Connective tissue growth factor (CTGF) in age-related vascular pathologies

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Abstract Connective tissue growth factor (CTGF, also known as CCN2) is a matricellular protein expressed in the vascular wall, which regulates diverse cellular functions including cell adhesion, matrix production, structural remodeling, angiogenesis, and cell proliferation and differentiation. CTGF is principally regulated at the level of transcription and is induced by mechanical stresses and a number of cytokines and growth factors, including TGF β . In this mini-review, the role of age-related dysregulation of CTGF signaling and its role in a range of macro- and microvascular pathologies, including pathogenesis of

aorta aneurysms, atherogenesis, and diabetic retinopathy, are discussed. A potential role of CTGF and TGF β in regulation and non-cell autonomous propagation of cellular senescence is also discussed.

Keywords CTGF · Extracellular matrix · Cerebromicrovascular · Vascular aging

Role of extracellular matrix in cardiovascular aging

The extracellular matrix (ECM) is critical for structural integrity of the cardiovascular system and is one of the most important regulators of cell adhesion, cell-to-cell communication, mechanotransduction, growth, remodeling, and differentiation. Tightly controlled ECM homeostasis is essential for normal homeostasis of the cardiovascular system. It regulates the dynamic behavior of the cells constituting the heart and vasculature and contributes to response to injury and tissue repair and ECM dysregulation. The importance of sustained dysregulation of ECM homeostasis in aging processes is illustrated by the fact that the ECM is dysregulated in many different types of diseases in multiple organs associated with aging, including a wide range of cardiovascular pathologies (for an excellent review see (Meschiari et al. 2017)). To uncover novel therapeutic targets and treatment strategies, it is important to understand the cellular and molecular mechanisms underlying ECM dysregulation in age-related pathological conditions.

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Connective tissue growth factor

Connective tissue growth factor (CTGF), also known as CCN2 (Table 1), is a small secreted matricellular protein of the CCN family of extracellular matrix-associated heparin-binding proteins (Ponticos 2013). CCN family member proteins are non-structural components of the ECM, characterized by rapid turnover, which plays regulatory roles by sequestering and modulating the activities of specific growth factors, are induced following injury and modulate cell–cell and cell–matrix interactions (Bradham et al. 1991; Frangogiannis 2012). CTGF has four conserved cysteine-rich domains: the insulin-like growth factor binding protein (IGFBP) domain, the von Willebrand type C repeats (vWC) domain, the thrombospondin type 1 repeat (TSR) domain, and a C-terminal (CT) domain with a cysteine knot motif. As a matricellular protein, it is thought to integrate diverse extracellular cues into complex biological responses (Ponticos et al. 2009, 2013). Accordingly, CTGF binds to various cell surface receptors, including integrin receptors and cell surface heparan sulfate proteoglycans (HSPGs), thus controlling cell signaling, cell–matrix recognition, and cell adhesion. It also binds growth factors (e.g., bone morphogenetic protein-4 (BMP4), transforming growth factor- β (TGF β), and vascular

endothelial growth factor (VEGF), thereby regulating their functions) and ECM proteins. CTGF has important roles in diverse biological processes in multiple tissue types, including cell adhesion, migration (Fan et al. 2000), angiogenesis (Brigstock 2002), wound repair, and ECM remodeling (Ponticos 2013; Sachdeva et al. 2017). Expression of CTGF is regulated by growth factors and cytokines (including angiotensin II (Gao et al. 2007), bone morphogenetic proteins, endothelin (Shi-Wen et al. 2007)), and mechanical stimuli, including hemodynamic forces (shear stress, high pressure/wall tension) (Ponticos 2013). Angiotensin II-induced hypertension is a strong inducer of vascular CTGF expression (Fig. 1a, b) (de las Heras et al. 2006, 2007; Ruperez et al. 2003a, b). There is also strong in vitro and ex vivo evidence that TGF β is a particularly important regulator of CTGF expression. Accordingly, vascular CTGF expression correlates with TGF β expression (Fig. 1a, b). VEGF also induces CTGF, which has an important clinical relevance for dysregulation of angiogenesis (Inoki et al. 2002; Lee et al. 2015), e.g., in diabetic retinopathy (Hinton et al. 2004).

