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Matrix Biology

Mini review

Type XIX Collagen: A new partner in the interactions between tumor cells and their microenvironment.

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Keywords:
FACIT
Collagen XIX
NC1 domain
Matrikine
Tumor invasion
Integrin
Signal transduction

ABSTRACT

Collagen XIX is a minor collagen associated with basement membranes zone, which belongs to the family of FACITs (Fibril Associated Collagens with Interrupted Triple Helices). The FACIT family is composed of collagens IX, XII, XIV, XVI, XX, XXI, XXII and XIX, which share many highly conserved structural motifs: short NC1 domain, a thrombospondin-like N-terminal domain (TspN), and numerous cysteine residues. The main role of FACITs is to ensure integrity and stability of the extracellular matrix and its fibrillar collagen network by regulating formation and size of collagen fibrils.

Collagen XIX was discovered in a human rhabdomyosarcoma cell line. The α1(XIX) chain is composed of 5 collagenous domains interrupted by 6 non-collagenous domains with a short C-terminal 19 amino-acid residues non-collagenous domain (NC1). This collagen is involved in the differentiation of muscle cells, central nervous system development and esophagus formation.

Collagen XIX is associated with the basement membrane zone like collagen XVIII and XV. Its short NC1(XIX) C-terminal domain inhibits migration and invasion of melanoma cells. It also exerts a strong anti-angiogenic effect *via* an inhibition of MMP-14 and VEGF expression. NC1(XIX) binding to αvβ3 integrin decreases protein phosphorylation of proteins involved in the FAK (Focal Adhesion Kinase) / PI3K (PhosphoInositide 3-Kinase) / Akt (proteine Kinase B) / mTOR (Mammalian Target Of Rapamycin) pathway. On the other hand, NC1(XIX) induces an increase in GSK3β activity by decreasing its degree of phosphorylation. The inhibition of this pathway could explain the anti-tumor properties of the NC1(XIX) domain.

Contents

1. Introduction

Collagen XIX is a minor collagen associated with basement membranes zone, which belongs to the FACITs family (Fibril Associated Collagens with Interrupted Triple Helices) (Myers et al., 1993; Yoshioka et al., 1992). Collagens are the most abundant proteins in mammals, with about 30% of the total protein weight. The collagen family is composed of 28 members numbered from I to XXVIII. Each collagen is composed of 3 polypeptide chains commonly referred as α chain. Currently, 42 genes coding for 46 different α chains have been characterized (Ricard-Blum, 2011; Ricard-Blum and Ruggiero, 2005). The lengths of the α chains vary from 662 amino acids for α1(X) to 3152 amino acids for α3(VI).

Some collagens are homotrimers composed of 3 identical α chains (types II, III, VII, VIII, X, XII, XXVIII). The others are heterotrimers composed of different α chains encoded by different genes or resulting from the alternative splicing of the same gene. The main characteristic of collagen is the presence of a triple helical structure resulting of three α chain coiling. The triple helix may represent more than 90% of intact molecule (as in collagen I) or less than 10% (as in collagen XII). It is composed of repeating triplets Gly - X - Aaa where X is predominantly a proline and Aaa a 4-hydroxyproline (van der Rest and Garrone, 1991). The collagen family may be divided into different subfamilies according to their supramolecular assemblies and their functions (for recent review of this classification, see (Ricard-Blum, 2011). In the current review, we will focus on knowledge about collagen XIX: its molecular structure, expression, function and its more recently described anti-tumor properties.

2. FACIT family

Collagen XIX belongs to the FACIT family. By definition, triple helix of FACIT collagens is interrupted by non-collagenous domains. They differ by their α chain composition and length, the number of their collagenous and non-collagenous domains (2 to 10). Nevertheless, the members of this family share many highly conserved structural motifs (Ricard-Blum and Ruggiero, 2005), as a short NC1 domain of a few tens of amino acids, a thrombospondin-like N-terminal domain (TspN), the presence of numerous cysteine residues, an especially highly conserved type Cys - $(Xaa)_4$ - Cys pattern at the junction of the Col1 and NC1 domains, and two exceptions to the repetition of the Gly - X - Aaa motif in the Col2 domain (Ricard-Blum, 2011; Ricard-Blum and Ruggiero, 2005). This family is composed of the collagens IX, XII, XIV, XVI, XX, XXI, XXII and XIX. The major role of FACITs is to ensure integrity and stability of the extracellular matrix and its fibrillar collagen network.

