Recent Progress in the Study of Vasoactive Modulators in Metabolic and Cardiovascular Diseases

Review

New Insights into the Role of Basement Membrane-Derived Matricryptins in the Heart

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The extracellular matrix (ECM), which contributes to structural homeostasis as well as to the regulation of cellular function, is enzymatically cleaved by proteases, such as matrix metalloproteinases and cathepsins, in the normal and diseased heart. During the past two decades, matricryptins have been defined as fragments of ECM with a biologically active cryptic site, namely the ‘matricryptic site,’ and their biological activities have been initially identified and clarified, including anti-angiogenic and anti-tumor effects. Thus, matricryptins are expected to be novel anti-tumor drugs, and thus widely investigated. Although there are a smaller number of studies on the expression and function of matricryptins in fields other than cancer research, some matricryptins have been recently clarified to have biological functions beyond an anti-angiogenic effect in heart. This review particularly focuses on the expression and function of basement membrane-derived matricryptins, including arresten, canstatin, tumstatin, endostatin and endorepellin, during cardiac diseases leading to heart failure such as cardiac hypertrophy and myocardial infarction.

Key words cardiac hypertrophy; cardiomyocyte; fibroblast; matricryptin; myocardial infarction; myofibroblast

1. INTRODUCTION

The extracellular matrix (ECM) surrounding cardiac cells acts as a structural framework for tissues and regulates the function of neighboring cells (e.g., cell adhesion, proliferation, migration and differentiation), and as such plays a key role in maintaining cellular homeostasis.^{1,2} ECM is composed of collagens, fibronectin, laminin, proteoglycans (e.g., versican, perlecan, syndecan and glypican), matricellular proteins, a family of non-structural glycoproteins (e.g., secreted protein acidic and rich in cysteine, thrombospondins, tenascins and periostin), and glycosaminoglycans (e.g., chondroitin sulfate, heparan sulfate and hyaluronan).¹ When a tissue is placed under a pathological condition, it is necessary for ECM to respond to the surrounding environment through binding a membrane receptor, integrin.^{2,3} Thereafter, various etiological stimuli, such as oxidative stress, pressure overload, growth factors and inflammatory cytokines, promote the degradation of ECM by proteases including matrix metalloproteinases (MMPs) and cathepsins.^{4,5} Matricryptin has been defined as a group of fragments cleaved from ECM which features matricryptic sites.⁶ In a broad sense, the fragments of ECM-associated enzymes (MMPs, a disintegrin and metalloproteinases and lysyl oxidase) and ECM-affiliated proteins (e.g., mucins, galectins and semaphorins) are also considered as matricryptins.^{7–9} Because most matricryptins have been clarified to be an endogenous anti-angiogenic and/or anti-tumor factor, they are expected to be novel anti-tumor drugs and are thus being widely investigated.¹⁰ Matricryptins also regulate fibrosis, wound-healing, inflammation and vasocontractility, and are considered to be associated with various diseases, such as cancer, arthritis,

pulmonary and kidney disease.^{11–21} Over the past decade, the expression level of matricryptins has been revealed to change in patients with cardiovascular diseases and/or in experimental models of cardiovascular diseases (Table 1). Moreover, a novel type I collagen $\alpha 1$ chain-derived matricryptin, p1158/59, has been reported to facilitate left ventricular remodeling in post-myocardial infarction by regulating scar formation.²² Thus, the relevance of matricryptins to cardiac diseases has been gaining attention.

The basement membrane communicates with adjacent cells to provide structural support, and serves as a storage site for soluble growth factors, which are then excised by proteases and endo- β -D-glucuronidase. It also mediates biological activities including migration, proliferation, adhesion and differentiation of marginal cells through interaction with integrins and other cell surface receptors.^{23–25} In addition, the glomerular basement membrane is essential for filtration.²⁶ The basement membrane is mainly composed of type IV collagen, laminin, entactin and perlecan. Moreover, types XV and XVIII collagens are also components of the basement membrane.²⁷ Type IV collagen, which has six types of α chains ($\alpha 1$ to $\alpha 6$), is a major basement membrane component and consists of a triple helical structure.^{28,29} The $\alpha 1$ and $\alpha 2$ chains are ubiquitously expressed in the basement membrane surrounding cardiomyocytes.^{30,31} The non-collagenous (NC) 1 domain of type IV collagens has been identified to contain several matricryptins: arresten ($\alpha 1$), canstatin ($\alpha 2$), tumstatin ($\alpha 3$), tetrastatin ($\alpha 4$), pentastatin ($\alpha 5$) and hexastatin ($\alpha 6$).^{32–35} The types XV and XVIII collagens, which belong to the multiplexin family, are proteoglycans found in the basement membrane.³⁶ Restin is a fragment from the NC domain of type XV collagen.³⁷

