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AGING AND ANGIOGENESIS

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1. ABSTRACT

Angiogenesis is impaired in aged tissues. It is probable that this deficit contributes to the increased severity of vascular diseases observed in older persons. The changes in angiogenesis that occur with aging have been noted at the molecular, cellular, and physiologic levels of regulation. Components of the neovascular process that are influenced by age include endothelial cells, the hemostatic cascade, neuro-chemical mediators, and growth factors and their cognate receptors. The structural and regulatory components of the matrix scaffold that surround newly formed vessels is also altered in aged tissues. These myriad changes result in delayed and impaired neovascularization. The clinical consequences of the decreased potential of aged tissues to form new vessels is detrimental during the revascularization of the ischemic heart and during the repair of injured tissues, but may be of benefit in slowing the growth of tumors. In this context, clinical strategies to improve the function of the aging vasculature in general, and the angiogenic response in particular, must be targeted to specific disease states in order to maximize the potential benefit to older individuals.

2. INTRODUCTION

Angiogenesis, the development of new vessels from pre-existing vasculature, is both delayed and altered with age (1-3). The age-related impairment in angiogenesis is detrimental during the revascularization of ischemic organs (such as the heart) and during the repair of injured tissues (such as the skin). Conversely, the impairment in angiogenesis with age may be of benefit in slowing the growth of tumors, thereby providing partial protection of older persons from rapid development of clinically significant cancers. This review will discuss the myriad components of the angiogenic response that are altered with age and the clinical significance of these changes to common age-related diseases.

3. MODELS FOR THE STUDY OF AGING AND ANGIOGENESIS

The study of vascular biology and angiogenesis in aged humans, in the absence of co-morbidities, remains a significant challenge. In addition to the high prevalence of diseases that are more common in the aged, such as diabetes and atherosclerosis, older individuals present with variations in nutritional status and exposure to drugs (both prescription and over-the-counter) that can affect vascular function. Moreover, it is well known that differences among individuals in a given cohort increase with age. Even a population that is matched for co-morbidities and environmental insults will have inherent differences in the

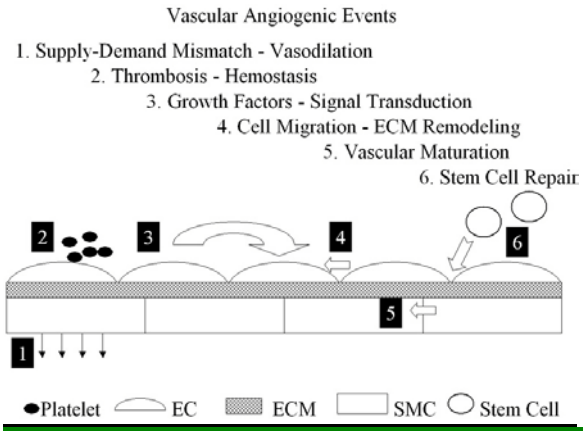

Figure 1. Cartoon illustrating the impact of aging on the sequential stages of the vascular angiogenic response.

rate at which their various organs and tissues age. This is especially the case for studies of the "old-old" (those over 85 years of age), who are much more difficult to study than "young-old" (65-74 years) and "middle-old" (75-84 years) humans (4).

In contrast to humans, animal model systems offer identical genetic backgrounds and controlled environmental exposures to define the role of aging in vascular impairment. Studies primarily in rodent models have yielded important, and largely reproducible, information on general changes in angiogenesis that occur with age. Published models have included 22-40 month-old mice and rats and 48-60-month-old rabbits. The age at which an animal is defined as “aged” is dependent upon the strain (hybrids often live longer) and environmental factors, such as diet (caloric-restricted mice and rats live longer). One accepted guideline is that an animal is definitively "aged" at the point in its lifespan that 50% of its age-matched cohort has expired.

