Biochimica et Biophysica Acta 1840 (2014) 2589–2598

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbagen

Matrikines from basement membrane collagens: A new anti-cancer strategy☆

Jean Claude Monboisse [a,b], Jean Baptiste Oudart [a,b], Laurent Ramont [a,b], Sylvie Brassart-Pasco [a], François Xavier Maquart [a,b,*]

[a] FRE CNRS/URCA 7369, Université de Reims Champagne Ardenne, UFR Médecine, 51 Rue Cognacq Jay, 51095 Reims Cedex, France
[b] Laboratoire Central de Biochimie, CHU de Reims, France

ARTICLE INFO

Article history:
Received 16 October 2013
Received in revised form 19 December 2013
Accepted 31 December 2013
Available online 6 January 2014

Keywords:
Cancer
Extracellular matrix
Angiogenesis
Matrikine
Preclinical trial

ABSTRACT

Background: Tumor microenvironment is a complex system composed of a largely altered extracellular matrix with different cell types that determine angiogenic responses and tumor progression. Upon the influence of hypoxia, tumor cells secrete cytokines that activate stromal cells to produce proteases and angiogenic factors. In addition to stromal ECM breakdown, proteases exert various pro- or anti-tumorigenic functions and participate in the release of various ECM fragments, named matrikines or matricryptins, capable to act as endogenous angiogenesis inhibitors and to limit tumor progression.
Scope of review: We will focus on the matrikines derived from the NC1 domains of the different constitutive chains of basement membrane-associated collagens and mainly collagen IV.
Major conclusions: The putative targets of the matrikine control are the proliferation and invasive properties of tumor or inflammatory cells, and the angiogenic and lymphangiogenic responses. Collagen-derived matrikines such as canstatin, tumstatin or tetrastatin for example, decrease tumor growth in various cancer models. Their anti-cancer activities comprise anti-proliferative effects on tumor or endothelial cells by induction of apoptosis or cell cycle blockade and the induction of a loss of their migratory phenotype. They were used in various preclinical therapeutic strategies: i) induction of their overexpression by cancer cells or by the host cells, ii) use of recombinant proteins or synthetic peptides or structural analogues designed from the structure of the active sequences, iii) used in combined therapies with conventional chemotherapy or radiotherapy.
General significance: Collagen-derived matrikines strongly inhibited tumor growth in many preclinical cancer models in mouse. They constitute a new family of anti-cancer agents able to limit cancer progression. This article is part of a Special Issue entitled Matrix-mediated cell behaviour and properties.

1. Introduction

Tumor microenvironment is a complex structure composed of a largely modified extracellular matrix (ECM) which closely interacts with various cell types to determine tumor angiogenesis and tumor progression. Cell–matrix interactions occurring in tumor progression and angiogenesis can be controlled by bioactive fragments revealed from ECM molecules by limited proteolysis or by cryptic site exposure. These fragments, named matrikines, are protein domains exerting a biological activity [1]. The term of matricryptin also defines a protein domain which exhibits a biological activity not carried by the native protein and unmasked by proteolysis [2]. Various matrikines have been described in the literature as endogenous inhibitors of angiogenesis [3]. In the present review, we will describe the anti-tumorigenic or anti-angiogenic matrikines derived from basement membrane-associated collagens, with a particular focus on matrikines derived from type IV and type XIX collagens.

2. Tumor microenvironment

During tumor progression, cancer cells create a local microenvironment characterized by a deregulated and disorganized ECM. Abnormal ECM affects cancer progression by directly promoting cell transformation and metastasis. ECM anomalies also deregulate behavior of stromal cells, facilitate tumor-associated angiogenesis and inflammation and thus lead to the generation of a tumorigenic microenvironment [4,5]. In tumor

Abbreviations: ECM, extracellular matrix; MMP, matrix metalloproteinases; SDF-1, Stroma-Derived Factor 1; CAFs, cancer-Associated Fibroblasts; ROS, reactive oxygen species; EMT, epithelial–mesenchymal transition; NC domain, non-collagenous domain; FACIT, fibril associated collagen with interrupted triple helix; FAK, Focal Adhesion Kinase; tPA, tissue plasminogen activator

☆ This article is part of a Special Issue entitled Matrix-mediated cell behaviour and properties.

* Corresponding authors at: Pr François Xavier Maquart, FRE CNRS/URCA 3481, Université de Reims Champagne Ardenne, UFR Médecine, 51 Rue Cognacq Jay, 51095 Reims Cedex, France. Tel.: +33 3 26 78 83 46.
E-mail address: fmaquart@chu-reims.fr (F.X. Maquart).

0304-4165/$ – see front matter © 2014 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.bbagen.2013.12.029

areas, ECM consists in a surrounding basement membrane, produced by epithelial, endothelial and stromal cells, and an interstitial matrix primarily made by stromal cells. Basement membrane is composed of type IV collagen, laminins, fibronectin, proteoglycans and some linker glycoproteins. In contrast, interstitial ECM is rich in fibrillar collagens, proteoglycans and glycoproteins such as fibronectin and tenascin C [5]. The stroma consists of a highly modified ECM embedding a variety of cell types (fibroblasts, endothelial cells, pericytes, inflammatory cells, etc. …) [6]. Tumor cells and stromal cells exert cross-talks that trigger cell activation, alterations in ECM biological properties and influence the proliferation of tumor cells, their invasive properties and their metastatic potential [7]. Inflammatory cells (macrophages, neutrophils, mast cells,…) secrete reactive oxygen species (ROS) and growth factors and cytokines that exert tumor-promoting actions [8]. Among them, various growth factors such as EGF, angiogenic factors such as VEGF or FGF2, chemokines and cytokines amplify the inflammatory state. ROS affect several cancer hallmarks. They are involved in the development of a more aggressive phenotype by triggering MMP secretion and epithelial–mesenchymal transition (EMT) program activation. ROS also sustain tumor angiogenesis by their implication in pericyte recruitment and activation of endothelial progenitors through VEGF and angiopoietin release [8]. ROS are also able to participate in the ECM breakdown by collagen degradation and by inducing an increase of its susceptibility to proteases [9,10].

In cancer microenvironment, fibroblasts usually constitute the preponderant cell population. When activated by TGFβ, Stroma-Derived Factor 1 (SDF1) or several MMPs, cancer-associated fibroblasts (CAFs) share phenotypic morphology with myofibroblasts, mainly the expression of α smooth muscle actin, [11,12]. They support tumor growth and promote ECM alterations by secreting ECM-modifying enzymes and ECM macromolecules, such as type I collagen, tenascin C, or fibronectin.

Tumor and stromal cells also produce extracellular proteases such as MMPs, the plasminogen activation cascade, cathepsins, which mediate many of the changes in the tumor microenvironment. The *in vivo* functions of proteases depend on the local balance between them and their physiological inhibitors (e.g. TIMPs or PAIs) [13–15]. MMPs have long been associated with the different stages of cancer progression for their ability to degrade ECM and thus seemed to be attractive cancer targets. Many drug development programs were initiated to block their ECM-degrading properties but the trials failed to reach their end points of increased survival [16]. In addition to their ECM-degrading activity, these proteases regulate a wide variety of physiological processes and signaling events and play a key role in the molecular cross-talk between tumor and stroma [17]. For instance, MMP proteolysis regulates cell signaling and functions by a highly specific and efficient proteolytic processing of substrates. They mediate receptor, chemokine and ligand shedding as well as activation or inactivation of growth factors and chemokines to precisely regulate cellular activities [16,18–21]. The knowledge of the diverse MMP activities are largely increased in an interconnected protease web, demonstrating new anti-tumorigenic and anti-inflammatory beneficial roles, that should not be inhibited, making them drug antitargets [20,22].

In addition to its mechanical properties, ECM can directly initiate signaling events, particularly by functioning as a precursor of biologically active fragments that are capable to regulate tumor cell invasive properties [4]. Proteolytic processing of some ECM substrates by MMPs or other proteases produces biologically active fragments, the matrikines. In turn, ECM components secreted by stromal cells also change in response to the invasive and metastatic potential of the tumor cells, indicating significant cross-talk between tumor and stromal cells [7]. In highly and poorly metastatic melanoma xenograft models, mass spectrometric analyses of the stromal ECM demonstrated that both tumor cells and stromal cells contribute to the secretion of proteins to form the tumor ECM. [7].

When the tumor increases in size, tumor cells face an increasing demand for nutrient and oxygen. Proliferating tumor cells distance themselves from the vasculature and colonize a microenvironment deficient in oxygen and nutrients. They need to reprogram their metabolism, a shift mediated by an increase in reactive oxygen species (ROS) levels generated by the mitochondria. ROS contribute to Hypoxia-Inducible-Factor (HIF) stabilization [8]. HIF can regulate cell migration by increasing lysyl oxydase (LOX) expression that leads to an increase in tissue stiffness by collagen fiber cross-linking.

Moreover, hypoxia, acidosis and nutrient deprivation lead to an "angiogenic switch" characterized by alterations in gene, microRNA, and growth factor expression and secretion [8,23]. Hypoxia is the main endogenous stimulus of tumor angiogenesis and lymphangiogenesis by stimulating VEGF secretion. Activated endothelial cells acquire a proliferative capacity and synthesize proteases that degrade the pre-existing basement membrane and allow them to migrate within the tumor. Furthermore, tumor vasculature, together with the lymphatic system, is the main route through which cancer cells metastasize and immune cells infiltrate [4].

3 . Basement membrane collagens

Basement membranes are highly specialized ECM that represent a barrier between the epithelium and the underlying ECM. They consist of type IV collagen and associated collagens (collagens XV, XVIII and XIX), structural glycoproteins (laminin, entactin, …) and proteoglycans, as perlecan [24]. They provide mechanical support for the cell. Glomerular basement membrane has a more specific role of filtration. Basement membranes also serve as a reservoir of growth factors and cytokines, which, after release by proteases such as MMPs, control various cellular functions. The interactions of the various components with the cells, *via* surface receptors as integrins, regulate biological activities such as migration, proliferation or cell differentiation [25].

3.1 . Collagen IV

Collagen IV is the major component of basement membranes. It is formed by the combination of three α(IV) chains among six possible, α1(IV) to α6(IV), each encoded by a different gene [26].

The tissue distribution of the α(IV) chains is variable. While α1(IV) and α2(IV) chains are ubiquitous, other chains in minor proportion are expressed in specialized basement membranes. The α3(IV) and α4(IV) chains occur only in α3α4α5 trimers in the aorta, the pulmonary alveoli, the cochlea, the glomerulus or the lens capsule. The α5(IV) chain is found in association with α3(IV) and α4(IV) chains in the same locations, but also in association with the α6(IV) chain in kidney basement membranes, lung, esophagus and skin [27].