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Table 1. Expression and Function of Basement Membrane-Derived Matricryptins in Heart

Matricryptins	Precursor protein	Expression and function	References
Arresten	Type IV collagen $\alpha 1$ chain	Increased expression in ischemia–reperfusion injury under mild hypothermic condition in pig	Lauten <i>et al.</i> , 2016
Canstatin	Type IV collagen $\alpha 2$ chain	Decreased expression in mouse myocardial infarction model Stimulation of proliferation, secretion of MMP-2 and MMP-9 and inhibition of contraction in myofibroblasts derived from rat myocardial infarction model Inhibition of H ₂ O ₂ apoptosis in H9c2 cardiomyoblasts Stimulation of migration in rat cardiac fibroblasts Inhibition of isoproterenol-induced apoptosis in differentiated H9c2 cardiomyoblasts	Sugiyama <i>et al.</i> , 2017 Sugiyama <i>et al.</i> , 2017 Kanazawa <i>et al.</i> , 2017 Okada <i>et al.</i> , 2017 Okada <i>et al.</i> , 2016
Tumstatin	Type IV collagen $\alpha 3$ chain	Stimulation of proliferation and migration in rat cardiac fibroblasts Inhibition of H ₂ O ₂ -induced apoptosis in H9c2 cardiomyoblasts Decreased expression of ischemia–reperfusion injury under mild and deep hypothermic condition in pig Increased expression of pressure-overload-induced hypertrophied myocardium in newborn rabbit	Yasuda <i>et al.</i> , 2017 ^b Yasuda <i>et al.</i> , 2017 ^a Lauten <i>et al.</i> , 2016 Nikolova <i>et al.</i> , 2012
Endostatin	Type XVIII collagen $\alpha 1$ chain	Cardioprotective role on MCT-induced RV disease through inhibiting I_{CaT} Inhibition of bradykinin-induced mouse atrial contraction Stimulation of proliferation and migration in rat cardiac fibroblasts Increased expression in pressure overload-induced mouse and newborn rabbit cardiac hypertrophy models Increased expression in mouse myocardial infarction model Protective role on rat myocardial infarction model Increased expression in serum of pulmonary arterial hypertension, CHD and chronic heart failure patients Decreased expression in pericardial fluid of CAD patients with collaterals No predictive information in CAD and CHD patients	Imoto <i>et al.</i> , 2016 Yasuda <i>et al.</i> , 2015 ^a Yasuda <i>et al.</i> , 2015 ^b Okada <i>et al.</i> , 2015 Givvimani <i>et al.</i> , 2013, 2012, 2011, 2010 Nikolova <i>et al.</i> , 2012 Qipshidze <i>et al.</i> , 2012 Isobe <i>et al.</i> , 2010 Damico <i>et al.</i> , 2015 Mitsuma <i>et al.</i> , 2007 Gouya <i>et al.</i> , 2014 Panchal <i>et al.</i> , 2004 Liou <i>et al.</i> , 2006 Eleuteri <i>et al.</i> , 2016 Ueland <i>et al.</i> , 2015
Endorepellin	Perlecan domain V	Increased expression in ischemia–reperfusion injury under mild hypothermic condition in pig	Lauten <i>et al.</i> , 2016

MMP: matrix metalloproteinase, MCT: monocrotaline, RV: right ventricular, I_{CaT} : T-type Ca^{2+} channel current, CHD: coronary heart disease, CAD: coronary artery disease.

Endostatin is cleaved from type XVIII collagen by MMPs and other proteases.^{38–44)} Endorepellin is released from a domain V of perlecan, which is a large basement membrane-type heparan sulfate proteoglycan.^{45,46)} Recently, we and others have focused on some of these basement membrane-derived matricryptins, which are proposed to contribute to regulate cardiac remodeling through modulating cardiac cells (Table 1). In this review, we would like to introduce and summarize those studies, and discuss their roles in cardiac remodeling.