In addition to studies in vivo, there are numerous *in vitro* models for the examination of changes in angiogenesis that are relevant to aging. These include vascularized tissue explants from the above-mentioned aged animals, as well as endothelial cells from aged donors. Endothelial cells aged *in vitro*, via serial passage in culture, provide additional information on cellular changes relevant to aging. Explants and cells in culture, in contrast to live animals, provide a more feasible method of examining specific cellular functions that are relevant to the angiogenic response, such as proliferation, migration, and biosynthesis (5-6).

4. PATHOGENESIS OF IMPAIRED ANGIOGENESIS IN AGING

The basic molecular and cellular events underlying age-related changes in angiogenesis are diverse and often tissue- and/or disease-specific. Aging is associated with an increased prevalence of conditions that contribute to general vascular pathology, such as hypertension, diabetes, and hypercholesterolemia (7). Moreover, prolonged exposure to environmental insults such as smoking also cause vascular damage. The resultant pathophysiology of impaired angiogenesis may be related to the production of reactive oxygen species (8) or advanced glycosylation end products (9). Indeed, clinical outcomes in older patients are attributable, in part, to an increased burden of disease and the cumulative effects of exogenous vascular insults. However, aging is also associated with primary vascular alterations. Clinical and experimental studies have demonstrated that systemic, as well as local, regulation of microvascular function becomes deficient with age, as will be discussed below. Thus, innate and environmentally mediated changes in the microvasculature combine to impair angiogenesis in aging.

4.1. The microvasculature in aging

The vascular mechanisms requisite for adequate angiogenesis are illustrated in Figure 1. Many of these mechanisms, including endothelial cell-mediated functions, are altered with age. Deficits in circulatory supply-demand, as well as increased prothrombotic potential, impair the early vascular responses in the aged endothelium. Dysregulation of angiogenic growth factors and their signaling pathways result in diminished endothelial proliferation and migration with impaired growth of new vascular supplies to compromised tissues. Furthermore, age-related increases in endothelial apoptosis, as well as impairment of maturation and stabilization of newly-formed vessels, can decrease the long-term protection of neovascularized tissue.

4.2. Pre-angiogenic vascular function

Normally, imbalances in circulatory supply-demand are initially counteracted by vascular rheologic shunting through vasodilation and the subsequent growth of new vessels to support the demands to the end-organ tissue. Disruption of end-organ blood flow due to vascular thrombosis triggers cascades that reduce intravascular obstruction to blood flow, inducing an

angiogenic response to create new conduits to compensate for the compromised vessels.

4.2.1. Vasodilation: Pre-angiogenic supply-demand vascular compensation

Regulation of flow through intact blood vessels is an integral function of the vasculature. Through expansion and constriction of lumenal diameters, vessels can dynamically adjust the delivery of oxygenated blood to specific organs. Dilation of pre-existing vascular capillary beds allows for the acute expansion of collateral vascular network to buttress the subsequent outgrowth of new blood vessels and delivery of systemic endothelial end-organ precursor cells. Thus, age-related impairment in vasodilation may prevent the vascular supply from meeting the immediate tissue needs and lead to organ dysfunction.

Age-associated changes in endothelial function also limit the capacity of older blood vessels to dynamically regulate the local blood flow. The altered rheology subsequently impairs vasodilation of both coronary and peripheral vascular beds in older persons. Prior studies have demonstrated differential effects of the age-related decrease in acetylcholine-induced vasodilation in mesenteric arteries, as compared to femoral and carotid arteries (10). Moreover, other studies have demonstrated that the mechanisms of age-related endothelial dysfunction may differ in the aortic and mesenteric vasculature (11). Such findings highlight that age-associated vascular changes do not occur uniformly and may reflect restricted events in the local vasculature. Clinical studies have often employed forearm brachial blood flow as a surrogate index for coronary vascular dynamics. Older persons have decreased brachial endothelium-dependent vasodilation, as compared to younger individuals with otherwise matched clinical characteristics (12). These data correlate with the impaired acetylcholine-induced endothelium-dependent coronary vasodilation observed with aging (13).