From the N-terminus to the C-terminus, each α(IV) chain comprises:

- an N-terminal domain comprising the 7S domain,
- a long central triple-helical domain with several interruptions, and
- a C-terminal NC1 domain of approximately 230 residues [24].

3.2 . Collagen XV

Collagen XV belongs to the family of multiplexins. From the N-terminus to the C-terminus, it consists of a non-collagenous domain (NC) of 529 amino acid residues, a collagen sequence comprising 579 residues and 8 interruptions of 7–45 residues and a C-terminal NC1 domain of 255 residues. It is mainly located in the basal neuronal membranes, mesenchymal, vascular, and some epithelial basement membranes [28]. Recombinant collagen XV inhibited fibrosarcoma cell adhesion and migration [29]. Proteolysis of the C-terminal NC1 domain gives rise to a matrikine, restin, which exerts an anti-tumor effect based on its anti-angiogenic properties [30]. Restin overexpression in human hepatoma cells strongly decreased tumor growth in mice [31]. Full length collagen XV was shown as a dose-dependent suppressor of tumorigenicity in cervical carcinoma cells independently of the presence of restin, whereas restin alone did not [32].

3.3 . Collagen XVIII

Collagen XVIII also belongs to the multiplexin family and shows a high structural homology with collagen XV. It was initially described as a heparan sulfate proteoglycan [33]. By cleavage with different MMPs (MMP-2, -7, -9, and -14) or other proteases, the C-terminal NC1 domain gave rise to a 20 kDa matrikine, endostatin [34]. Anti-tumor and anti-angiogenic activities of endostatin have been widely described in numerous reviews and will not be described here [35,36]. Collagen XVIII also contains cryptic polypeptide modules, such as an N-terminal variant containing a frizzled module (FCZ 18), sharing a structural identity with the extracellular cysteine-rich domain of the frizzled receptors. This domain inhibited *in vivo* cell proliferation and tumor growth in mouse through the Wnt/β-catenin signaling pathway [37,38].

3.4 . Collagen XIX

Collagen XIX is a minor FACIT collagen associated with basement membranes. It is formed by the combination of three chains α1(XIX). From the N-terminus to the C-terminus, it comprises:

- an N-terminal NC6 domain of 268 residues,
- a discontinuous collagenous domain of 832 residues, comprising 5 collagenous domains interrupted by 4 non-collagenous domains of 20 to 40 residues, and
- a C-terminal non-collagenous NC1 domain of 19 residues [39].

It is present in the vascular, neural and epithelial membranes and seems to play an important role in muscle differentiation and angiogenesis [40]. The cleavage of the NC1 domain gives rise to a matrikine whose anti-tumor effects have been described in a murine melanoma model [41,42].

4 . Matrikines derived from basement membrane collagens

These matrikines inhibit *in vivo* tumor growth through anti-angiogenic and/or anti-tumor activities at different levels. The first putative target is directly the cancer cells, with inhibition of their proliferation by induction of cell cycle blockade [43–45]. The inhibition can also be exerted on the migratory properties of cancer cells by inhibiting proteolytic cascades (MMPs, plasminogen activation system). The inhibition may occur through an alteration of protease location at the cell migration front by induction of their endocytosis and a reduction of their expression at the cell surface [45–47]. A second putative target of the matrikines is tumor angiogenesis, with action on endothelial cells. The inhibitory activity can be exerted on proliferation and apoptosis of endothelial cells or their migratory properties [43,47]. A third target consists in ROS production and cytokine secretion by inflammatory and immune cells, although few studies have described the action of matrikines on these cells [48]. Finally, the secretion of pro-angiogenic growth factors or cytokines by stromal fibroblasts can also be controlled by matrikines [49].

Many matrikines derive either from the triple helix domain or from NC1 domains of collagen IV. Various sequences of the collagen α1(IV) chain promote adhesion and migration of melanoma cells by binding to integrin α3β1 [50,51].

Table 1 shows the main matrikines derived from the NC1 domain of the different chains of basement membrane-associated collagens and their main biological targets.

Table 1
Matrikines derived from NC1 domains of basement membrane-associated collagens and their main targets in anti-cancer strategy. The two first columns of the table show the NC1 domains of the different basement membrane-associated collagen chains and the corresponding matrikines. The other columns indicate the anti-angiogenic and/or anti-tumoral activities of matrikines.

NC1 domain	Matrikine	Anti-angiogenic	Anti-tumor
NC1 α1(IV)	Arresten	+	+
NC1 α2(IV)	Canstatin	+	+
NC1 α3(IV)	Tumstatin	+	+
NC1 α4(IV)	Tetrastatin	−	+
NC1 α5(IV)	Pentastatin	+	−
NC1 α6(IV)	Hexastatin	+	−
NC1 α1(XV)	Restin	+	+
NC1 α1(XVIII)	Endostatin	+	+
NC1α1(XIX)	NC1 α1(XIX)	+	+

4.1 . Arresten

Arresten, the NC1 domain of the α1(IV) collagen chain, inhibited FGF-2 or VEGF-induced proliferation of endothelial cells and increased apoptosis by down-regulating anti-apoptotic Bcl family members, as Bcl-2 and Bcl-xL. It also inhibited migration and tube formation, as well as matrigel neovascularization. The active site of arresten was localized within the C-terminal half of the matrikine [52]. In endothelial cells, FGF-2-stimulated MMP-2 mRNA expression and MMP-2 secretion were not affected by arresten but MMP-2 activation was suppressed [53]. Its anti-angiogenic activity occurred *via* binding to α1β1 integrin and an intracellular signal transduction leading to the inhibition of HIF-1α factor [54,55]. Migration and invasion of human squamous carcinoma cells overexpressing arresten were inhibited and a marked increase in the expression and the localization of E-cadherin in cell–cell contact was observed [56]. Arresten caused a decrease in tumor growth with a strong decrease in tumor vasculature in various cancer models and liver metastases [57,58].

4.2 . Canstatin

Canstatin, the NC1 domain of the α2(IV) collagen chain, is an endogenous inhibitor of angiogenesis. *In vitro*, it inhibited the proliferation and migration of endothelial cells and pseudotube formation on matrigel [59,60]. The N-terminal domain (residues 1–89) of canstatin contained the sequence responsible for the induction of apoptosis, while the C-terminal sequence (residues 157–227) specifically inhibited the proliferation of endothelial cells [61,62]. The induction of apoptosis involved two signaling pathways, a first one depending on a Fas/Fas ligand pathway with activation of caspases 8 and 9, and a second depending on the binding of canstatin to αVβ5 integrin which triggered the FAK/PI3K/Akt cascade [63,64]. Canstatin inhibited angiopoietin-1-induced angiogenesis and lymphangiogenesis by inducing a decrease in the expression of angiopoietin-1 in endothelial cells and lymphatic endothelial cells under hypoxia and by decreasing their proliferative and migratory properties. It also inhibited the expression of Tie-2 and VEGFR3 [64]. When overexpressed by tumor cells, it inhibited their proliferation via a mitochondrial apoptotic mechanism [63,66]. Canstatin inhibited *in vivo* tumor growth in various cancer models by inducing senescence of tumor cells [59,63,64,66].

4.3 . Tumstatin

Tumstatin, the NC1 domain of the α3(IV) collagen chain, was shown to exert both anti-angiogenic and anti-tumor activities through two distinct sequences [67]. The sequence 54–132, called Tum-5, was responsible for the anti-angiogenic activity which resulted in induction of endothelial cell apoptosis. Its binding to the αvβ3 integrin, independently of the RGD sequence, induced a transduction pathway similar to that induced by canstatin. Tumstatin, through its 69–98 sequence, inhibited CAP-dependent protein translation *via* down-regulation of the mTOR pathway in proliferating endothelial cells [68]. The efficiency of tumstatin treatment appeared to depend on the PTEN/Akt pathway

[69]. Injection in mice of different tumstatin-overexpressing tumor cells induced a strong decrease in tumor growth [70,71].

In the C-terminal portion of tumstatin, a second sequence corresponding to residues 185–203, had a strong anti-tumor activity, demonstrated in murine and human melanoma models, with its overexpression in cancer cells or with synthetic peptides reproducing the sequence [44,46,72]. The sequence also bound to the αvβ3 integrin, independently of the RGD sequence, and induced an intracellular transduction pathway involving the phosphorylation of the early activation of FAK and PI3 kinase [73]. It reduced tumor progression by inhibiting proteolytic cascades, mainly the activation of pro-MMP-2 and the plasminogen activation system by down-regulating the secretion of tissue plasminogen activator (tPA) [46,72]. Two other recombinant peptides derived from this tumstatin sequence (peptide 19 and peptide 21) also induced apoptosis in human gastric carcinoma or in human hepatoma cells both *in vitro* and *in vivo*, as well as in endothelial cells [74,75].

4.4 . Tetrastatin

No anti-angiogenic activity has been demonstrated for the NC1domain of the α4 (IV) chain in the model of the chick chorioallantoic membrane [76]. However, its overexpression in human melanoma cells induced an anti-proliferative effect on these cells and a significant inhibition of their invasive properties. The decrease of invasive properties was due, at least in part, to a reduction in the amount of active MMP-14 and the loss of its location at the migration front, inducing a non-migratory cell phenotype. In a xenograft model of human melanoma in mouse, the NC1α4 (IV) domain, now called tetrastatin, induced a decrease in tumor growth of more than 80% [45]. Peptides reproducing several tetrastatin sequences and named tetrastatin-1, -2 and -3 respectively, strongly inhibited endothelial cell migration without any significant effect on their proliferation [77,78].

4.5 . Pentastatin

Few studies have been devoted to the NC1 domain of the α5(IV) collagen chain, although its strong anti-angiogenic activity was demonstrated in the model of the chick chorioallantoic membrane [76]. Several peptide sequences in this domain, named pentastatins 1, 2 and 3 respectively, inhibited endothelial cell *in vitro* proliferation and migration [77]. Among these peptides, pentastatin 1, corresponding to residues 1516–1535 of the α5(IV) chain had a strong anti-angiogenic activity in an *in vivo* angiogenesis model in mouse and greatly reduced tumor growth in xenograft models of breast and non-small cell lung cancers [79,80].

The recombinant NC1α5(IV) domain, named Lamstatin, and a 17-amino acid peptide derived from its sequence, decreased the tumor-associated lymphatic network in a lung adenocarcinoma xenograft mouse model [81].

4.6 . Hexastatin

Hexastatin, the NC1 domain of the α6(IV) chain, inhibited endothelial cell proliferation and neovascularization in an *in vivo* angiogenesis model in mouse. Similarly, it reduced tumor growth in different murine cancer models, Lewis lung carcinoma and pancreatic insulinoma [82]. Two peptides reproducing hexastatin sequences and named hexastatin-1 and -2, strongly inhibited endothelial cell migration without any significant effect on their proliferation [77].