2. BASEMENT MEMBRANE-DERIVED MATRICRYPTINS

2.1. Arresten Arresten, a 26 kDa fragment from the NC1 domain of the type IV collagen $\alpha 1$ chain, was first isolated from human placenta.³²⁾ Membrane type 1 (MT1)-MMP and MT2-MMP are thought to be involved in the release of arresten⁴⁷⁾ which is degraded by cathepsin S.⁴⁸⁾ Arresten exerts anti-angiogenic and subsequent anti-tumor activity by inhibiting proliferation, migration, tube formation and matrigel

neovascularization, as well as promoting apoptosis in endothelial cells and by suppressing the migration and invasion of tumor cells.^{32,49–51)} These activities are mediated through binding $\alpha_1\beta_1$ integrin,^{32,49–51)} and the C-terminus half of arresten is considered to be an active site.⁵⁰⁾ Arresten also suppresses the b-fibroblast growth factor-induced MMP-2 activation without suppressing its mRNA expression and secretion in mouse retinal endothelial cells.⁵²⁾ Nanoparticle-mediated arresten gene-delivery inhibits intimal hyperplasia in the murine vascular graft model.⁵³⁾ The expression level of arresten increased in ischemia–reperfusion injury under hypothermic conditions in female pigs.⁵⁴⁾ However, the biological activity of arresten in the heart has not yet been studied.

2.2. Canstatin Canstatin, a 24 kDa peptide derived from the NC1 domain of the $\alpha 2$ chain of type IV collagen, was identified by Kamphaus *et al.*³³⁾ It is suggested that canstatin is released from the $\alpha 2$ chain by MT1-MMP and MT2-MMP, while arresten is released from the $\alpha 1$ chain.⁴⁷⁾ Canstatin inhibits angiogenesis and tumor growth through the inhibition of migration and tube formation in endothelial cells,^{33,55)} and

through the induction of apoptosis in endothelial cells and cancer cells.^{56–58)} Both N-terminal (1–89 amino acid) fragments and C-terminal fragments (157–227 amino acid) of canstatin can inhibit *in vivo* tumor growth, mainly through the induction of apoptosis and the suppression of the proliferation in endothelial cells, respectively.^{59,60)} These anti-angiogenic and anti-tumor effects of canstatin are proposed to be mediated by binding its predicted receptor, $\alpha_v\beta_3$ and/or $\alpha_v\beta_5$ integrin.⁵⁶⁾ Although the expression level of canstatin in healthy individuals and patients with cardiac diseases has not yet been clarified, we recently clarified that canstatin is constitutively expressed in the normal myocardium, and that the expression level in the infarcted area is significantly decreased at 2 weeks after myocardial infarction in rats.⁶¹⁾ Because canstatin is degraded by cathepsin S,⁴⁸⁾ and the expression and activity of cathepsin S increases in the experimental mouse myocardial infarction model,⁶²⁾ canstatin might be degraded after myocardial infarction. Recently, we clarified that canstatin prevents isoproterenol-induced apoptosis through the inhibition of mitochondrial fission by suppressing the dephosphorylation of dynamin related protein 1 at Ser637 in differentiated H9c2 cardiomyoblasts.⁶³⁾ We also found in H9c2 cardiomyoblasts that canstatin suppressed hypoxia-induced apoptosis through the activation of the focal adhesion kinase (FAK)/Akt signaling pathway by binding $\alpha_v\beta_3$ and/or $\alpha_v\beta_5$ integrin.⁶⁴⁾ Furthermore, it has been shown that canstatin stimulates the migration of cardiac fibroblasts by the secretion of MMP-2,⁶⁵⁾ and that it promotes the proliferation and secretion of MMPs, as well as inhibits collagen gel contraction in myofibroblasts derived from the infarcted area of the myocardial infarction model rat.⁶¹⁾ Collectively, it is suggested that canstatin plays multiple roles in several types of cardiac cells, and potentially regulates cardiac remodeling.