In addition to altered patterns of endothelium-mediated vasodilation, aging is associated with defects in vasoconstriction. Two integral mediators of these respective functions are nitric oxide and endothelin-1. Coronary basal and stimulated nitric oxide production is altered with aging (14). Nitric oxide-dependent aortic vasodilation is diminished with aging (15). Endothelin-1-induced coronary vasoconstriction is increased in association with aging (16-17). *Ex vivo* studies of aged rodent hearts have demonstrated diminished resistance to myocardial ischemia and reperfusion injury in association with decreased coronary nitric oxide production (18). Moreover, age-related depression in hypoxia-induced factor-1 mediated expression of vascular endothelial growth factor (VEGF) may impair the co-dependent vasodilatory actions of nitric oxide and VEGF (19). The compromise in early compensatory actions in the vasculature illustrates that age-associated alterations in the regulation of vascular tone contribute to impaired angiogenic responses with subsequent risk of apoptosis and necrosis of dependent tissues.

Clinical strategies aimed at promoting vasodilation have a profound impact on vascular function. Indeed, pharmacotherapies aimed at decreasing peripheral vascular resistance may have proangiogenic actions, thereby preventing the development of myocardial ischemia due to coronary artery disease (20). Thus, interventions specifically targeted at restoring age-related vasodilation may have a marked impact on the protection of older persons from clinically significant cardiovascular events.

4.2.2. Hemostatic proteolytic cascades: Pre-angiogenic vascular self-defense

Age-related changes in coagulation and fibrinolytic pathways can also contribute to impaired angiogenesis in the aging vasculature. A shift in the dynamic proteolytic cascades increases the predisposition to thrombotic occlusion of older blood vessels. Furthermore, as the regulation of hemostasis extends beyond maintaining vascular integrity and is critical in the initiation of angiogenesis, alterations in aging proteolyic pathways may have profound deleterious results on the aged vasculature.

Previous studies have demonstrated that aging is associated with increased coagulability and decreased fibrinolysis, suggesting a potential basis for the predisposition of older individuals to coronary and cerebrovascular events. Large-scale population-based studies have demonstrated a correlation between advanced age and increased levels of d-dimer and plasma coagulation factors (21-22). The expression of plasminogen activator inhibitor-1, a fibrinolytic enzyme inhibitor associated with coronary thrombosis (23) and myocardial infarction, (24) is upregulated in aged human endothelial cells (25). In addition, the production of coagulation inhibitors, such as activated protein C and antithrombin III, demonstrate age-dependent changes in various populations (26-27). These changes correlate with an age-associated increase in thrombotic event rates. Indeed, advanced age has been shown to be an associated risk factor for stroke incidence and complications (28-29). These clinical observations provide evidence of a potential age-associated increase in systemic vascular thrombotic potential. Moreover, the combination of enhanced coagulability and alterations in the regulation of coronary vascular blood flow may provide a pathophysiologic synergism that increases myocardial susceptibility to vascular disease in the aging heart.

Age-related alterations in hemostatic cascades may also impair the subsequent induction of growth-factor-mediated angiogenesis. Proteases of the coagulation and fibrinolytic pathways have a significant role in the cleavage and activation of angiogenic growth factors (30). The same cascades have direct actions on the activation of matrix metalloproteases that are critical in the latter maturation stages of angiogenesis (31-32). In addition, proteolytic processing of precursor molecules result in anti-angiogenic regulation through the generation of angiostatin, as well as endostatin (33). Moreover, direct actions of proteases on activation of cellular receptors can modulate the angiogenic function of endothelial cells (34-35). Taken together these

data highlight that changes in hemostatic function in the aging vasculature may have a profound effect on the induction of new vessels.