4.7 . NC1(XIX) domain

The short NC1 (XIX) C-terminal domain is composed of 19 amino acid residues. It inhibited the migration and invasion capacities of melanoma cells *in vitro* without affecting their proliferation [41]. It also exerted a strong inhibition of *in vivo* tumor growth in a murine melanoma model with a decrease in tumor vascularization. NC1(XIX) inhibited *in vitro* pseudotube formation in matrigel by human microvascular endothelial cells. This effect was accompanied by an intense inhibition of MMP-14 and VEGF expression [42].

5 . Matrikine receptors and intracellular transduction

Matrikines derived from basement membrane collagens exert their anti-angiogenic or anti-tumor activities by binding to cell surface receptors, belonging to the integrin family, on tumor or endothelial cells (Table 2):

- arresten exerted its effects through α1β1 integrin liganding [55].
- canstatin bound to αVβ3 and αVβ5 integrins on endothelial or tumor cells [63,66].
- tumstatin bound to α3β1 and αvβ3 integrins on a site distinct of the RGD binding site and induced a conformational change of the αvβ3 integrin [73,83].
- tetrastatin bound to αVβ3 integrin with a moderate affinity as demonstrated in our laboratory by SPR assays [45].

Different proteins such as tropomyosin, glypicans, laminin or MMP-2 were described as putative endostatin receptors to mediate its anti-angiogenic activities [84]. As well, cell surface nucleolin was also demonstrated to serve as an endostatin receptor which mediates anti-angiogenic and anti-tumor activities of endostatin [85]. Up to now, no such interactions have been described for the other collagen-derived matrikines.

Arresten binding to α1β1 integrin inhibited phosphorylation of Focal Adhesion Kinase (FAK), c-Raf, MEK, ERK1/2 and p38 MAPK, but had no effect on the PI3K/Akt pathway. Arresten also strongly inhibited the expression of HIF-1α and VEGF in endothelial cells cultured under hypoxic conditions [43,55].

Canstatin triggered intracellular transduction pathways through integrin binding, which resulted in inhibition of the FAK/PI3K/Akt pathway. Canstatin increased the amount of Fas ligand and decreased that of FLIP protein, suggesting that canstatin-induced apoptosis is mediated through a death receptor-dependent pathway [59,60]. This pathway was amplified in the mitochondria with a reduction of the membrane potential and increased caspase 3, 8 and 9 activities [63]. Canstatin also inhibited the phosphorylation of FAK, Akt and downstream targets as the mTOR pathway [43].

Tumstatin also induced endothelial cell apoptosis [43]. In tumstatin-treated endothelial cells, cap-dependent protein synthesis was strongly inhibited through a decreased activation of downstream regulators of αVβ3, such as FAK, PI3K and Akt [68]. Tumstatin also triggered an inhibition of the NF-κB pathway by down-regulation of the hypoxia-induced expression of the transcription factor COX-2 [86]. In melanoma cells, we showed that the tumstatin 185–203 peptide induced the early phosphorylation of FAK and PI3K. A pretreatment of melanoma cells with wortmannin, a PI3-kinase inhibitor, reverted the effect of the

Table 2
Receptors of matrikines derived from NC1 domains of basement membrane-associated collagens. The two first columns of the table summarize the integrins implicated in the anti-angiogenic or anti-tumoral activities of the different matrikines derived from the α(IV) collagen chains. The third column indicates the bibliographic references used. Nd: matrikine receptor not described up to now.

Matrikines	Receptor	References
Arresten	α1β1 integrin	[55]
Canstatin	αVβ3 and αVβ5 integrins	[63,135]
Tumstatin	αVβ3 integrin	[73]
	α3β1 integrin	[83]
Tetrastatin	αVβ3 integrin	[45]
Pentastatin	nd	
Hexastatin	nd	
Restin	nd	
Endostatin	α3β1 and αVβ3 integrins	[35]
NC1α1(XIX)	nd	

tumstatin-derived peptide on cell proliferation as well as MMP-14 gene expression [73].

Pentastatin-1 or modified-related peptides blocked the VEGF pathway and FAK activation and stimulated Akt phosphorylation [87].

6 . Matrikine generation

Tumstatin was produced *in vivo* by cleavage of collagen IV by MMP-2 and MMP-9, as demonstrated by using MMP-2 and MMP-9-null mice [88]. The serum concentration of circulating tumstatin in mice was determined by ELISA and found to be between 300 and 360 ng/mL [88]. In addition, COL4A3 null mice, deficient in tumstatin, showed an increased pathological angiogenesis and accelerated tumor growth which could be reversed by exogenous tumstatin administration at physiological concentrations [88]. In our laboratory, we found tumstatin concentrations in mouse serum of approximately 600 ng/mL [89]. The concentration of circulating tumstatin was determined in human serum as 10 to 150 ng/mL [90]. In addition, in patients with lung carcinoma, tumstatin levels in poorly differentiated tumor tissues were significantly lower than in non-tumor tissues and well-differentiated tumor tissues. As well, patients with metastases had serum tumstatin levels 50% lower than patients without metastases [90]. In renal carcinoma tissues, tumstatin levels were also significantly lower than in normal tissues [91]. These data confirm the *in vivo* production of tumstatin and its potential interest in the control of tumor growth.

A competitive ELISA assay was developed in our laboratory to determine the NC1 (XIX) concentration in patient sera. We showed that NC1(XIX) was also detectable in human serum at very variable concentrations (0 to 800 ng/mL) [92].

The tumor suppressor functions of p53 derive from its ability to act as a specific transcription factor which negatively regulates angiogenesis and tumor growth by both inhibiting the production of proangiogenic factors such as VEGF and by increasing production of anti-angiogenic factors such as thrombospondin-1 or basement membrane collagen-derived matrikines such as endostatin, arresten and tumstatin. It was demonstrated that p53 activated collagen XVIII and IV synthesis *via* up-regulation of α(II) collagen prolyl-4 hydroxylase and led to an increased release of anti-angiogenic matrikines [93–95].

7 . Basement membrane-associated collagen chains as biomarkers

The expression of various basement membrane-associated collagens is modulated during tumor invasion. The destruction of the basement membrane is the first step in epithelial cancer invasion and metastasis. The alteration of α1(IV) and α2(IV) chain expression was correlated with tumor differentiation degree and preceded the loss of the other α(IV) chains in various cancer types [96,97].

Tumor sections from patients with lung carcinoma showed tumstatin expression around some cancer clusters [98]. In 34 patients with lung carcinoma, a strong expression of tumstatin was associated with a lesser degree of tumor vascularization [99]. The expression of tumstatin was** down-regulated in renal carcinoma tissues, as demonstrated by RT-PCR and western blot analyses [91]. In patients with non-small lung cancer, tumor tumstatin-mRNA expression was significantly correlated with tumor pathologic stage and patients with low tumstatin-mRNA expression had poorer overall survival and disease-free survival than those with high expression [100].

Nevertheless, in gastric carcinomas, an overexpression of COL4A3 appeared associated with a poor prognosis and an increased COL4A3 expression might play an important role in the pathogenesis and subsequent progression of gastric carcinoma [101]. As well, after treatment of patients with advanced non-small cell lung cancer with a combination of gemcitabine and cisplatin, median overall survival was significantly longer in patients with low COL4A3 expression compared to patients with high expression. Accordingly, a high COL4A3 expression appeared to be a negative predictive factor for survival [102].

As well, the loss of α5(IV) and α6(IV) chains from the epithelial basement membrane at the early stage of cancer invasion was reported in several types of cancer. In colorectal cancer, it was associated with an hypermethylation of their promoter [103–106]. The down-regulation of α6(IV) chain expression by siRNA induced a slight increase in cancer cell invasiveness [106]. In hepatic bile duct carcinoma, the absence of α2(IV) and α6(IV) chains corresponded to a significantly poorer prognosis [107].

Collagen XIX also disappeared from the basement membrane during breast cancer progression at invasive stages [108].

8 . Preclinical therapeutic strategies

Matrikines have been tested in animal cancer models under different experimental protocols:

- matrikine *in vivo* overexpression, using constructions with viral vectors or plasmid DNA electrotransfer
- use of recombinant proteins
- use of synthetic peptides reproducing the active sequences or structural analogues.

8.1 . In vivo overexpression of matrikines

In vivo overexpression of canstatin in mouse was obtained by DNA transfer after construction of an adenovirus encoding a fusion protein canstatin-human albumin. This construction reduced the clearance of canstatin by increasing the molecular mass. The intratumoral injection of this adenovirus caused a decrease in tumor growth in a model of mammary carcinoma by inducing apoptosis of tumor and endothelial cells [63]. The intratumoral injection of an adenovirus combining canstatin with a fluorescent protein (GFP-canstatin) led to similar results in a model of esophagus carcinoma [109]. Another strategy was developed from the overexpression of tumstatin induced by lentivirus in mesenchymal stem cells. In a model of prostate, these stem cells also led to a strong decrease in tumor growth [110]. In a hepatocarcinoma model, the injection at regular intervals of a plasmid containing the cDNA encoding the Tum-1 fragment of tumstatin induced a significant decrease in tumor growth [111].

However, therapeutic strategies using viral vectors can cause side effects. To avoid these side effects or the need for repeated injections, DNA electrotransfer technique in the muscle cells can be used: it is simple to develop and does not induce inappropriate immune response. A plasmid containing the cDNA encoding the matrikine is injected into the mouse muscle and induces high production rate for several months. Such a strategy demonstrated anti-angiogenic and anti-tumor effects of canstatin in models of breast carcinoma or prostate cancer in nude mice and in a syngeneic melanoma model [112]. Similarly, we showed in our laboratory that the *in vivo* overexpression of tumstatin induced after DNA electrotransfer led to a strong decrease in tumor growth and increased survival in a mouse syngeneic melanoma model. Endostatin overexpression in mouse led to side effects such as cataract and induced structural alterations in basement membrane [113,114] while tumstatin-overexpressing mice were kept alive for 10 months without detectable side effects [89].

8.2 . Recombinant matrikines

Matrikines derived from NC1 domains of α(IV) collagen chains were produced in different cell systems. Their peri- or intratumoral injections induced a decrease in tumor growth in mouse experimental cancer models [58,115,116]. However, these strategies required large amounts of the recombinant matrikine in a soluble form. The prokaryotic system *in E. coli*, for example, produced notable amounts of recombinant matrikine, but for the most part in an insoluble form. In addition, the presence of enterotoxin in the soluble fraction limited their use *in vivo*.

A eukaryotic system, in particular HEK cells, provided soluble matrikines, but in too small amount, requiring excessively long production process. The baculovirus system seems to offer the best compromise for the production of soluble recombinant matrikines in sufficient quantities [116].