2.3. Tumstatin Tumstatin, a 28 kDa NC fragment cleaved from the C-terminal region of the type IV collagen $\alpha 3$ chain, was named by Maeshima *et al.* based on its unique property of causing “tumor stasis.”³⁵⁾ In adult humans, the type IV collagen $\alpha 3$ chain mRNA is highly expressed in the kidney, skeletal muscle and lung, but not in the heart, brain, placenta, liver or pancreas.⁶⁶⁾ Tumstatin is produced by MMPs, especially MMP-9.⁶⁷⁾ Its physiological concentration is approximately 336 ng/mL in normal mice⁶⁷⁾ and 68.5 ng/mL in the healthy human.¹⁵⁾ The serum concentration of tumstatin increases in human lung carcinoma patients with metastases (*ca.* 90.4 ng/mL), and conversely decreases in patients without metastases (*ca.* 45.2 ng/mL).¹⁵⁾ Tumstatin is well known to have both anti-angiogenic and anti-tumor properties through the inhibition of proliferation and the induction of apoptosis in endothelial cells and tumor cells.^{35,67–73)} These effects of tumstatin are exerted through the regulation of the FAK/phosphatidylinositol 3-kinase (PI3K)/Akt/mammalian target of the rapamycin pathway *via* binding its receptor, $\alpha_v\beta_3$ integrin, in an RGD (Arg-Gly-Asp)-independent manner.^{67,68,70–72,74,75)} Tumstatin also binds $\alpha_3\beta_1$ integrin, which inhibits $\alpha_v\beta_3$ integrin activity.⁷⁶⁾ The fragment of 54–132 amino acids of tumstatin is important in its anti-angiogenic activity, and the cleaved fragments, such as T3 peptide (69–88 amino acids) and T7 peptide (74–98 amino acids), also preserve the activity.^{71,77)} It was reported that tumstatin suppresses vascular remodeling and inflammation in the experimental asthma model of mouse and sheep.^{11,78)} It also inhibits glomerular hypertrophy

in experimental diabetic mice.⁷⁹⁾ The expression level of tumstatin was significantly increased in pressure-overload-induced hypertrophied myocardium in the newborn rabbit.⁸⁰⁾ On the other hand, the expression of tumstatin decreased in the myocardium after ischemia–reperfusion injury under mild and deep hypothermic conditions in pig.⁵⁴⁾ Recently, we have shown that the T3 peptide prevents H₂O₂-induced apoptosis in H9c2 cardiomyoblasts.⁸¹⁾ We also found that it stimulates the proliferation and migration of adult rat cardiac fibroblasts,⁸²⁾ indicating that tumstatin might participate in the process of cardiac remodeling during cardiac disease.

2.4. Endostatin Endostatin, a C-terminal fragment of the collagen XVIII $\alpha 1$ chain localized in the vessel wall and basement membrane, was originally isolated from the conditioned media of a non-metastatic murine hemangioendothelioma cell line, EOMA, by O'Reilly *et al.*⁴²⁾ After the type XVIII collagen is enzymatically cleaved in the hinge region of the C-terminal NC 1 domain by proteinases, including MMPs (-2, -3, -7, -9, -12, -13 and -20), MT1-MMP, cathepsins (B, L, S and V) and elastase, then 20 and 24–30 kDa endostatin-containing peptides were released.^{38–41,43,44)} Endostatin is the most studied matricryptin, having potent anti-angiogenic properties and anti-tumor effects through the inhibition of neovascularization. The anti-angiogenic effect of endostatin has been clarified to be dependent on the inhibition of proliferation and migration, and the induction of autophagy and apoptosis in endothelial cells, while its molecular mechanisms are complex because endostatin is able to bind various cell surface receptors such as $\alpha_v\beta_3$, $\alpha_v\beta_5$, $\alpha_5\beta_1$ integrins, vascular endothelial growth factor (VEGF) receptor (VEGFR) 1, VEGFR2, glypican-1 and glypican-4.^{72,83–88)} Furthermore, endostatin has a variety of cellular functions in addition to its anti-angiogenic and anti-tumor effects, such as the prevention of proliferation and migration of osteoblastoma through inhibition of the T-type calcium ion channel,⁸⁹⁾ the reduction of blood pressure *via* nitric oxide (NO) production,^{19,90,91)} the inhibition of dermal and pulmonary fibrosis,^{20,92,93)} and the prevention of inflammatory arthritis.^{14,21,94,95)} Based on these studies, endostatin-derived recombinant proteins are expected to be used as anti-tumor, anti-fibrotic and anti-rheumatoid arthritis drugs. Among these, a modified human endostatin with a zinc binding peptide in the N-terminus (ZBP-endostatin; its trade name is Endostar), which is more stable and effective than native endostatin, has been approved by the State Food and Drug Administration of China for the treatment of non-small-cell lung cancer.^{96–98)} Several studies have shown the upregulation of endostatin expression in heart tissue and/or serum level in a variety of cardiovascular diseases.^{80,99–106)} The median serum concentration of endostatin in healthy subjects ranges from 14–78 ng/mL.^{107–109)} A high serum level of endostatin seems to be correlated with a higher risk of mortality in patients with pulmonary arterial hypertension (*ca.* 95 ng/mL) and chronic heart failure (*ca.* 245 ng/mL).^{99,104)} On the other hand, several studies suggest that the serum endostatin level adds no predictive information in patients with coronary artery disease and chronic heart failure.^{110–112)} Thus, it remains unclear whether the upregulation of endostatin in cardiac diseases plays a role in the progression of the cardiac disease. Givvimani *et al.* have reported that the transition from compensatory to decompensate heart failure is associated with the upregulation of endostatin following the activation of MMP-9 in the mouse