4.3. Growth factors

Age-related impairment of angiogenesis is also due to dysregulation of vascular cytokine pathways. Induction of endothelial growth factors is one of the most well-described phases in angiogenesis and there are significant decreases in the availability and release of these factors with aging. In addition, age-related deficits in cytokine receptors and their signaling pathways also contribute to delayed angiogenesis. Experimental models have demonstrated impairments in the expression and function of angiogenic factors, such as VEGF (3, 19), basic fibroblast growth factor (b-FGF) (36-37), transforming growth factor beta (TGF-beta) (38), and platelet-derived growth factor (PDGF) (39) in aging. Restoration of growth factors through delivery of protein or induced expression has been shown to promote angiogenesis in aged tissues (3, 40).

4.3.1. Vascular endothelial growth factor

The expression of VEGF, a critical component of both physiologic and pathophysiologic angiogenesis (41), is diminished in aged animal models. The lack of VEGF in aging has been noted in both basal and stimulated tissues (3). At the cellular level, both macrophages and endothelial cells from aged mice produce significantly less VEGF than cells from young mice (42-43). Indeed, hypoxia-induced VEGF expression is also reduced with advanced age (19). Wounds from aged mice contain significantly less VEGF than wounds from young animals (42). In a rabbit model of hindlimb ischemia, age-associated deficits in angiogenic activity were improved with delivery of recombinant VEGF, suggesting that strategies aimed at restoration of VEGF may improve angiogenesis in the peripheral vasculature of older persons (3).

4.3.2. Platelet-derived growth factor

Whereas age-related deficiencies in VEGF contribute to impaired angiogenesis in the peripheral vasculature, in the cardiac vasculature, it is dysregulation of PDGF B pathways that mediate depressed angiogenesis in the heart. Previous studies have demonstrated that the phenotypic angiogenic regulation of cardiac microvascular endothelial cells is governed in part by PDGF-B mediated communication with surrounding cardiac myocytes. In the aged rodent heart, levels of PDGF-B are diminished (39) due, in part, to impaired expression by cardiac microvascular endothelial cells (43). In addition to lower levels of PDGF-B in older cardiac endothelial cells, endothelial precursor cells from the aging murine bone marrow do not express PDGF-B in the presence of cardiac myocytes.

The dysregulation in PDGF-B expression and induction contributes to the age-related impairment in cardiac angiogenesis and subsequent function. In *ex vivo* cardiac allograft transplant studies, which recapitulate organ bed-specific regulation by host endothelial cells that are incorporated during neovascularization of the cardiac tissue (44), older mice lack the capacity to vascularize and engraft the transplanted hearts (43). VEGF was not capable of independently promoting cardiac angiogenesis. In contrast, restoration of the PDGF pathways, by injection of recombinant cytokine, enhanced angiogenesis and protected the aging heart from myocardial infarction (43).

4.3.3. Fibroblast growth factor

Age-related alterations in vascular growth factor pathways are also linked to changes in FGF (36). Previous studies have demonstrated that levels of FGF-2 decrease more rapidly than those of PDGF with age (45). Indeed, wounds from aged mice contained significantly less FGF-2 than wounds from young animals (42). Decreases in FGF levels can impair angiogenesis directly, as well as indirectly through PDGF-dependent pathways (46).

4.4. Aging and impaired growth-factor responses

In addition to deficits in growth factor levels, the cellular response to replacement of these factors may be altered with aging. Age-related shifts in signal transduction pathways range from a diminished response to growth factor stimulation to excess activation of inhibitory cascades. The response to exogenous growth factors may only be "deficient" in that aged tissues do not attain the response manifested by young tissues. For example, it is generally accepted that aged cells and tissues benefit significantly from stimulation with exogenous replacement of VEGF and, to a lesser extent, TGF-beta 1 (3, 47). In contrast, the response to both PDGF and FGF is variable with increased age (45). FGF-induced endothelial migration (48) and capillary growth demonstrate age-dependent declines. PDGF-mediated cell proliferation is down-regulated in aged cardiac fibroblasts (49), and PDGF induced chemotaxis is depressed in aged rodent endothelial cells (50). Conversely, as noted previously, injection of PDGF restores the angiogenic response in the myocardium of aged rats (43).