FACITs regulate formation and size of collagen fibrils (Ivanova and Krivchenko, 2014) but also the training and control of cell organization in the extracellular matrix (Tao et al., 2012). They possess domains promoting adhesion and interaction with collagen fibrils, a rigid domain which projects outside the fibrils and multiple domains for interactions with other components of the extracellular matrix. These interactions have been demonstrated in cartilage, where collagen IX binds to fibronectin (Parsons et al., 2011) and in the skin, where collagen XII interacts with collagen I and tenascin X (Chiquet et al., 2014). Moreover, FACIT collagens can interact with cells (Inoguchi et al., 1995). Considering the restrictive and specific expression of FACITs in some tissues, their fine regulation of expression during development and the serious functional alterations caused by their mutations, they are probably involved in specific functions in different organs. For example, during the embryonic period, collagen XIV is involved in the formation of interstitial myocardial tissues (Tao et al., 2012). Collagen XII is involved in the regulation of the organization and properties of the collagen fibers in the muscle and bone (Chiquet et al., 2014).

3. Collagen XIX

3.1 Discovery

Collagen XIX was discovered in 1992 by Yoshioka et al., 1992, in Marion Gordon's team. It was characterized by cDNA sequencing in a human rhabdomyosarcoma cell line (CCL-136, American Type Culture Collection). Because of its isolation in the HY-67 clone, this new collagen chain was at first called α1(Y) (Yoshioka et al., 1992). In 1993, Myers et al described in turn a new collagen that they called "RH COL" because of its isolation in human Rhabdomyosarcoma cell lines (RH) (Myers et al., 1993). In fact, these two discoveries were identical and the name collagen XIX was used to replace α1(Y) and RH COL terms in 1994 (Myers et al., 1994). Then, structural analysis were conducted on collagen XIX and allowed its classification in the FACIT family. (Inoguchi et al., 1995; Khaleduzzaman et al., 1997)

3.2 Chromosomal localization and molecular structure

The human COL19A1 gene, encoding the α1(XIX) chain, is mapped on human chromosome 6 in the q12-q14 region. Genes encoding the α1 chain of two other FACIT collagens, collagen XII (COL12A1) (Gerecke et al., 1997; Oh et al., 1992) and collagen IX (COL9A1) (Kimura et al., 1989) are also located In the same region. Collagen XIX gene comprises 250 kb and 51 exons. The exons encoding the NC1, Col1, NC2, Col2, NC3, Col3, NC4 domains of the α1(XIX) chain share high homology with those encoding the NC1, Col1, NC2, Col2, NC3 domains of the α2(IX) chain (Khaleduzzaman et al., 1997). Similarly, there

are significant homologies between the exons encoding the Col5 - NC6 domains of the α1(XIX) chain and the exons encoding the Col3 and NC4 domains of the α1(IX) chain. These data suggest the existence of an ancestral gene encoding the different FACIT α chains (Khaleduzzaman et al., 1997). COL19A1 encodes a messenger RNA of 10.4 kb, with a long 3'-untranslated region of 5.3 kb (Inoguchi et al., 1995). There is 82% sequence homology between the human and murine α1(XIX) chains. The strongest homologies are located at the C-terminal side (Sumiyoshi et al., 1997).

Collagen XIX is composed of three α1(XIX) chains, forming a homotrimer of 400 kDa. Each α chain consists of 1142 amino acids with a molecular mass of 112 kDa. The first 23 amino acids constitute the signal peptide (Inoguchi et al., 1995). The amino acid composition is comparable to other collagens, although collagen XIX is the richest in leucine (10.7%) (Nassa et al., 2012). Each chain contains 5 collagenous domains (COL1 to COL5) interrupted by 6 non-collagenous domains (NC1 to NC6), composed of 19, 44, 23, 31, 20, 291 amino acid residues, respectively (Inoguchi et al., 1995).

From a structural point of view, collagen XIX is close to another FACIT, collagen XVI. The NC2 α1(XIX) and NC4 α1(XIX) domains are similar to the corresponding domains of collagen XVI (Myers et al., 1994). The NC6 α1(XIX) domain shows very strong homologies with the NC4 α1(XII), NC3 α1(XII), NC3 α1(XIV) and NC11 α1(XVI) domains (Khaleduzzaman et al., 1997). NC6 α1(XIX) domain also shows very strong homologies with two domains of others proteins, the PARP (Proline Arginine Rich Peptide) domain of collagen V and XI and the N terminal module of thrombospondin (Khaleduzzaman et al., 1997). Phylogenetic analysis shows that collagen XIX also has similarities with collagens IX and XXII, which also belong to the FACIT family (Nassa et al., 2012). The NC2 α1(XIX) domain has a stable helix structure and is probably responsible for the trimerization of the α1(XIX) chains (Boudko et al., 2008).This domain is also involved in the formation of disulfide inter-chain bonds at the junction between Col1 and NC1 domains (Boudko et al., 2008). The α1(XIX) chain also contains 14 cysteine residues, 2 at the junction of Col1 and NC1 domains, 2 others in the NC4 domain and the 10 others in the NC6 domain (thrombospondin-like domain) (Inoguchi et al., 1995). The presence of cysteine promotes the formation of inter-chain and intra-chain disulfide bonds (Myers et al., 2003; Myers et al., 1994), allowing the formation and the stabilization of the triple helix. The presence of inter-chain disulfide bonds was confirmed by rotatory shadowing electron microscopy. In addition, electron microscopy showed that collagen XIX has a rod-like structure (240nm) with a globular domain at the N-terminal end (Myers et al., 2003). This technique also showed the presence of 45% collagen XIX monomers and 55% oligomers (dimers, trimers, tetramers, pentamers, hexamers and other multimers) connected and stabilized by N-terminus inter-molecular disulfide bonds (Myers et