Fig. 1. Basement Membrane-Derived Matricryptins in the Development of Cardiac Diseases

Pathological cardiac remodeling associated with myocardial infarction and cardiac hypertrophy, which are caused by ischemic stress and pressure overload, respectively, eventually results in decompensated heart failure. Matricryptins cleaved from the extracellular matrix (ECM) by proteases exert various biological functions on cardiac cells, and might regulate the pathological cardiac remodeling both positively and negatively. Endothelial angiogenesis mediates enhanced cardiomyocyte viability and adaptive hypertrophy. Matricryptins can inhibit endothelial angiogenesis. After myocardial infarction, cardiac fibroblasts migrate into the infarcted area, proliferate, secrete matrix metalloproteinases (MMPs) and differentiate into myofibroblasts. Myofibroblasts are involved in wound healing by the secretion of ECM proteins, and stabilize the scar tissue by contraction. On the other hand, chronic activation of fibroblasts/myofibroblasts induces excessive fibrosis. Matricryptins probably have a key role in these fibrotic processes through the regulation of fibroblast/myofibroblast functions. Ischemia-induced cell death and pressure overload-induced cardiomyocyte hypertrophy accompanied by functional and electrical remodeling cause contractile dysfunction and arrhythmia. Matricryptins might exert a cytoprotective effect partly through activating a survival pathway and modulating ion channel activity.

aortic stenosis model.¹⁰³⁾ In the rabbit pressure overload-induced left ventricular hypertrophy model, the suppression of endostatin-release by an MMP-9 specific inhibitor-treatment preserved cardiac contractility.⁸⁰⁾ In patients with coronary arterial disease, high pericardial endostatin level is related to the decreases in coronary collateral formation, which is beneficial for diminishing the infarcted area and improving the prognosis of the patients.¹¹³⁾ These studies indicate that endostatin can be harmful in the pathology of cardiac disease. On the other hand, several studies have reported cardioprotective effects of endostatin. Isobe *et al.* reported that an anti-endostatin antibody treatment exacerbates the pathology of left ventricular myocardial infarction through the activation of MMP-2, -9 and collagen production.¹¹⁴⁾ The T-type calcium ion channel, which participates in the process of cardiac remodeling, including cardiac hypertrophy and arrhythmia, is re-expressed in the pressure overload-induced hypertrophied heart.^{115–119)} We have recently shown that endostatin inhibits the T-type calcium ion channel in a ventricular cardiomyocyte from guinea pig,¹²⁰⁾ as well as in a cardiomyocyte from hypertrophied right ventricles of monocrotaline-induced pulmonary hypertensive rats.¹²¹⁾ In addition, treatment with small interfering (si)RNA against collagen XVIII decreases the survival rate and exacerbates right ventricular remodeling.¹²¹⁾ Endostatin also has biological effects in the heart, such as the inhibition of bradykinin-induced atrial contractions, per-

haps through inhibition of the L-type calcium channel¹²²⁾ and the stimulation of the proliferation and migration of cardiac fibroblasts through activation of the PI3K/Akt signaling pathway.¹²³⁾ Further study is required to clarify whether endostatin exacerbates or prevents cardiac remodeling.

2.5. Endorepellin Perlecan, a large heparan sulfate proteoglycan, is ubiquitously expressed in the basement membrane.⁴⁵⁾ Endorepellin, a fragment of domain V of perlecan's core protein (*ca.* 85 kDa), named by Mongiat *et al.*,⁴⁶⁾ has an anti-angiogenic function through mechanisms which include the inhibition of endothelial cell migration, tube formation and the induction of autophagy in endothelial cells.^{46,124–128)} These effects were mediated through antagonism to its dual receptors, $\alpha_2\beta_1$ integrin and VEGFR2.^{124–128)} On the other hand, endorepellin exerts a pro-angiogenic effect on brain endothelial cells through VEGF production by binding the $\alpha_3\beta_1$ integrin.¹²⁹⁾ Although the precise mechanism of endorepellin cleavage from perlecan has not been clarified, cathepsins, MMPs and chymase are thought to be candidate molecules responsible for its cleavage.¹³⁰⁾ Endorepellin is further cleaved into LG3 fragment, a C-terminus fragment cleaved by cathepsin L, and bone morphogenetic protein-1-Tolloid-like proteases,^{131,132)} which promotes neointima formation in the vascular rejection of allograft through the migration and survival of vascular smooth muscle cells *via* the extracellular signal-regulated kinase-dependent pathway.¹³³⁾ Both perlecan and endorepellin