8.3 . Synthetic peptides and structural analogues

Different sequences are responsible for the anti-angiogenic and anti-tumor activity of NC1 domains of collagen IV chains. For example, tumstatin contains two distinct sequences:

- the 74–90 sequence, called T7 peptide, responsible for the anti-angiogenic activity [117]
- the 185–203 sequence, responsible for anti-tumor activity [44].

In the T7 peptide, mutagenesis experiments showed that the amino acids Leu (L78), Val (V82) and Asp (D84) were essential for biological activity and were directly involved in the binding of tumstatin on αvβ3 integrin [117]. A synthetic peptide, the peptide 21, reproducing a portion of the T7 peptide, induced apoptosis in endothelial cells and inhibited tumor growth of gastric carcinoma in mice [74,118].

The 185–203 peptide inhibited in vivo tumor growth in a murine melanoma by intratumoral or intra-peritoneal injections [72,119]. By intravenous injection, tumor growth of gastric carcinoma was decreased in mice; tumor section showed an increased apoptosis with high levels of Bad and caspase 3 and PTEN and a decrease in MMP-2 level [74,120]. Within the 185–203 sequence, biological activity was contained in the 7 N-terminal amino acids of the sequence (CNYYSNS) [119,121]. Amino acids Tyr (Y188) Ser (S189) and Ser (S191) were essential, their replacement by Ala or Phe for Y188 to S189 and S191 abolished the biological activity [44]. By molecular dynamics simulation, we showed that these residues form a β turn [119]. Based on these results, the cyclopeptide YSNSG, forming a constrained β turn, was designed. In a melanoma model, it had higher *in vitro* and *in vivo* anti-tumor and anti-angiogenic activities than those of the native linear peptide and showed increased stability [47,122].

Pentastatins, reproducing sequences of the NC1 α5(IV) domain, showed anti-angiogenic activity *in vitro* [77]. Pentastatin-1, a 20 amino acid peptide, significantly reduced tumor growth of breast carcinoma and small cell lung cancer in mice when intraperitoneally injected [79,80]. From these peptides, an analysis of the structure–activity relationship was determined in an attempt to optimize the performance of collagen IV peptides capable of exerting anti-angiogenic activities [123]. In a mouse pancreatic cancer model, the prevention or intervention treatment with trombospondin-1, endostatin or tumstatin peptides, as well as suppression of their endogenous production by gene deletion, showed a stage-specific effect of these matrikines on tumor progression. For example, tumstatin showed an effect in a prevention treatment but effectively blocked tumor growth in an intervention treatment. These data demonstrated that matrikines could exert differential inhibitory effects in the initial phase of angiogenesis induction or its maintenance or amplification [124].

8.4 . Combined therapies

Xenograft tumors obtained in mouse with colorectal and renal carcinoma cells overexpressing thrombospondin 1, endostatin or tumstatin, showed a delayed tumor growth. Nevertheless, after three weeks, tumors escape growth suppression and undergo logarithmic growth. In response to matrikine overexpression, tumor cells up regulate proangiogenic factor secretion such as VEGF, PDGF-A or FGF-2. The combination of matrikine treatment with a VEGF-2 signaling blockade resulted in a synergistic effect and strongly delayed tumor growth escape [125].

Tables 3 and 4 present the main preclinical trials in mice of treatments with a matrikine in combination with chemotherapy or radiotherapy. Therapeutic transgenes constructed with a conditionally replicating adenoviral vector (CRAd vector) and an arresten cassette decreased tumor growth in a melanoma model [126]. *In vivo* overexpression of canstatin, combined with that of apoptosis-inducing ligand under the control of hTERT promoter (TRAIL) to target proliferating cells, potentiated the inhibitory effects of each treatment used alone [127]. Overexpression of canstatin induced by adenovirus or after recombinant DNA electrotransfer also enhanced the effects of radiation by increasing apoptosis of endothelial cells in breast cancer models [63,112].

Similarly, overexpression of tumstatin induced by intramuscular injection of a recombinant DNA significantly increased anti-tumor effects of gemcitabine, an inhibitor of DNA synthesis in two cancer models [128]. *In vitro* and *in vivo*, the peptides 185–191 of tumstatin potentiated the effects of cisplatin [121]. The use of another fragment of tumstatin, the T7 peptide, also increased the effects of treatment with bevacizumab, an anti-VEGF monoclonal antibody [117]. A recombinant protein combining tumstatin 45–132 and TNF-α, obtained in a baculovirus expression system, inhibited xenograft tumor in mice [129].

9 . Clinical trials

To increase survival in cancer, many clinical trials with MMP inhibitors have been developed, but unsuccessfully because of the complexity of the multiple roles of these proteases [22]. Anti-cancer therapeutic strategies using matrikines gave efficient results in preclinical cancer models in mouse, even though, in some cases, matrikine-treated tumors might escape to growth suppression [125]. Nevertheless, their application to human appears more difficult and only a few examples of clinical trials with matrikines have been reported. Several phase I and II clinical trials were completed in the U.S.A. with human recombinant endostatin (rhendostatin). They showed a safe and good tolerance following various administration protocols but they generally proved ineffective [35,84]. This might be due to a rapid degradation of the matrikine used in the trials by circulating proteases, or to a misrefolding of the recombinant endostatin. The poor clinical responses and the cost for the production of high amounts of recombinant protein led to the stop of phase II trials. The use of shorter peptides and/or cyclic derivatives could provide a better stability *in vivo*. In 2005, ZBP-endostatin, corresponding to an N-terminal modified endostatin and known as its trade name Endostar, was approved by the Chinese State Food and Drug Administration as a cancer drug. Both phase I and IIa studies showed that Endostar had a good tolerance in clinical use. The various phase IIb clinical trials also showed an increased response rate [84]. Currently, phase III and IV clinical trials demonstrated the efficiency of Endostar in combination with chemotherapy in advanced non-small cell lung cancer, and in other advanced stage cancers, with no specific side effects [130–133]. A phase II multicenter trial with Endostar and dacarbazine is also in progress in patients with metastatic melanoma [134].

In contrast, little or no clinical trials have so far been successfully conducted with collagen IV-derived matrikines and have not yet been subject to any publication.

10 . Conclusions

Experimental studies *in vitro* and in various preclinical cancer models in mice highlight the strong potential of matrikines for anti-cancer agents design and development. Their ability to inhibit the proliferative and invasive properties of cancer cells and their anti-angiogenic activities are opening new opportunities to limit tumor progression. In addition, their endogenous origin contributes to a better tolerance, limiting side effects. The characterization of active minimal sequences will promote structural analogues design to improve their bioavailability and pharmacokinetic properties. The binding of these matrikines or their analogues on cell surface receptors such as αvβ3

Table 3
Main preclinical trials conducted with matrikines derived from NC1 domains of basement membrane-associated collagens. The columns of the table show different preclinical cancer models with matrikines. The first column indicates the different collagen IV-derived matrikines and the second one the form of matrikine used. The third one indicates the mouse cancer model and the latter summarizes the corresponding bibliographic references.

Matrikine	Used form	Cancer model	References
Arresten	Overexpression: recombinant DNA	Colorectal cancer	[58]
	Overexpression: recombinant DNA	Squamous cell carcinoma	[56]
	Recombinant domain	Teratocarcinoma	[55]
	Recombinant domain	Colon carcinoma	[136]
Canstatin	Canstatin-GFP adenovirus	Esophagus carcinoma	[109]
	Canstatin-HSA adenovirus	Breast cancer–melanoma	[63]
	Recombinant domain	Prostate adenocarcinoma	[58]
	Recombinant domain	Melanoma	[61,62,66]
	Recombinant domain	Colon carcinoma	[64]
Tumstatin	Overexpression: recombinant DNA	Melanoma	[72]
	Overexpression: recombinant DNA	Hepatocellular carcinoma	[111]
	hTERT/RGD NC1α3(IV) adenovirus	Prostate carcinoma	[137]
	Recombinant domain	Renal–prostate carcinomas	[67]
	Recombinant domain	Lewis lung carcinoma Squamous cell carcinoma	[116]
	Recombinant domain	Sarcoma 180	[115]
	Recombinant domain	Ovarian cancer	[138]
	Recombinant peptide P21	Melanoma	[118]
	Synthetic peptide	Gastric carcinoma	[119]
	Synthetic peptide	Melanoma	[74,120]
	Structural analogues		[47,122]
Tetrastatin	Overexpression: recombinant DNA	Melanoma	[45]
Pentastatin	Synthetic peptide (Pentastatin-1)	Breast cancer Non-small cell lung cancer	[79,80]
Hexastatin	Recombinant domain	Lewis lung Carcinoma	[82]
NC1α1(XIX)	Synthetic peptide	Melanoma	[41,42]

integrin for example, will also permit the proposal of strategies to specifically target cancer cells or activated endothelial cells, to address chemotherapeutic molecules and increase their efficiency and specificity. Matrikines and derived analogues may constitute a new family of potent anti-cancer agents to be used in combination with conventional chemotherapy or radiation, which opens real prospects to effectively limit tumor progression.

Acknowledgements

This work was supported by funds from the Centre National de la Recherche Scientifique (UMR 7369), University of Reims Champagne Ardenne, the Region Champagne-Ardenne and the FEDER (Contract State-Region 2007–2013), the Ligue contre le Cancer

Table 4
Combined therapies with matrikines. The columns of the table show preclinical cancer models treated with matrikines in combination with other cancer therapies. The first column indicates the different collagen IV-derived matrikines or matrikine-derived peptides and the second one describes the associated therapy. The third one indicates the mouse cancer model used and the latter summarizes the corresponding bibliographic references.