Aging is also associated with a shift in endothelial cell cytokine signaling pathways that results in enhanced apoptosis (51). The age-related pathophysiology may be due, in part, to an apoptotic shift in TNF alpha signaling (52, 53). Indeed, TNF alpha normally mediates a diverse array of molecular and cellular cardiovascular actions, including the induction of PDGF pathways in endothelial cells (54, 55) and the enhancement of angiogenic activity (56, 57). With aging there are alterations in signaling pathways, with subsequent increases in TNF receptor-associated death domain (TRADD) and (FASDD) proapoptic cascades (58, 59). These changes, coupled with increased systemic levels of TNF alpha (60, 61), favor apoptosis in aged endothelial cells (62) and concurrent inhibition of angiogenesis.

4.5. The matrix and angiogenesis in aging

Angiogenesis requires the invasion and migration of endothelial cells into the area of new vessel formation. As such, the proteins of the extracellular matrix (ECM), as well as their regulated degradation, are requisite for adequate angiogenesis. The relationship between the

components of the ECM and the endothelial cell can be structural (such as collagens) or regulatory (such as thrombospondins). Cellular attachment to the ECM is mediated largely by the integrins, a large family of heterodimeric receptors that serve as the transmembrane bridge between extracellular molecules and intracellular signaling pathways. The proteolysis of ECM is regulated primarily by matrix metalloproteinases (MMPs) that are secreted by both endothelial cells and the cells that provide the support network for the angiogenic response. The latter include mural cells (pericytes and smooth muscle cells) and fibroblasts. Changes in ECM proteins, integrins, MMPs, and regulatory growth factors can disrupt endothelial growth-factor pathways and migration, further contributing to age-related impairments in angiogenesis.

4.5.1 Structural matrix proteins

Matrix proteins, such as types I, III, and IV collagen, fibronectin, and laminin, provide the scaffold upon which angiogenesis occurs. Changes in many of these proteins have been reported with aging. For example, in the dermis, protein content with age includes a decrease in collagen, the primary structural protein, as a result of both decreased production and increased degradation. The physical properties are also altered; whereas collagen in young skin is oriented in rope-like bundles, in aged skin the protein becomes disorganized with random orientation that may fail to support endothelial migration (63).

Fibronectin is a large extracellular protein that has also been examined in aging and angiogenesis. It functions to regulate inflammation and cell adhesion by providing a provisional matrix, along with collagen III, for cell migration during the early phases of tissue repair and angiogenesis. Although aged cells have been associated with an increase in fibronectin synthesis in culture, studies of aged tissues and wounds from aged animals have shown a decrease in fibronectin expression in association with reduced levels of collagen (64). Less is known about the changes that occur in the deposition of basement membrane proteins such as laminin and type IV collagen in aging. Excess deposition of these proteins is found in the age-associated pathologic states of diabetes and atherosclerosis, but there are no differences in the expression of these proteins in the microvasculature during angiogenesis in healthy young and aged tissues (65).

Little is known about changes in integrin expression and function with aging. A lack of available integrins at the cell surface has been noted in fibroblasts aged *in vitro* (66). However, recent studies have found that age-related declines in integrin- mediated functions, such as cellular attachment and adhesion to the ECM, are likely due to defects in communication between the integrin complex and intracellular signaling pathways. In support of a decline in integrin function, but not expression (67), are data showing that integrin protein content is significantly increased in the myocardium of aged mice. In addition, aged fibroblasts that demonstrate impaired migration show preserved integrin expression but are deficient in integrin-mediated coordination of the actin cytoskeleton (68).