have key roles in the development of cardiovascular and musculoskeletal function in zebrafish embryos.¹³⁴⁾ Ischemia–reperfusion injury under mild hypothermic condition in female pig caused a significant increase in LG3 expression.⁵⁴⁾ Currently, there have been no further reports determining the precise roles of endorepellin in the mammalian heart tissue and cells.

3. PERSPECTIVES ON THE ROLES OF BASEMENT MEMBRANE-DERIVED MATRICRYPTINS IN CARDIAC DISEASES

In the development of cardiac diseases, matricryptins cleaved from the basement membrane are expected to exert various biological functions on cardiac cells, and to contribute to the regulation of pathological cardiac remodeling processes (Fig. 1).

Following myocardial infarction, adequate angiogenesis is necessary for the survival and adaptive hypertrophy of the remaining cardiomyocytes to gain oxygen and nutrients.^{135,136)} Therefore, the induction of angiogenesis is expected to be one therapeutic method for myocardial infarction.^{137,138)} On the other hand, inadequate angiogenesis mediates the transition from compensated cardiac hypertrophy to decompensated heart failure.¹³⁶⁾ All of the basement membrane-derived matricryptins focused on in this article have an anti-angiogenic effect. The disruption of angiogenesis by arresten and endorepellin increased in the injured myocardium after ischemia–reperfusion,⁵⁴⁾ which might exacerbate the pathological condition. It is also presumed that the decreases in canstatin in the area of myocardial infarction,⁶¹⁾ and in tumstatin in the endothelial basement membrane of the injured myocardium after ischemia–reperfusion⁵⁴⁾ potentially promote adaptive cardiac remodeling through the promotion of angiogenesis. Conversely, increases in tumstatin in the pressure-overload-induced hypertrophied myocardium⁸⁰⁾ might contribute to pathological cardiac hypertrophy. The endostatin level in pericardial fluid is decreased in patients with coronary artery disease with collateral formation, in comparison to those without it.¹¹³⁾ Exogenous hydrogen sulfide inhibits endostatin expression and exerts a cardioprotective effect in the mouse myocardial infarction model.¹⁰⁶⁾ These observations indirectly suggest that endostatin is detrimental in cardiac remodeling by its anti-angiogenic effect. However, anti-endostatin antibody-treatment leads to a deterioration in mortality, cardiac hypertrophy and myocardial fibrosis in the rat myocardial infarction model, despite promoting angiogenesis.¹¹⁴⁾ Moreover, knockdown of the endostatin gene worsens monocrotaline-induced right ventricular remodeling.¹²¹⁾ Thus, increased endostatin during myocardial infarction and pressure overload-induced hypertrophy potentially exerts a cardioprotective effect through the suppression of pathological cardiac remodeling, regardless of its anti-angiogenic effect.

After myocardial infarction, cardiac fibroblasts migrate into the infarcted area, proliferate and differentiate into myofibroblasts, which are involved in wound healing by the secretion of collagens and the maturation of scar tissue through contraction. On the other hand, chronic activation of myofibroblasts induces excessive fibrosis, which causes cardiac dysfunction.^{139,140)} We have clarified that canstatin promotes the migration of cardiac fibroblasts,⁶⁵⁾ stimulates the proliferation and secretion of MMPs, and inhibits collagen gel contraction