Role of CTGF in age-related vascular pathologies

CTGF is widely expressed during development in the cardiovascular system, and CTGF-deficient embryos die shortly after birth due to complex developmental defects. In adulthood, CTGF likely plays a role in the pathogenesis of several diseases affecting the heart, including heart failure, cardiac fibrosis, and scar formation post-myocardial infarction (Ponticos 2013). In the vasculature, upregulated CTGF expression has been linked to atherogenesis (Cicha et al. 2005, 2006; Oemar et al. 1997), smooth muscle cell apoptosis (Hishikawa et al. 1999), and aneurysm formation (Branchetti et al. 2013; Meng et al. 2014; Muratoglu et al. 2013; Sachdeva et al. 2017; Wang et al. 2006). Experimental studies indicate that angiotensin II and increased pressure/wall tension result in upregulation of CTGF, which promote changes in the synthetic phenotype in vascular smooth muscle cells (Branchetti et al. 2013). The resulting remodeling of the ECM compromises the structural integrity of vascular wall, likely promoting aneurysm formation and aorta dissection/rupture (Meng et al. 2014; Muratoglu et al. 2013; Wang et al. 2006). Upregulation of CTGF may also be involved in the pathogenesis of hypertension-induced cerebral microhemorrhages by weakening the

Table 1 Pathological role of CCN family member matricellular proteins in the vasculature

CCN member	Aliases	Biological role in the vasculature
CCN1	IGFBP10	<ul style="list-style-type: none"> • Angiogenesis • Regulation of cell migration and cell adhesion • Atherogenesis
CCN2	CTGF, IGFBP8, HCS24	<ul style="list-style-type: none"> • Atherogenesis • Angiogenesis • Remodeling • Aneurysm formation
CCN3	Nov, IGFBP9	<ul style="list-style-type: none"> • Angiogenesis • Regulation of cell adhesion, migration, proliferation, differentiation, and survival • Atherogenesis (Shi et al. 2017)
CCN4	WISP-1, Elm-1	unknown
CCN5	WISP-2, CTGF-L	• Remodeling
CCN6	WISP-3	unknown

Fig. 1 Hypertension and aging upregulates vascular CTGF expression. **a** Correlation between mRNA expression of TGF β and CTGF in aorta of young (3 months old) and aged (24 months old) normotensive and hypertensive mice. Hypertension was induced by infusion of angiotensin II (Ang II; 1000 ng/min/kg in sterile saline, via subcutaneously implanted osmotic minipumps (Toth et al. 2013a, b)). **b** Correlation between mRNA expression of TGF β and CTGF in middle cerebral arteries of control and IGF-

1 deficient normotensive and hypertensive mice. To mimic endocrine changes associated with aging adult-onset circulating IGF-1 deficiency was induced in *Igf1^{fl/y}* mice by adeno-associated virus (AAV8)-mediated expression of Cre recombinase in the liver at 4 months of age, as reported (Ashpole et al. 2015, 2017; Bailey-Downs et al. 2012; Mitschelen et al. 2011; Tarantini et al. 2016a, b, c; Toth et al. 2014, 2015a). mRNA expression was assessed by qPCR as described (Tarantini et al. 2015, 2016a, c, 2017a, b)

microvascular wall (Tarantini et al. 2017b; Toth et al. 2015b; Ungvari et al. 2017). CTGF is also expressed in atherosclerotic plaques and is thought to play a role in regulation of plaque stability (Game et al. 2007; Ponticos 2013) as well as chemotaxis of leukocytes (Cicha et al. 2005). In diabetic retinopathy, CTGF contributes to thickening of the retinal capillary basal lamina and is involved in loss of pericytes (Klaassen et al. 2015). Interestingly, recent data suggest that downregulation of CTGF via IGF-1 signaling in dilated cardiomyopathy attenuates myocardial fibrosis and improves cardiac function (Touvron et al. 2012).

The available evidence suggests that aging is associated with upregulation of CTGF expression both in the vasculature (Fig. 1a) and the heart (Chiao et al. 2012), which may contribute to age-related remodeling of the ECM (Fleenor et al. 2010). Age-related upregulation of CTGF is potentially interesting as the biological processes regulated by CTGF (ECM remodeling) are involved in the development of a wide range of age-related vascular pathologies, from atherosclerosis and aorta dissection to cerebral microhemorrhages. The molecular mechanisms underlying age-related increases in CTGF expression are not well understood. Although IGF-1 signaling is considered as an evolutionarily conserved endocrine longevity assurance pathway (Ashpole et al. 2017; Bennis et al.

2017; Podlutzky et al. 2017) and there is a strong evidence that age-related decline in circulating IGF-1 levels promote cardiovascular aging (Sonntag et al. 2013; Tarantini et al. 2016b; Ungvari and Csiszar 2012), experimentally induced IGF-1 deficiency does not result in marked upregulation of CTGF in the vasculature (Fig. 1b). There are reports suggesting that in cardiac myocytes aging-associated downregulation of microRNAs 18a, 19a, and 19b, all of which target *Ctgf*, is causally linked to upregulation of CTGF (van Almen et al. 2011). These CTGF targeting miRNAs are also known to be downregulated in aged vascular cells (Csiszar et al. 2014), but their role in dysregulation of vascular CTGF expression remains to be elucidated.