4.5.2. Matricellular Proteins

In contrast to traditional structural matrix proteins, other proteins in the extracellular space have been termed "matricellular." These components of the extracellular matrix do not function as structural proteins, but rather act as modulators of the angiogenic response (69). Matricellular proteins, including tenascin, secreted protein acidic and rich in cysteine (SPARC, BM-40, osteonectin), thrombospondin-1 (TSP-1), and Thrombospondin-2 (TSP-2), provide an additional point of regulation in the local balance among pro-angiogenic (such as VEGF) and anti-angiogenic (such as angiostatin) factors. The net activity of these factors, in conjunction with the expression and function of the matricellular proteins, is critical in determining whether blood vessels will develop within a tissue. Unfortunately, studies of the expression of matricellular proteins with age have primarily been examined in pathologic models that may not reflect normal aging. For example, in a study of glomerulonecrosis in the rat model, alterations in the expression of TSP-1, an inhibitor of angiogenesis, were reported in the aged animal relative to controls (70). We have found that the expression of TSP-2, also an inhibitor of angiogenesis, is increased in aged mice compared with younger mice during neovascular invasion into a sponge *in vivo* (65). These data suggest that over-expression of anti-angiogenic proteins may also contribute to delayed angiogenesis in aging.

SPARC (osteonectin) is a multifunctional matricellular protein that regulates tissue remodeling and endothelial cell proliferation (71). The adult SPARC null mouse exhibits increased fibrovascular invasion into a sponge implant relative to the wild-type mouse (72). The enhanced angiogenic response is associated with increases in VEGF expression in sponges from the SPARC null mouse. However, this phenotype disappears as the mouse ages, such that the aged null mouse has an angiogenic response that is identical to its wild-type counterpart. The mechanism of this loss of phenotype is unclear and may result from a global diminution in VEGF production in the aged mice. Changes in the expression and function of SPARC in tissues with normal aging are controversial. Whereas early reports found an increase in SPARC mRNA in fibroblasts aged *in vitro*, recent studies indicate that SPARC expression may be reduced in aged organs. Further studies are needed to clarify the role of SPARC in impaired angiogenesis in aging.

4.5.3. Matrix metalloproteinases

Synthesis and activation of MMPs during angiogenesis are controlled by several mechanisms, including growth-factor stimulation, exposure to the extracellular matrix (ECM), changes in pH, and the hemostatic proteolytic cascades described above. Among MMPs, the expression of collagenases (MMP-1, 13), stromelysins (MMP-3), gelatinases (MMP-2, 9), and membrane type MMPs contribute significantly to angiogenic activity (73). Collagenases degrade intact collagen and gelatinases cleave previously denatured collagen (gelatin). The membrane-type MMPs (MT-MMPs) activate MMP-2 and mediate pericellular proteolytic activity to support endothelial cell migration.

The expression and activity of MMPs in the aging vasculature is dysregulated. Increased expression of matrix metalloproteinase-2 is associated with intimal thickening in the aging rat (74). Excess MMP activity contributes to age-related aortic remodeling (75), suggesting that enhanced degradation of the vascular matrix contributes to the vascular dysfunction of aging. Conversely, it is an age-related impairment in MMP activity that mediates diminished angiogenesis in the microvasculature. Previous studies have revealed that MMP activation and subsequent migration on collagen is reduced in aged endothelial cells (6). Aged microvascular endothelial cells in a 3D collagen milieu show a deficit in MMP activity and excess TIMP activity that prevents their formation of capillary-like networks (76). Taken together, these studies demonstrate that dysregulation of MMPs, with both excess and deficient activity, is a key factor in age-related vascular pathology and impaired angiogenesis.