in myofibroblasts isolated from the area of myocardial infarction.⁶¹⁾ In addition, the expression of canstatin in the infarcted area is significantly decreased at 2 weeks after myocardial infarction in rats.⁶¹⁾ In the acute phase of myocardial infarction, canstatin likely induces the migration of cardiac fibroblasts into the infarcted area. In the late phase, decreased canstatin in the area of myocardial infarction leads to the suppression of proliferation and ECM degradation through the inhibition of MMPs secretion, as well as the promotion of contraction in myofibroblasts, which latently contribute to the maturation of scar tissue. Tumstatin is significantly increased in the pressure-overload-induced hypertrophied myocardium during both compensated and decompensated stages.⁸⁰⁾ We have shown that tumstatin stimulates the proliferation and migration of cardiac fibroblasts.⁸²⁾ Thus, tumstatin might play a pro-fibrotic role in the development of decompensated cardiac hypertrophy. It was demonstrated that an anti-endostatin antibody increased fibrosis in the non-infarcted area of the myocardial infarction model rat.¹¹⁴⁾ In addition, an endostatin-derived peptide, E4, prevented bleomycin-induced pulmonary fibrosis.^{20,92)} In contrast, we have shown the pro-fibrotic effects of endostatin, including the stimulation of proliferation, migration and wound healing in cardiac fibroblasts.¹²³⁾ Although endostatin is assumed to play a key role in myocardial fibrotic responses through the regulation of fibroblast functions, further study is needed to clarify whether it worsens or suppresses myocardial fibrosis.

Since cardiomyocytes have poor regenerative capacity, ischemia (hypoxia)-induced cardiomyocyte death associated with myocardial infarction results in contractile dysfunction and subsequent decompensated heart failure.^{141,142)} Pressure overload causes electrical remodeling, which is associated with cardiac hypertrophy, contractile disorder and arrhythmia through the functional alteration of ion channels, such as transient receptor potential channels, voltage-dependent calcium channels, voltage-gated sodium channels, inositol 1,4,5-trisphosphate receptor and potassium channels, in cardiomyocytes.^{115,143–151)} Most matricryptins exhibit a pro-apoptotic effect on endothelial cells. On the other hand, canstatin exerts a cytoprotective effects against cell death-inducing stress.^{63,64)} An active fragment of tumstatin, the T3 peptide, also prevents oxidative stress-induced apoptosis⁸¹⁾ in H9c2 cardiomyoblasts. From experiments using a neutralizing antibody,¹¹⁴⁾ endostatin is supposed to have a cardioprotective effect. Cav3.2, a T-type calcium channel, plays an important role in the pathogenesis of cardiac hypertrophy *via* the activation of the calcineurin/nuclear factor of activated T cells pathways.¹¹⁵⁾ Pharmacological inhibition of the T-type calcium channel reduces arrhythmia in a mouse model of dilated cardiomyopathy, and reduces sudden death in the mouse myocardial infarction model.¹¹⁷⁾ It was previously reported that endostatin prevented the proliferation and migration of osteoblastoma through the inhibition of T-type calcium channel.⁸⁹⁾ We have shown that endostatin inhibits the T-type calcium channel expressed in a ventricular cardiomyocyte from guinea pig, and in a hypertrophied cardiomyocyte from the right ventricle of monocrotaline-induced pulmonary hypertensive rat.^{120,121)} We also showed that a knockdown of the collagen XVIII (endostatin) gene by siRNA-treatment decreases the survival rate and exacerbates the monocrotaline-induced right ventricular remodeling.¹²¹⁾ Thus, the cardioprotective effect of endostatin might be ex-

erted through the inhibition of T-type calcium channel activity. Collectively, it is conceivable that basement membrane-derived matricryptins are likely endogenous cardioprotective peptides.

4. POSSIBLE APPLICATION OF BASEMENT MEMBRANE-DERIVED MATRICRYPTINS TO THE TREATMENT OF CARDIAC DISEASES

Finally, we discuss the possibility of basement membrane-derived matricryptins as a novel therapeutic target for cardiac diseases. While the N-terminal fragment of canstatin inhibits angiogenesis, mainly through the induction of endothelial apoptosis, the anti-angiogenic activity of the C-terminal fragment occurs specifically through the inhibition of endothelial proliferation.^{59,60} The N-terminal fragment of endostatin has both anti-angiogenic and anti-tumor effects.¹⁵² On the other hand, the C-terminal fragment of endostatin, named E4, exerts an anti-fibrotic effect.²⁰ While the LG1/2 region of endorepellin causes autophagy in endothelial cells *via* VEGFR2 downregulation, LG3 does not have such a function.¹²⁷ Thus, there might be various active sites which exert different physiological effects in each matricryptin. However, the specific regions which exert cardioprotective and/or pro/anti-fibrotic effects have not yet been clarified. Therefore, it is necessary to elucidate the specific active sites, and to produce applicable short fragments of matricryptins with higher efficacy in order to apply these fragments to the treatment of specific cardiac diseases.