More recently, upregulation of CTGF expression has been demonstrated in senescent cells (Kim et al. 2004). Vascular aging and pathological conditions characterized by accelerated vascular aging are characterized by the increased presence of senescent cells in the vascular wall (Ungvari et al. 2010b). For example, the presence of senescent vascular cells has been demonstrated in human atherosclerotic lesions. Senescent vascular cells develop a distinct senescence-associated secretory phenotype (SASP) (Ungvari et al. 2013), which likely contributes to the chronic low-grade sterile inflammation of the aged vasculature (Ungvari et al. 2010b). The SASP is thought

to induce paracrine senescence in healthy neighboring cells. Importantly, there are in vitro data suggesting that CTGF, similar to TGF β , may contribute to the induction of paracrine senescence (Jun and Lau 2017).

Another potentially important mechanism that may exacerbate age-related CTGF expression is infection with cytomegalovirus (CMV) (Inkinen et al. 2001). In the USA, 54% of adults 30–39 years of age and 91% of adults 80 years of age or older are CMV seropositive (Aiello et al. 2017; Jackson et al. 2017; Leng et al. 2017; Nikolich-Zugich and van Lier 2017; Souquette et al. 2017; Staras et al. 2006) and CMV infection has been linked to upregulation of CTGF (Inkinen et al. 2001).

There is growing evidence that cerebrovascular pathologies play a critical role in the pathogenesis of Alzheimer's disease (AD). Recent studies suggest that CTGF expression in the brain of AD patients correlates with the progression of clinical dementia and amyloid burden (Ueberham et al. 2003; Zhao et al. 2005). In mouse models of AD-type amyloid neuropathology, consumption of a diabetogenic diet resulted in a significant elevation in CTGF in the brain, which was associated

with an increased AD-type amyloid plaque burden (Zhao et al. 2005). In that context, it is interesting that increases in brain CTGF expression induced by consumption of a high-fat diet (Fig. 2) are exacerbated in the Nrf2-deficient mouse model of accelerated microvascular aging (Ungvari et al. 2010a, 2011a, b, c). Although the effects of high-fat diet specifically on microvascular CTGF still have to be demonstrated, there are strong data showing that high-fat diet upregulates CTGF expression in large arteries (Martinez-Martinez et al. 2014, 2016).

Important insight into the role of CTGF in cerebrovascular pathologies and neurodegenerative diseases comes from studies of the rare, recessively inherited CARASIL (cerebral autosomal recessive arteriopathy with subcortical infarcts and leukoencephalopathy) syndrome (also known as Maeda syndrome (Maeda et al. 1976)). In CARASIL, the clinical picture and white matter changes are similar to those observed in CADASIL, but the onset of cognitive decline begins much earlier (Tikka et al. 2014). CARASIL is caused by mutations in the HTRA1 gene, which impairs the function of the serine protease HTRA1 as repressor of TGF β signaling (Hara et al. 2009) (HTRA1 inhibits TGF β signaling by cleaving proTGF β 1 in the ER). Increased TGF β levels in the vascular wall promote extracellular matrix accumulation and vascular remodeling via upregulating the production of CTGF and other downstream targets of TGF β signaling. As a

Fig. 2 Obesity upregulates CTGF expression. Synergistic effects of Nrf2 deficiency and obesity on cerebral mRNA expression of *Ctgf* in wild type control and Nrf2^{-/-} mice fed a high-fat diet (HFD) or standard diet (SD). Obesity-induced upregulation of CTGF (Elmarakby and Sullivan 2012; Tan et al. 2013) has been demonstrated in the vascular wall (Martinez-Martinez et al. 2016). Nrf2 is a transcription factor that orchestrates cellular defenses against oxidative stress by upregulating the expression of genes whose protein products are involved in the detoxification of reactive oxygen species. There is strong evidence that activation of the Nrf2-antioxidant response element signaling pathway in blood vessels protects the vasculature from obesity-induced adverse effects (Ungvari et al. 2010a, 2011c). mRNA expression was assessed by qPCR

Fig. 3 Potential role of increased CTGF expression in the vascular wall in age-related micro- and macrovascular pathologies

consequence, small cerebral arteries in patients with CARASIL exhibit marked arteriosclerotic changes. Interestingly, a decrease in CTGF expression in HtrA1-deficient mice was reported (Beaufort et al. 2014).

Perspectives

In conclusion, CTGF is a key mediator that modulates the actions of many other growth factors in the vascular wall and regulates the formation and remodeling of the ECM, and thereby contributes to a range of macro- and microvascular pathologies associated with aging (Fig. 3). There is increasing evidence that CTGF is upregulated in senescence. Our current understanding of its critical role in the pathogenesis of diabetic retinopathy, including its role in regulation of basal lamina thickening, pericyte apoptosis, angiogenesis, and structural reorganization of the extracellular matrix, suggests that it may play an equally important role in microvascular pathophysiology in aging. Further studies are warranted to understand the mechanistic role of CTGF and other matricellular proteins in remodeling and altered homeostasis of the extracellular matrix in vascular aging.

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