Matrikine	Associated therapy	Cancer model	References
Arresten	IL-24	Melanoma	[126]
Canstatin	TRAIL	Breast	[127]
Canstatin	Radiotherapy (I^{131})	Breast	[65]
Canstatin	Ionizing radiation	Breast Prostate Melanoma	[112]
Tumstatin	Gemcitabine	Lung Kidney	[128]
Tumstatin 185–191	Cisplatin	Lung	[121]
Tumstatin T7	Bevacizumab	Kidney	[117]
Tumstatin 45–132	TNF α	F6 tumor	[129]

References

[1] F.X. Maquart, A. Siméon, S. Pasco, J.C. Monboisse, Régulation de l'activité cellulaire par la matrice extracellulaire: le concept de matrikine, J. Soc. Biol. 193 (1999) 423–428.
[2] G.E. Davis, K.J. Bayless, M.J. Davis, G.A. Meininger, Regulation of tissue injury responses by the exposure of matricryptic sites within extracellular matrix molecules, Am. J. Pathol. 156 (2000) 1489–1498.
[3] P. Nyberg, L. Xie, R. Kalluri, Endogenous inhibitors of angiogenesis, Cancer Res. 65 (2005) 3967–3979.
[4] P. Lu, V.M. Weaver, Z. Werb, The extracellular matrix: a dynamic niche in cancer progression, J. Cell Biol. 196 (2012) 395–406, http://dx.doi.org/10.1083/jcb.201102147.
[5] D. Hanahan, R.A. Weinberg, Hallmarks of cancer: the next generation, Cell 144 (2011) 646–674.
[6] M. Egeblad, E.S. Nakasone, Z. Werb, Tumors as organs: complex tissues that interface with the entire organism? Dev. Cell 18 (2010) 884–901.
[7] A. Naba, K.R. Clauser, S. Hoersch, H. Liu, S.A. Carr, R.O. Hynes, The Matrisome: *in silico* definition and *in vivo* characterization by proteomics of normal and tumor extracellular matrices, Mol. Cell. Proteomics 11 (2012) 1–18.
[8] V. Catalano, A. Turdo, S. Di Franco, F. Dieli, M. Todaro, G. Stassi, Tumor and its microenvironment: a synergistic interplay, Semin. Cancer Biol. 23 (2013) 522–532.
[9] J.C. Monboisse, P. Braquet, A. Randoux, J.P. Borel, Non-enzymatic degradation of acid-soluble calf skin collagen by superoxide ion: protective effect of flavonoids, Biochem. Pharmacol. 32 (1983) 53–58.
[10] J.C. Monboisse, M. Gardès-Albert, A. Randoux, J.P. Borel, C. Ferradini, Collagen degradation by superoxide anion in pulse and gamma radiolysis, Biochim. Biophys. Acta 965 (1988) 29–35.
[11] R. Kalluri, M. Zeisberg, Fibroblasts in cancer, Nat. Rev. Cancer 6 (2006) 392–401.
[12] M. Shimoda, K.T. Mellody, A. Orimo, Carcinoma-associated fibroblasts are a rate-limiting determinant for tumor progression, Semin. Cell Dev. Biol. 21 (2010) 19–25.
[13] E.I. Deryugina, J.P. Quigley, Matrix metalloproteinases and tumor metastasis, Cancer Metastasis Rev. 25 (2006) 9–34.
[14] E.I. Deryugina, J.P. Quigley, Cell surface remodeling by plasmin: a new function for an old enzyme, J. Biomed. Biotechnol. 2012 (2012) 564259, http://dx.doi.org/10.1155/2012/564259.
[15] J.M. Rothberg, K.M. Bailey, J.W. Wojtkowiak, Y. Ben-Nun, M. Bogyo, E. Weber, K. Moin, G. Blum, R.R. Mattingly, R.J. Gillies, B.F. Sloane BF, Acid-mediated tumor proteolysis: contribution of cysteine cathepsins, Neoplasia 15 (2013) 1125–1137.
[16] C.M. Overall, O. Kleifeld, Tumour microenvironment — opinion: validating matrix metalloproteinases as drug targets and anti-targets for cancer therapy, Nat. Rev. Cancer 6 (2006) 227–239.
[17] K. Kessenbrock, V. Plaks, Z. Werb, Matrix metalloproteinases: regulators of the tumor microenvironment, Cell 141 (2010) 52–67, http://dx.doi.org/10.1016/j.cell.2010.03.015.