5. CLINICAL CONSEQUENCES OF IMPAIRED ANGIOGENESIS IN AGING

5.1. Cardiovascular disease

Ischemic cardiovascular disease is a common cause of morbidity and mortality in the United States population over age 65 (77). Clinical outcomes following myocardial infarction are poorer with higher related mortality rates for geriatric patients. In addition, those surviving initial cardiac events are more likely to develop congestive heart failure, suggesting that age-related changes in the cardiovascular system may predispose older individuals to increased cardiac pathology. Indeed, both clinical and animal studies have demonstrated that the protective role of ischemic preconditioning, as manifested by pre-infarct angina, is depressed in the aged heart (78-80). Furthermore, therapeutic interventions that are directed at reducing the morbidity and mortality of cardiovascular disease are associated with decreased efficacy, as well as higher complication rates in older individuals (81-82). Overall, these differences support the hypothesis that age is a risk factor for poor cardiovascular outcomes. Indeed, angiographic studies have suggested that sub-groups of older patients with diabetes demonstrate decreased collateral circulation than do younger counterparts otherwise matched for clinical characteristics (83). This age-associated clinical risk may be partially attributable to changes in the biology of the aging cardiovascular system. Thus, as the population ages and the incidence of cardiovascular disease increases, the need for therapies optimally tailored for older patients is an increasingly important public health issue. Indeed, a comprehensive understanding of the pathogenesis of age-related impairment in angiogenesis is essential for the development of improved treatment strategies for cardiovascular disease in older persons.

5.2. Wound healing

Wound healing has traditionally been divided into three different phases: inflammatory, granulation tissue formation/proliferative, and remodeling (84). Within each of these phases, there are myriad components (cells, matrix molecules, and growth factors) that contribute to wound repair. In this complicated context, it is generally accepted that wound healing is altered in aged individuals (47). However, whether the alterations represent a slowing of each of the stages or reflect true impairments (i.e., new events and/or the absence of events that are present during wound healing in young individuals) is still debated.

In the basal state, there are fewer capillaries in aged skin and other tissues (63). The density and function of fibroblasts and other stromal cells that surround blood vessels are also diminished. The decrease in cell and vessel density is due to diminished proliferation and increased apoptosis. Changes with age in the mural cell population, which stabilize small vessels, are less clear. Smooth muscle cells obtained from large vessels and arterioles of aged mice do not demonstrate global decreases in proliferation and function. Indeed, preservation of the proliferative and migratory responses in these cell types may predispose the aged vasculature to atherosclerotic changes (85).

After an injury, angiogenesis occurs during the deposition of granulation tissue in the wound bed. The highly vascularized area subsequently promotes the migration of fibroblasts and other connective tissue cells that are critical for tissue integrity and remodeling. As such, it has generally been accepted that delayed angiogenesis contributes to slowed wound healing in aging. Holm-Pedersen was among the first to report that the rate of capillary growth in wounded tissues was decreased in older animals (86). The decline in neovascularization has been attributed to decreases in both the proliferation and migration of endothelial cells in the wound bed (87). Other age-related defects in endothelial cell behavior during wound repair include increased adhesion to leukocytes, enhanced response to TNF-alpha, and greater IL-1 production (42, 64, 88, 89). Several studies have examined delayed angiogenesis during tissue repair in aged animals after injury and have found reduced levels of angiogenic factors, such as the growth factors transforming growth factor-ß1 (TGF- beta 1) and VEGF (2, 3, 40).

A recent histologic analysis has examined neovascularization into subdermal implants in a “wound” model of angiogenesis in young and aged mice. In addition to the previously reported delay in angiogenesis in aged mice relative to the young mice, there were multiple impairments in the neovascular response. Alterations found in the aged mice included a decrease in vessel density and less collagen deposition in the extracellular matrix; a deficient inflammatory response; a lack of the angiogenic growth factors, VEGF and TGF-beta 1; and an increase in thrombospondin-2 (TSP-2), an inhibitor of angiogenesis (65).