Endostatin has a very short half-life of within 1–2 h. Fc-endostatin, a recombinant human endostatin conjugated to the Fc domain of immunoglobulin G, prolongs the half-life to more than one week, which allows it to exert its anti-tumor activity at a lower concentration than the intact endostatin.¹⁵³ The terminal elimination half-life of endostar-loaded polyethylene glycol-modified poly DL-lactide-co-glycolide nanoparticles is more than one day, and the anti-tumor effect of endostar-loaded nanoparticles is stronger than endostar-alone.¹⁵⁴ Thus, the adjustment of their pharmacokinetic behavior is also thought to be necessary for the clinical application of matricryptins.

An anti-VEGF monoclonal antibody, bevacizumab, which is used as an anti-tumor drug, increases the risk of hypertension.¹⁵⁵ Fc-conjugated endostatin, which has anti-angiogenic and anti-tumor effects, also lowers blood pressure through NO production, and counteracts the elevated blood pressure induced by an anti-VEGF antibody-treatment in mice.⁹¹ Thus, there might be an advantage in combination treatment using endostatin and anti-VEGF antibody for anti-tumor therapy. On the other hand, the potential interaction between drugs traditionally used to treat heart failure and basement membrane-derived matricryptins has not been clarified. β -Adrenergic receptor antagonists, such as carvedilol, bisoprolol and metoprolol, are widely used as therapeutic agents for heart failure.¹⁵⁶ Stimulation of the β -adrenergic receptor causes cardiomyocyte apoptosis, which is suppressed by ECM through binding β_1 integrin and activating the FAK/PI3K/Akt pathway.¹⁵⁷ Thus, matricryptins such as arresten, tumstatin, endostatin and endorepellin, which can bind β_1 integrin, might mediate the cardioprotective effect of β -adrenergic receptor antagonists.

Inhibition of the renin–angiotensin–aldosterone system by angiotensin converting enzyme (ACE) inhibitors and angiotensin II type 1 receptor (AT1R) blockers (ARBs) is widely used in the treatment of cardiovascular diseases.¹⁵⁸ Various ACE inhibitory peptides derived from animals and plants have been found.¹⁵⁹ An amino acid sequence of the NC1 domain of type XVIII collagen in the vicinity of endostatin resembles the snake venom-derived bradykinin-potentiating peptides, which strongly inhibit ACE activity.¹⁶⁰ Isobe *et al.* reported that an anti-endostatin antibody increased ACE activity in the infarcted myocardium.¹¹⁴ Thus, it is presumed that an XVIII-type collagen fragment which includes endostatin may act as an endogenous ACE inhibitor. Both AT1R and β_1 integrin act as mechanosensors for pressure-overload in cardiomyocytes, and are involved in myocardial hypertrophy and contraction *via* activation of the mitogen-activated protein kinase pathway, FAK, Akt and integrin-linked kinase (ILK).¹⁶¹ Angiotensin II can activate ILK, a serine/threonine kinase, which binds the C-terminus of β_1 integrin, and is associated with cardiomyocyte hypertrophy.¹⁶² Therefore, β_1 integrin-binding matricryptins may mediate the therapeutic effect of ARBs *via* modulating the mechanical stress-induced signaling pathway in pressure overload-induced cardiac hypertrophy.

5. CONCLUSION

Many kinds of matricryptins have been identified during the past two decades. The functional analysis of matricryptins has been performed, and almost all of them have been clarified to exhibit anti-angiogenic and anti-tumor effects. Based on this experimental groundwork, the application of matricryptins-derived proteins as an anti-tumor, anti-arthritis and anti-fibrotic drug is expected. Actually ZBP-endostatin has already been approved in China. On the other hand, during the past decade, several studies have demonstrated that expression level of basement membrane-derived matricryptins changes in cardiac disease patients and/or in experimental models of cardiac diseases. In particular, we and several other groups have recently proposed that matricryptins exhibit various biological functions on cardiac cells, and contribute to regulate cardiac remodeling. These findings indicate that the matricryptins function as endogenous regulatory factors in cardiac remodeling, and are thus potential targets for the development of novel drugs against heart failure. However, there are still many unsolved points in the role of matricryptins in the normal and diseased heart. The blood concentration of endostatin in patients with cardiac diseases has been well documented, while little is known about the concentration of other basement membrane-derived matricryptins. For this reason, further studies are needed on basement membrane-derived matricryptins other than endostatin. Their elucidation is important to future therapeutic pathways in the treatment of cardiac diseases. It is expected that further research in this field will progress in the near future.

Conflict of Interest The authors declare no conflict of interest.

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