[18] C.M. Overall, R.A. Dean, Degradomics: systems biology of the protease web. Pleiotropic roles of MMPs in cancer, Cancer Metastasis Rev. 25 (2006) 69–75.
[19] M.D. Martin, L.M. Matrisian, The other side of MMPs: protective roles in tumor progression, Cancer Metastasis Rev. 26 (2007) 717–724.
[20] C. López-Otín, L.H. Palavalli, Y. Samuels, Protective roles of matrix metalloproteinases: from mouse models to human cancer, Cell Cycle 8 (2009) 3657–3662.
[21] J. Decock, S. Thirkettle, L. Wagstaff, D.R. Edwards, Matrix metalloproteinases: protective roles in cancer, J. Cell. Mol. Med. 15 (2011) 1254–1265, http://dx.doi.org/10.1111/j.1582-4934.2011.01302.x.
[22] A. Dufour, C.M. Overall, Missing the target: matrix metalloproteinase antitargets in inflammation and cancer, Trends Pharmacol. Sci. 34 (2013) 233–342, http://dx.doi.org/10.1016/j.tips.2013.02.004.
[23] J. Chou, P. Shahi, Z. Werb, MicroRNA-mediated regulation of the tumor microenvironment, Cell Cycle 12 (2013) 20.
[24] R. Kalluri, Basement membranes: structure, Nat. Rev. Cancer 3 (2003) 422–433.
[25] J. Kruegel, N. Miosge, Basement membrane components are key players in specialized extracellular matrices, Cell. Mol. Life Sci. 67 (2010) 2879–2895.
[26] S. Ricard-Blum, The collagen family, Cold Spring Harb. Perspect. Biol. 3 (2011) a004978.
[27] V. LeBleu, M. Sund, H. Sugimoto, G. Birrane, K. Kanasaki, E. Finan, C.A. Miller, V.H. Gattone II, H. McLaughlin, C.F. Shield III, R. Kalluri, Identification of the NC1 domain of α3 chain as critical for α3α4α5 type IV collagen network assembly, J. Biol. Chem. 285 (2010) 41874–41885.
[28] D. Li, C.C. Clark, J.C. Myers, Basement membrane zone type XV collagen is a disulfide-bonded chondroitin sulfate proteoglycan in human tissues and cultured cells, J. Biol. Chem. 275 (2000) 22339–22347.
[29] M. Hurskainen, F. Ruggiero, P. Hägg, T. Pihlajaniemi, P. Huhtala, Recombinant human collagen XV regulates cell adhesion and migration, J. Biol. Chem. 285 (2010) 5258–5265, http://dx.doi.org/10.1074/jbc.M109.033787.
[30] R. Ramchandran, M. Dhanabal, R. Volk, M.J.F. Waterman, M. Segal, H. Lu, B. Knebelmann, V.P. Sukhatme, Antiangiogenic activity of restin, NC10 domain of human collagen XV: comparison to endostatin, Biochem. Biophys. Res. Commun. 255 (1999) 735–739.
[31] R. Xu, L. Xin, J.M. Zhang, Z.P. Li, R.B. Gan, Restin expressed in vivo suppresses the growth of tumors in nude mice? Sheng Wu Hua Xue Yu Sheng Wu Wu Li Xue Bao (Shanghai) 34 (2002) 571–575.
[32] M.J. Mutolo, K.J. Morris, S.H. Leir, T.C. Caffrey, M.A. Lewandowska, M.A. Hollingsworth, A. Harris, Tumor suppression by collagen XV is independent of the restin domain, Matrix Biol. 31 (2012) 285–289, http://dx.doi.org/10.1016/j.matbio.2012.03.003.
[33] M. Rehn, T. Pihlajaniemi, α1(XVIII), a collagen with frequent interruptions in the collagenous sequence, a distinct tissue distribution, and homology with type XV collagen, Proc. Natl. Acad. Sci. U. S. A. 91 (1994) 4234–4238.
[34] M.S. O'Reilly, T. Boehm, Y. Shing, N. Fukai, G. Vasios, W.S. Lane, E. Flynn, J.R. Birkhead, B.R. Olsen, J. Folkman, Endostatin: an endogenous inhibitor of angiogenesis and tumor growth, Cell 88 (1997) 277–285.
[35] J. Folkman, Antiangiogenesis in cancer therapy-endostatin and its mechanisms of action, Exp. Cell Res. 312 (2006) 594–607.
[36] S. Ricard-Blum, L. Ballut, Matricryptins derived from collagens and proteoglycans, Front. Biosci. 16 (2011) 674–697.
[37] D. Quélard, E. Lavergne, I. Hendaoui, H. Elamaa, U. Tiirola U, R. Heljasvaara, T. Pihlajaniemi, B. Clément, O. Musso, A cryptic frizzled module in cell surface collagen 18 inhibits Wnt/beta-catenin signaling, PLoS One 3 (2008) e1878.
[38] I. Hendaoui, E. Lavergne, H.S. Lee, S.H. Hong, H.Z. Kim, C. Parent, N. Heuzé-Vourc'h, B. Clément, O. Musso, Inhibition of Wnt/β-catenin signaling by a soluble collagen-derived frizzled domain interacting with Wnt3a and the receptors frizzled 1 and 8. Inhibition of Wnt/β-catenin signaling by a soluble collagen-derived frizzled domain interacting with Wnt3a and the receptors frizzled 1 and 8, PLoS One 7 (2012) e30601.
[39] J.C. Myers, D. Li, A. Bageris, V. Abraham, A.S. Dion, P.S. Amenta, Biochemical and immunohistochemical characterization of human type XIX defines a novel class of basement membrane zone collagens, Am. J. Pathol. 151 (1997) 1729–1740.
[40] J.C. Myers, D. Li, P.S. Amenta, C.C. Clark, C. Nagaswami, J.W. Weisel, Type XIX collagen purified from human umbilical cord is characterized by multiple sharp kinks delineating collagenous subdomains and by intermolecular aggregates via globular, disulfide-linked, and heparin-binding amino termini, J. Biol. Chem. 278 (2003) 32047–32057(Epub 2003 Jun 3).
[41] L. Ramont, S. Brassart-Pasco, J. Thevenard, A. Deshorgue, L. Venteo, J.Y. Laronze, M. Pluot, J.C. Monboisse, F.X. Maquart, The NC1 domain of type XIX collagen inhibits in vivo melanoma growth, Mol. Cancer Ther. 6 (2007) 506–514.
[42] A. Toubal, L. Ramont, C. Terryn, S. Brassart-Pasco, D. Patigny, J. Sapi, J.C. Monboisse, F.X. Maquart, The NC1 domain of type XIX collagen inhibits melanoma cell migration, Eur. J. Dermatol. 20 (2010) 712–718.
[43] V.G. Cooke, R. Kalluri, Molecular mechanism of type IV collagen-derived endogenous inhibitors of angiogenesis, Methods Enzymol. 444 (2008) 1–19, http://dx.doi.org/10.1016/S0076-6879(08)02801-2.
[44] J. Han, N. Ohno, S. Pasco, J.C. Monboisse, J.P. Borel, N.A. Kefalides, A cell binding domain from the α3 chain of type IV collagen inhibits proliferation of melanoma cells, J. Biol. Chem. 272 (1997) 20395–20401.
[45] S. Brassart-Pasco, J. Thevenard, K. Senechal, L. Ramont, J. Devy, L. Di Stefano, A. Dupont-Deshorgue, S. Brezillon, J.F. Jazeron, S. Ricard-Blum, F.X. Maquart, J.C. Monboisse, A novel anti-tumor matrikine: the NC1 domain of the α4 (IV) collagen chain, PLoS One 7 (2012) e29587.
[46] S. Pasco, J. Han, P. Gillery, G. Bellon, F.X. Maquart, J.P. Borel, N.A. Kefalides, J.C. Monboisse, A specific sequence of the noncollagenous domain of the α3(IV) chain of type IV collagen inhibits expression and activation of matrix metalloproteinases by tumor cells, Cancer Res. 60 (2000) 467–473.
[47] J. Thevenard, L. Ramont, J. Devy, B. Brassart, A. Dupont-Deshorgue, N. Floquet, L. Schneider, F. Ouchani, C. Terryn, F.X. Maquart, J.C. Monboisse, S. Brassart-Pasco, The YSNSG cyclopeptide derived from tumstatin inhibits tumor angiogenesis by down-regulating endothelial cell migration, Int. J. Cancer 126 (2010) 1055–1066.
[48] J.C. Monboisse, R. Garnotel, G. Bellon, N. Ohno, C. Perreau, J.P. Borel, N.A. Kefalides, The alpha 3 chain of type IV collagen prevents activation of human polymorphonuclear leukocytes, J. Biol. Chem. 269 (1994) 25475–25482.
[49] H. Liu, B. Chen, B. Lilly, Fibroblasts potentiate blood vessel formation partially through secreted factor TIMP-1, Angiogenesis 11 (2008) 223–234.
[50] M.K. Chelberg, J.B. Mc Carthy, A.P.N. Skubitz, L.T. Furcht, E.C. Tsilibary, Characterization of a synthetic peptide from type IV collagen that promotes melanoma cell adhesion, spreading and motility, J. Cell Biol. 111 (1990) 262–270.
[51] A.J. Miles, A.P. Skubitz, L.T. Furcht, G.B. Fields, Promotion of cell adhesion by single-stranded and triple-helical peptide models of basement membrane collagen α1(IV)531–543. Evidence for conformationally dependent and conformationally independent typeIV collagen cell adhesion sites, J. Biol. Chem. 269 (1994) 30939–30945.
[52] P. Nyberg, L. Xie, H. Sugimoto, P. Colorado, M. Sund, K. Holthaus, A. Sudhakar, T. Salo, R. Kalluri, Characterization of the anti-angiogenic properties of arresten, an alpha1beta1 integrin-dependent collagen-derived tumor suppressor, Exp. Cell Res. 314 (2008) 3292–3305, http://dx.doi.org/10.1016/j.yexcr.2008.08.011.
[53] C.S. Boosani, N. Nalabothula, N. Sheibani, A. Sudhakar, Inhibitory effects of arresten on bFGF-induced proliferation, migration, and matrix metalloproteinase-2 activation in mouse retinal endothelial cells, Curr. Eye Res. 35 (2010) 45–55, http://dx.doi.org/10.3109/02713680903374208.
[54] P.C. Colorado, A. Torre, G. Kamphaus, Y. Maeshima, H. Hopfer, K. Takahashi, R. Volk, E.D. Ramborsky, S. Herman, P.K. Sarkar, M.B. Ericksen, M. Dhanabal, M. Simons, M. Post, D.W. Kufe, R.R. Weichselbaum, V.P. Sukhatme, R. Kalluri, Anti-angiogenic cues from vascular basement membrane collagen, Cancer Res. 60 (2000) 2520–2526.
[55] A. Sudhakar, P. Nyberg, V.G. Keshamouni, A.P. Mannam, J. Li, H. Sugimoto, D. Cosgrove, R. Kalluri, Human α1 type IV collagen NC1 domain exhibits distinct antiangiogenic activity mediated by α1β1 integrin, J. Clin. Invest. 115 (2005) 2801–2810.
[56] M. Aikio, I. Alahuhta, S. Nurmenniemi, J. Suojanen, R. Palovuori, S. Teppo, T. Sorsa, C. López-Otín, T. Pihlajaniemi, T. Salo, R. Heljasvaara, P. Nyberg, Arresten, a collagen-derived angiogenesis inhibitor, suppresses invasion of squamous cell carcinoma, PLoS One 7 (2012) e51044.
[57] C.R. Lv, L. Chen, C.Q. Dou, W.B. Chen, C. Lin, Arresten expressed in vivo suppresses the growth of SGC-7901 tumor xenografts in nude mice, Zhonghua Wai Ke Za Zhi 43 (2005) 1391–1394.
[58] M.Y. Long, H.H. Li, J.Y. Xu, D.M. Lai, Z.H. Weng, Inhibitory effects of transfection of arresten gene on liver metastasis from colorectal cancer in nude mice, Chin. J. Cancer 27 (2008) 312–315.
[59] G.D. Kamphaus, P.C. Colorado, D.J. Panka, H. Hopfer, R. Ramchandran, A. Torre, Y. Maeshima, J.W. Mier, V.P. Sukhatme, R. Kalluri, Canstatin, a novel matrix-derived inhibitor of angiogenesis and tumor growth, J. Biol. Chem. 275 (2000) 1209–1215.
[60] D.J. Panka, J.W. Mier, Canstatin inhibits Akt activation and induces Fas dependent apoptosis in endothelial cells, J. Biol. Chem. 278 (2003) 37632–37636.
[61] G.A. He, J.X. Luo, T.Y. Zhang, F.Y. Wang, R.F. Li, Canstatin-N inhibits in vitro endothelial cell proliferation and suppresses in vivo tumor growth, Biochem. Biophys. Res. Commun. 312 (2003) 801–805.
[62] G.A. He, J.X. Luo, T.Y. Zhang, Z.S. Hu, F.Y. Wang, The C-terminal domain of canstatin suppresses in vivo tumor growth associated with proliferation of endothelial cells, Biochem. Biophys. Res. Commun. (2004) 354–360.
[63] C. Magnon, A. Galaup, B. Mullan, V. Rouffiac, C. Bouquet, J.M. Bidart, F. Griscelli, P. Opolon, M. Perricaudet, Canstatin acts on endothelial and tumor cells via mitochondrial damage initiated through interaction with αvβ3 and αvβ5 integrins, Cancer Res. 15 (2005) 4353–4361.
[64] J. Hwang-Bo, K.H. Yoo, H.S. Jeong, I.S. Chung, Recombinant canstatin inhibits angiopoeitin-1 induced angiogenesis and lymphangiogenesis, Int. J. Cancer 13 (2011) 298–309.
[65] C. Magnon, P. Opolon, M. Ricard, E. Connault, P. Ardouin, A. Galaup, D. Métivier, J.M. Bidart, S. Germain, M. Perricaudet, M. Schlumberger, Radiation and inhibition of angiogenesis by canstatin synergize to induce HIF-1α-mediated tumor apoptotic switch, J. Clin. Invest. 117 (2007) 1844–1855.
[66] J.M. Roth, A. Akalu, A. Zelmanovich, D. Policarpio, B. Ng, S. MacDonald, S. Formenti, L. Liebes, P.C. Brooks, Recombinant α2(IV)NC1 domain inhibits tumor cell-extracellular matrix interactions, induces cellular senescence, and inhibits tumor growth in vivo, Am. J. Pathol. 166 (2005) 901–911.
[67] Y. Maeshima, P.C. Colorado, A. Torre, K.A. Holthaus, J.A. Grunkemeyer, M.B. Ericksen, H. Hopfer, Y. Xiao, I.E. Stillman, R. Kalluri, Distinct antitumor properties of a type IV collagen domain derived from basement membrane, J. Biol. Chem. 275 (2000) 21340–21348.
[68] Y. Maeshima, A. Sudhakar, J.C. Lively, K. Ueki, S. Kharbanda, C.R. Kahn, N. Sonenberg, R.O. Hynes, R. Kalluri, Tumstatin, an endothelial cell-specific inhibitor of protein synthesis, Science 295 (2002) 140–143.
[69] T. Kawaguchi, Y. Yamashita, M. Kanamori, R. Endersby, K.S. Bankiewicz, S.J. Baker, G. Bergers, R.O. Pieper, The PTEN/Akt pathway dictates the direct alphaVbeta3-dependent growth-inhibitory action of an active fragment of tumstatin in glioma cells in vitro and in vivo, Cancer Res. 66 (2006) 11331–11340.
[70] H.X. Ye, Y. Yao, X.J. Jiang, X.R. Yuan, Tumstatin transfected into human glioma cell line U251 represses tumor growth by inhibiting angiogenesis, Chin. Med. J. (Engl.) 126 (2013) 1720–1725.