Replacement of deficient angiogenic growth factors, such as FGF and VEGF, improves the migration and proliferation of aged endothelial cells in culture (5, 90). Moreover, treatment of aged tissues, both in the basal state and after ischemic injury, with angiogenic growth factors increased subsequent capillary density (3). The extrapolation to enhanced wound repair, as a direct result of

increased neovascularization (as opposed to the numerous other changes induced by the application of these angiogenic factors), remains to be proven. Indeed, in animal models, treatment with an inhibitor of angiogenesis had inconsistent effects on cutaneous wound repair (91, 92). The importance of angiogenesis to wound healing may be tissue specific: a lack of newly formed vessels has been shown to be detrimental to repair of intestinal anastomoses (93). It is important to note that none of the studies were performed on aged animals. In this context, it is likely that a limitation on capillary growth that is not detrimental to healing in a young animal may have significant consequences for an aged animal, in which multiple other impairments co-exist.

5.3. Cancer

Although aging is associated with an increased prevalence of cancer (94), tumor growth in older animals tends to be less rapid, compared with histologically similar tumors in younger animals (83, 95, 96). Indeed, deaths due to primary malignancies are lower in the geriatric population (97), resulting in individuals dying with, rather than from, cancer. The biological basis of these associations are multifactorial, including the development of cancers in at-risk populations at younger ages (94), as well as the increased manifestation of cardiovascular diseases as an alternative cause of mortality in older persons (77). In addition, primary physiological changes due to aging, such as decreases in cellular mitotic and migratory activity, may contribute to decreased tumor growth in older persons.

In addition to the above changes, it is likely that impaired angiogenesis in tumors contributes to the diminished impact of cancers on the geriatric population. In post-menopausal patients with breast cancer, who were otherwise matched for extent of disease and estrogen receptor status, angiogenesis in tumor beds decreased with advanced age (98). Animal studies have also demonstrated less neovascularization in cancers in aged mice as compared to genetically identical younger counterparts (83, 95, 96). Given the correlation between microvessel density and the prognosis of many tumors, a decrease in vessel density with aging may be of benefit in this clinical context (96). Some have proposed that the myriad changes in the components of tumor tissue with age, in particular diminished growth factor availability with subsequent decreased angiogenesis, arose as a protective response against the increased incidence of tumors with aging (99).

6. FUTURE THERAPIES FOR IMPAIRED ANGIOGENESIS IN AGING

The challenging environment of aged tissues underscores the need for therapies that will be effective in the context of the complicated scenario discussed above. To this end, stem cell technologies may have an important role in the restoration of organ function after vascular injury. Recent studies have demonstrated that adult bone marrow is a source for generating an array of non-hematopoietic cells, including specialized cells of the heart and brain cells. Bone marrow-derived cardiac myocytes populate heart after cardiac transplantation (100) and myocardial infarction (101, 103). Similarly, bone marrow cells can generate neurons in both the central (104, 105) and peripheral nervous (106) systems. Moreover, these bone marrow-derived cells can improve end-organ activity, resulting in significant increases in cardiac (107, 108), as well as sensory and motor function (109, 110). Unfortunately the availability and function of adult stem cells may decline with increased age (111, 112). However, transplantation with young bone marrow cells can partially reverse this decline, giving rise to young donor-derived endothelial precursor cells that can restore cardiac angiogenic activity in aging mice (113). Harnessing the full clinical potential of these cells for the treatment of older persons may require approaches to promote stem cell numbers and function, in addition to enhancing angiogenesis in order to increase the delivery of restorative stem cells to the injured tissue.

It is important to note that future therapies directed at restoring angiogenesis, with or without stem cell approaches, should be optimally tailored and focused for age-associated disease states. Strategies aimed at minimizing the burden of cardiovascular diseases should target the prevention of ischemia in the heart in aged individuals. Local treatments to enhance angiogenesis in the wounds of surgical and trauma patients are the best methods to improve age-related impairments in wound healing. Such targeted approaches would ideally preserve the potential protective actions of decreased angiogenesis on pathologic processes such as tumor formation and growth.

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