓ ~21% ^{‡‡}	not reported	not reported	?	
T6-T12 Spinal cord injury	(Sex not reported) Humans	Blood Plasma	not reported	n.s.	not reported	?	[103]

n.s. = non-significant change or no change compared to control
? = Not able to be determined from the data available
‡ compared to exercise trained levels
‡ femoral artery diameter
§ no quantitative data are provided in the report
‡‡ decrease in nuclei/microvessel fragment
‡‡‡ decrease in patent microvessel in epicenter and penumbra vasculature after SCI
KO = knockout mouse