[71] Y.P. Yang, C.X. Xu, G.S. Hou, J.X. Xin, W. Wang, X.X. Liu, Effects of eukaryotic expression plasmid encoding human tumstatin gene on endothelial cells in vitro, Chin. Med. J. (Engl.) 123 (2010) 2269–2273.
[72] S. Pasco, L. Ramont, L. Venteo, M. Pluot, F.X. Maquart, J.C. Monboisse, In vivo overexpression of tumstatin domains by tumor cells inhibits their invasive properties in a mouse melanoma model, Exp. Cell Res. 301 (2004) 251–265.
[73] S. Pasco, J.C. Monboisse, N. Kieffer, The α3(IV) 185-5203 peptide from noncollagenous domain 1 of type IV collagen interacts with a novel binding site on the β3 subunit of integrin αVβ3 and stimulates focal adhesion kinase and phosphatidylinositol-3-kinase phosphorylation, J. Biol. Chem. 275 (2000) 32999–33007.
[74] Y.J. Li, L.C. Sun, Y. He, X.H. Liu, Q.M. Wang, X.M. Jin, The anti-tumor properties of two tumstatin peptide fragments in human gastric carcinoma, Acta Pharmacol. Sin. 30 (2009) 1307–1315.
[75] Y. Liu, J. Li, H. Xu, Y. Zhang, Y. Liu, X. Liu, Mitochondria-mediated tumstatin peptide-induced HepG2 cell apoptosis, Int. J. Mol. Med. 24 (2009) 653–659.
[76] E. Petitclerc, A. Boutaud, A. Prestayko, J. Xu, Y. Sado, Y. Ninomiya, M.P. Jr Sarras, B.G. Hudson, P.C. Brooks, New functions for non-collagenous domains of human collagen type IV. Novel integrin ligands inhibiting angiogenesis and tumor growth in vivo, J. Biol. Chem. 275 (2000) 8051–8061.
[77] E.D. Karagiannis, A.S. Popel, Identification of novel short peptides derived from the α4, α5, and α6 fibrils of type IV collagen with anti-angiogenic properties, Biochem. Biophys. Res. Commun. 354 (2007) 434–439.
[78] E.D. Karagiannis, A.S. Popel, A systematic methodology for proteome-wide identification of peptides inhibiting the proliferation and migration of endothelial cells, Proc. Natl. Acad. Sci. U. S. A. 105 (2008) 13775–13780, http://dx.doi.org/10.1073/pnas.0803241105.
[79] J.E. Koskimaki, E.D. Karagiannis, E.V. Rosca, F. Vesuna, P.T. Winnard Jr., V. Raman, Z.M. Bhujwalla, A.S. Popel, Peptides derived from type IV collagen, CXC chemokines, and thrombospondin-1 domain containing proteins inhibit neovascularization and suppress tumor growth in MBA-MB-231 breast cancer xenografts, Neoplasia 11 (2009) 1285–1291.
[80] J.E. Koskimaki, E.D. Karagiannis, B.C. Tang, H. Hammers, D.N. Watkins, R. Pili, A.S. Popel, Pentastatin-1, a collagen IV-derived 20-mer peptide, suppresses tumor growth in a small cell lung cancer xenograft model, BMC Cancer 10 (2010) 29–35.
[81] M. Weckmann, L.M. Moir, C.A. Heckman, J.L. Black, B.G. Oliver, J.K. Burgess, Lamstatin–a novel inhibitor of lymphangiogenesis derived from collagen IV, J. Cell. Mol. Med. 16 (2012) 3062–3073, http://dx.doi.org/10.1111/j.1582-4934.2012.01648.x.
[82] T.M. Mundel, A.M. Yliniemi, Y. Maeshima, H. Sugimoto, M.K. Kieran, R. Kalluri, Type IV collagen α6 chain-derived noncollagenous domain 1 (α6(IV) NC1) inhibits angiogenesis and tumor growth, Int. J. Cancer 122 (2008) 1738–1744.
[83] Y. Maeshima, U.L. Yerramalla, M. Dhanabal, K.A. Holthaus, S. Barbashov, S. Kharbanda, C. Reimer, M. Manfredi, W.M. Dickerson, R. Kalluri, Extracellular matrix-derived peptide binds to αVβ3 integrin and inhibits angiogenesis, J. Biol. Chem. 276 (2001) 31959–31968.
[84] Y. Fu, H. Tang, Y. Huang, N. Song, Y. Luo, Unraveling the mysteries of endostatin, IUBMB Life 61 (2009) 613–626, http://dx.doi.org/10.1002/iub.215.
[85] H. Shi, Y. Huang, H. Zhou, X. Song, S. Yuan, Y. Fu, Y. Luo, Nucleolin is a receptor that mediates antiangiogenic and antitumor activity of endostatin, Blood 110 (2007) 2899–2906.
[86] C.S. Boosani, A.P. Mannam, D. Cosgrove, R. Silva, K.M. Hodivala-Dilke, A. Sudhakar, Regulation of COX-2 mediated signaling by α3 type IV noncollagenous domain in tumor angiogenesis, Blood 110 (2007) 1168–1177.
[87] J.E. Koskimaki, E. Lee, W. Chen, C.G. Rivera, E.V. Rosca, N.B. Pandey, A.S. Popel, Synergy between a collagen IV mimetic peptide and a somatotropin-domain derived peptide as angiogenesis and lymphangiogenesis inhibitors, Angiogenesis 16 (2013) 159–170, http://dx.doi.org/10.1007/s10456-012-9308-7.
[88] Y. Hamano, M. Zeisberg, H. Sugimoto, J.C. Lively, Y. Maeshima, C. Yang, R.O. Hynes, Z. Werb, A. Sudhakar, R. Kalluri, Physiological levels of tumstatin, a fragment of collagen IV α3 chain, are generated by MMP-9 proteolysis and suppress angiogenesis via αVβ3 integrin, Cancer Cell 3 (2003) 589–601.
[89] J. Thevenard, L. Ramont, L.M. Mir, A. Dupont-Deshorgue, F.X. Maquart, J.C. Monboisse, S. Brassart-Pasco, A new anti-tumor strategy based on in vivo tumstatin overexpression after plasmid electrotransfer in muscle, Biochem. Biophys. Res. Commun. 432 (2013) 549–552, http://dx.doi.org/10.1016/j.bbrc.2013.02.074.
[90] Y.Q. Luo, L.J. Yao, L. Zhao, A.Y. Sun, H. Dong, J.P. Du, S.Z. Wu, W. Hu, Development of an ELISA for quantification of tumstatin in serum samples and tissue extracts of patients with lung carcinomas, Clin. Chim. Acta 411 (2010) 510–515.
[91] C.X. Xu, X.X. Liu, G.S. Hou, Y.F. Yan, S.M. Chen, W. Wang, G.S. Jiang, B. Liu, J.X. Xin, The expression of tumstatin is down-regulated in renal carcinoma, Mol. Biol. Rep. 37 (2010) 2273–2277.
[92] J.B. Oudart, S. Brassart-Pasco, E. Luczka, A. Dupont-Deshorgue, G. Bellon, S.P. Boudko, H.P. Bächinger, J.C. Monboisse, F.X. Maquart, L. Ramont, Analytical methods for measuring collagen XIX in human cell cultures, tissue extracts, and biological fluids, Anal. Biochem. 437 (2013) 111–117, http://dx.doi.org/10.1016/j.ab.2013.03.007.
[93] J.G. Teodoro, A.E. Parker, X. Zhu, M.R. Green, p53-mediated inhibition of angiogenesis through up-regulation of a collagen prolyl hydroxylase, Science 313 (2006) 968–971.
[94] J.G. Teodoro, S.K. Evans, M.R. Green, Inhibition of tumor angiogenesis by p53: a new role for the guardian of the genome, J. Mol. Med. 85 (2007) 1175–1186.
[95] S. Assadian, W. El-Assaad, X.Q. Wang, P.O. Gannon, V. Barrès, M. Latour, A.M. Mes-Masson, F. Saad, Y. Sado, J. Dostie, J.G. Teodoro, p53 inhibits angiogenesis by inducing the production of Arresten, Cancer Res. 72 (2012) 1270–1279, http://dx.doi.org/10.1158/0008-5472.CAN-11-2348.
[96] Y. Oka, I. Naito, K. Manabe, Y. Sado, H. Matsushima, Y. Ninomiya, M. Mizuno, T. Tsuji, Distribution of collagen type IV alpha1-6 chains in human normal colorectum and colorectal cancer demonstrated by immunofluorescence staining using chain-specific epitope-defined monoclonal antibodies, J. Gastroenterol. Hepatol. 17 (2002) 980–986.
[97] H. Nagatsuka, R. Santos Rivera, M. Gunduz, Y.J. Lee, R. Tamamura, E. Gunduz, I. Naito, Y. Sado, N. Nagai, Immunolocalization and distribution patterns of type IV collagen alpha chains in oral mucosal melanoma, Virchows Arch. 447 (2005) 710–716.
[98] M. Polette, J. Thiblet, D. Ploton, A.C. Buisson, J.C. Monboisse, J.M. Tournier, P. Birembaut, Distribution of α1(IV) and α3(IV) chains of type IV collagen in lung tumors, J. Pathol. 182 (1997) 185–191.
[99] S. Caudroy, J. Cucherousset, M. Lorenato, J.M. Zahm, C. Martinella-Catusse, M. Polette, P. Birembaut, Implication of tumstatin in tumor progression of human bronchopulmonary carcinomas, Hum. Pathol. 35 (2004) 1218–1222.
[100] Y.Q. Luo, Z. Ming, L. Zhao, L.J. Yao, H. Dong, J.P. Du, S.Z. Wu, W. Hu, Decreased tumstatin-mRNA is associated with poor outcome in patients with NSCLC, IUBMB Life 64 (2012) 423–431, http://dx.doi.org/10.1002/iub.1016.
[101] X.C. Nie, J.P. Wang, W. Zhu, X.Y. Xu, Y.N. Xing, M. Yu, Y.P. Liu, Y. Takano, H.C. Zheng, COL4A3 expression correlates with pathogenesis, pathologic behaviors, and prognosis of gastric carcinomas, Hum. Pathol. 44 (2013) 77–86, http://dx.doi.org/10.1016/j.humpath.2011.10.028.
[102] C.P. Jiang, B.H. Wu, S.P. Chen, M.Y. Fu, M. Yang, F. Liu, B.Q. Wang, High COL4A3 expression correlates with poor prognosis after cisplatin plus gemcitabine chemotherapy in non-small cell lung cancer, Tumor Biol. 34 (2013) 415–420, http://dx.doi.org/10.1007/s13277-012-0565-2.
[103] Y. Hiki, K. Iyama, J. Tsuruta, H. Egami, T. Kamio, S. Suko, I. Naito, Y. Sado, Y. Ninomiya, M. Ogawa, Differential distribution of basement membrane type IV collagen alpha1(IV), alpha2(IV), alpha5(IV) and alpha6(IV) chains in colorectal epithelial tumors, Pathol. Int. 52 (2002) 224–233.
[104] K. Ikeda, K. Iyama, N. Ishikawa, H. Egami, M. Nakao, Y. Sado, Y. Ninomiya, H. Baba, Loss of expression of type IV collagen alpha5 and alpha6 chains in colorectal cancer associated with the hypermethylation of their promoter region, Am. J. Pathol. 168 (2006) 856–865.
[105] Y. Baba, K. Iyama, K. Ikeda, S. Ishikawa, N. Hayashi, N. Miyanari, Y. Honda, Y. Sado, Y. Ninomiya, H. Baba, Differential expression of basement membrane type IV collagen alpha chains in gastric intramucosal neoplastic lesions, J. Gastroenterol. 42 (2007) 874–880.
[106] Y. Baba, K. Iyama, K. Ikeda, S. Ishikawa, N. Hayashi, N. Miyanari, Y. Sado, Y. Ninomiya, H. Baba, The expression of type IV collagen alpha6 chain is related to the prognosis in patients with esophageal squamous cell carcinoma, Ann. Surg. Oncol. 15 (2008) 555–565.
[107] K. Hirashima, K. Iyama, Y. Baba, Y. Honda, Y. Sado, Y. Ninomiya, M. Watanabe, H. Takamori, T. Beppu, H. Baba, Differential expression of basement membrane type IV collagen α2 and α6 chains as a prognostic factor in patients with extrahepatic bile duct carcinoma, J. Surg. Oncol. 107 (2013) 402–407.
[108] P.S. Amenta, S. Hadad, M.T. Lee, N. Barnard, D. Li, J.C. Myers, Loss of types XV and XIX collagen precedes basement membrane invasion in ductal carcinoma of the female breast, J. Pathol. 199 (2003) 298–308.
[109] X.W. Zheng, Y. Li, F.A. Tang, J. Ma, P.Y. Zheng, G.F. Lu, In vivo antitumor effect of canstatin gene on human œsophagal carcinoma xenografts in nude mice, Chin. J. Cancer 28 (2009) 1–7.
[110] X. Zhang, W. Xu, H. Qian, W. Zhu, R. Zhang, Mesenchymal stem cells modified to express lentivirus TNF-α tumstatin (45–132) inhibit the growth of prostate cancer, J. Cell. Mol. Med. 15 (2011) 433–444.
[111] T. Goto, H. Ishikawa, K. Matsumoto, D. Nishimura, N. Kusaba, N. Taura, H. Shibata, H. Miyaaki, T. Ichikawa, K. Hamasaki, K. Nakao, Y. Maeshima, K. Eguchi, Tum-1, a tumstatin fragment, gene delivery into hepatocellular carcinoma suppresses tumor growth through inhibiting angiogenesis, Int. J. Oncol. 33 (2008) 33–40.
[112] C. Magnon, P. Opolon, E. Connault, L.M. Mir, M. Perricaudet, D. Martel-Renoir, Canstatin gene electrotransfer combined with radiotherapy: preclinical trials for cancer treatment, Gene Ther. 15 (2008) 1436–1445.
[113] H. Elamaa, R. Sormunen, M. Rehn, R. Soininen, T. Pihlajaniemi, Endostatin overexpression specifically in the lens and skin leads to cataract and ultrastructural alterations in basement membranes, Am. J. Pathol. 166 (2005) 221–229.
[114] L. Seppinen, R. Sormunen, Y. Soini, H. Elamaa, R. Heljasvaara, T. Pihlajaniemi, Lack of collagen XVIII accelerates cutaneous wound healing, while overexpression of its endostatin domain leads to delayed healing, Matrix Biol. 27 (2008) 535–546, http://dx.doi.org/10.1016/j.matbio.2008.03.003.
[115] I.S. Chung, Y.I. Son, Y.J. Ko, C.H. Baek, J.K. Cho, H.S. Jeong, Peritumor injections of purified tumstatin delay tumor growth and lymphatic metastasis in an orthotopic oral squamous cell carcinoma model, Oral Oncol. 44 (2008) 1118–1126.
[116] C.S. Boosani, A.K. Varma, A. Sudhakar, Validation of different systems for tumstatin expression and its in vitro and in vivo activities, J. Cancer Sci. Ther. 2009 (2010) 8–18.
[117] H.P. Eikesdal, H. Sugimoto, G. Birrane, Y. Maeshima, V.G. Cooke, M. Kieran, R. Kalluri, Identification of amino acids essential for the antiangiogenic activity of tumstatin and its use in combination antitumor activity, Proc. Natl. Acad. Sci. U. S. A. 105 (2008) 15040–15045.
[118] G.M. Zhang, Y.M. Zhang, S.B. Fu, X.H. Liu, X. Fu, Y. Yu, Z.Y. Zhang, Effects of cloned tumstatin-related and angiogenesis-inhibitory peptides on proliferation and apoptosis of endothelial cells, Chin. Med. J. 121 (2008) 2324–2334.
[119] N. Floquet, S. Pasco, L. Ramont, P. Derreumaux, J.Y. Laronze, J.M. Nuzillard, F.X. Maquart, A.J. Alix, J.C. Monboisse, The antitumor properties of the α3(IV)-(185–203) peptide from the NC1 domain of type IV collagen (tumstatin) are conformation-dependent, J. Biol. Chem. 279 (2004) 2091–2100.

[120] Y. He, Y. Jiang, Y.J. Li, X.H. Liu, L. Zhang, J.J. Liu, H. Shi, H.N. Li, Y.C. Ma, X.M. Jin, 19-peptide, a fragment of tumstatin, inhibits the growth of poorly differentiated gastric carcinoma cells in vitro and in vivo, J. Gastroenterol. Hepatol. 25 (2010) 935–941.
[121] W. Wang, P. Chen, J.L. Li, Y.F. Pei, Q.C. Shuang, C.H. Liu, S. Cai, S.K. Liu, L.Y. Zhu, R. Zhou, The effects of tumstatin 185-191 on lung adenocarcinoma cell lines and the association with protein kinase B and extracellular regulated protein kinase activation, Zhonghua Jie He He Hu Xi Za Zhi 33 (2010) 123–127.
[122] J. Thevenard, N. Floquet, L. Ramont, E. Prost, J.M. Nuzillard, M. Dauchez, H. Yezid, A.J. Alix, F.X. Maquart, J.C. Monboisse, S. Brassart-Pasco, Structural and antitumor properties of the YSNSG cyclopeptide derived from tumstatin, Chem. Biol. 13 (2006) 1307–1315.
[123] C.G. Rivera, E.V. Rosca, N.B. Pandey, J.E. Koskimaki, J.S. Bader, A.S. Popel, Novel peptide-specific quantitative structure-activity relationship (QSAR) analysis applied to collagen IV peptides with antiangiogenic activity, J. Med. Chem. 54 (2011) 6492–6500.
[124] L. Xie, M.B. Duncan, J. Pahler, H. Sugimoto, M. Martino, J. Lively, T. Mundel, M. Soubasakos, K. Rubin, T. Takeda, M. Inoue, J. Lawler, R.O. Hynes, D. Hanahan, R. Kalluri, Counterbalancing angiogenic regulatory factors control the rate of cancer progression and survival in a stage-specific manner, Proc. Natl. Acad. Sci. U. S. A. 108 (2011) 9939–9944, http://dx.doi.org/10.1073/pnas.1105041108.
[125] N.T. Fernando, M. Koch, C. Rothrock, L.K. Gollogly, P.A. D'Amore, S. Ryeom, S.S. Yoon, Tumor escape from endogenous, extracellular matrix-associated angiogenesis inhibitors by up-regulation of multiple proangiogenic factors, Clin. Cancer Res. 14 (2008) 1529–1539, http://dx.doi.org/10.1158/1078-0432.CCR-07-4126.
[126] L. Chai, S. Liu, Q. Mao, D. Wang, X. Li, X. Zheng, H. Xia, A novel conditionally replicating adenoviral vector with dual expression of IL-24 and arresten inserted in E1 and the region between E4 and fiber for improved melanoma therapy, Cancer Gene Ther. 19 (2012) 47–54, http://dx.doi.org/10.1038/cgt.2011.84.
[127] W.B. Wang, Y.L. Zhou, D.F. Heng, C.H. Miao, Y.L. Cao, Combination of tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) and canstatin gene suppression therapy on breast tumor xenograft growth in mice, Breast Cancer Res. Treat. 110 (2008) 283–295.
[128] B. Yao, Q.M. He, L. Tian, F. Xiao, Y. Jiang, R. Zhang, G. Li, L. Zhang, J.M. Hou, X.C. Cheng, Y.J. Wen, B. Kan, J. Li, X. Zhao, B. Hu, Q. Zhou, L. Zhang, Y.Q. Wei, Enhanced antitumor effect of the combination of tumstatin gene therapy and gemcitabine in murine models, Hum. Gene Ther. 16 (2005) 1075–1086.
[129] Y. Yan, W. Xu, H. Qian, W. Zhu, F. Mao, X. Zhang X, Tumstatin45–132-TNFalpha suppresses tumor growth through anti-angiogenic effects and cytotoxicity, Biotechnol. Appl. Biochem. 56 (2010) 119–127, http://dx.doi.org/10.1042/BA20100038.
[130] J. Wang, Y. Sun, Y. Liu, Q. Yu, Y. Zhang, K. Li, Y. Zhu, Q. Zhou, M. Hou, Z. Guan, W. Li, W. Zhuang, D. Wang, H. Liang, F. Qin, H. Lu, X. Liu, H. Sun, Y. Zhang, J. Wang, S. Luo, R. Yang, Y. Tu, X. Wang, S. Song, J. Zhou, L. You, J. Wang, C. Yao, Results of randomized, multicenter, double-blind phase III trial of rh-endostatin (YH-16) in treatment of advanced non-small cell lung cancer patients, Zhongguo Fei Ai Za Zhi 8 (2005) 283–290, http://dx.doi.org/10.3779/j.issn.1009-3419.2005.04.07.
[131] J. Wang, Y. Sun, S. Qin, Results of phase IV clinical trial of combining endostar with chemotherapy for treatment of advanced non-small cell lung cancer (NSCLC), J. Clin. Oncol. 28 (Suppl. 15) (2010) 7598.
[132] H.X. Xu, X.E. Huang, Z.Y. Qian, X. Xu, Y. Li, C.G. Li, Clinical observation of Endostar® combined with chemotherapy in advanced colorectal cancer patients, Asian Pac. J. Cancer Prev. 12 (2011) 3087–3090.
[133] R. Biaoxue, Y. Shuanying, L. Wei, Z. Wei, Z.S. Ming, Systematic review and meta-analysis of Endostar (rh-endostatin) combined with chemotherapy versus chemotherapy alone for treating advanced non-small cell lung cancer, World J. Surg. Oncol. 10 (2012) 170, http://dx.doi.org/10.1186/1477-7819-10-170.
[134] C. Cui, L. Mao, Z. Chi, L. Si, X. Sheng, Y. Kong, S. Li, B. Lian, K. Gu, M. Tao, X. Song, T. Lin, X. Ren, S. Qin, J. Guo, A phase II, randomized, double-blind, placebo-controlled multicenter trial of Endostar in patients with metastatic melanoma, Mol. Ther. 21 (2013) 1456–1463, http://dx.doi.org/10.1038/mt.2013.79.
[135] V. Pedchenko, R. Zent, B.G. Hudson, Alpha(v)beta3 and alpha(v)beta5 integrins bind both the proximal RGD site and non-RGD motifs within noncollagenous (NC1) domain of the alpha3 chain of type IV collagen: implication for the mechanism of endothelia cell adhesion, J. Biol. Chem. 279 (2004) 2772–2780.
[136] Y. Lv, J.P. Zheng, The inhibitory effects of arresten protein on tumor formation, Chin. Med. Sci. J. 27 (2012) 11–17.
[137] T. Miyoshi, S. Hirohata, H. Ogawa, M. Doi, M. Obika, T. Yonezawa, Y. Sado, S. Kusachi, S. Kyo, S. Kondo, Y. Shiratori, B.G. Hudson, Y. Ninomiya, Tumor-specific expression of the RGD-alpha3(IV)NC1 domain suppresses endothelial tube formation and tumor growth in mice, FASEB J. 20 (2006) 1904–1906.
[138] Y. You, X. Xue, M. Li, X. Qin, C. Zhang, W. Wang, C. Giang, S. Wu, Y. Liu, W. Zhu, Y. Ran, Z. Zhang, W. Han, Y. Zhang, Inhibition effect of pcDNA-tum-5 on the growth of S180 tumor, Cytotechnology 56 (2008) 97–104, http://dx.doi.org/10.1007/s10616-007-9117-9.