al., 2003). Collagen XIX presents no glycosylation site (Inoguchi et al., 1995). However, it contains a binding site for heparin in the NC6 domain (Inoguchi et al., 1995).

The use of polyclonal anti-sera against the C- or N-terminal domains of collagen XIX, under reducing conditions, in a sample of human rhabdomyosarcoma cells, allowed to highlight a Mr 165 kDa band corresponding to the α1(XIX) chain. In non-reducing conditions, a band with Mr arround 230 kDa was observed, which confirms the presence of disulfide inter-chain bonds (Myers et al., 1997). Experimentally, the 165 kDa band was sensitive to the action of bacterial collagenase, which released a fragment of 34 kDa. Moreover, amino-acid sequence analysis revealed a presumed cleavage site for bacterial collagenase capable of releasing a fragment of a potential molecular weight of 37 kDa, which corresponds to the fragment experimentally obtained (Myers et al., 1997).

Despite its structural similarities with other members of the FACIT family, collagen XIX has also been classified within the multiplexin family, with collagens XV and XVIII. Indeed, it is not associated with the fibers and possesses a thrombospondin-like N-terminal domain, like collagen XIII and XV. Moreover, immunohistochemistry localized collagen XIX in the basement membrane zone of different organs, like other multiplexin (Myers et al., 1997).

3.3 Cell and tissue expression

3.3.1 Expression in mouse

The COL19A1 gene encoding the α1 chain of XIX collagen is mapped on mouse chromosome 1 (Khaleduzzaman et al., 1997). Sumiyoshi *et al* have examined tissue expression of Col19A1 gene by RT-PCR during mouse embryogenesis and in adult mice. COL19A1 murine transcripts are detectable as soon as the 11^{th} day of gestation in almost all organs studied (except liver). In adult mice, the tissue expression of the messenger RNA is restricted to the central nervous system (cerebrum and cerebellum), testis and eye, with traces in the aorta, lung or kidney. The central nervous system is the only organ where COL19A1 gene expression gradually increases after birth. It is ten fold higher in adult mice compared to the embryonic period (Sumiyoshi et al., 1997). The locations of the transcripts are summarized in Table 1 .

3.3.2 Expression in Human

During the embryonic period, human collagen XIX has a broad tissue distribution. However, it represents only a tiny proportion of tissues, for example 10^{-6} % of the weight of the umbilical cord (Myers et al., 1997). In adult, its tissue distribution is restricted to specific

areas, mainly vascular, neuronal and epithelial basement membranes (Sumiyoshi et al., 1997). Some biochemical and immunohistochemical studies have studied the distribution of collagen XIX in human organs (Myers et al., 1997; Su et al., 2010) (Table 2).

4. Physiopathology

Functions of collagen XIX currently remain unclear. Like other FACITs, it participates in the formation and maintenance of extracellular matrix, mainly during embryonic period (Sumiyoshi et al., 1997). However, many other functions of collagen XIX are probably yet to be discovered.

4.1 Collagen XIX in myogenic differentiation

In situ hybridization analysis of the transcripts of the murine COL19A1 gene in the muscle shows gene expression in the myotome as soon as the 9th day of gestation. This expression is superposed with that of the regulatory gene of muscle differentiation: myf-5 (myogenic factor 5). Afterwards, muscle expression of COL19A1 gene gradually decreases (Sumiyoshi et al., 2001). In other words, collagen XIX seems to play a role in the early stages of embryonic muscle differentiation. Rhabdomyosarcoma cell lines were used to study embryogenesis of skeletal muscle and differentiation of mesenchymal cells. Some cells of these lines produce collagen XIX and muscle proteins such as myosin and α-actinin. Under culture conditions, collagen XIX and muscle protein production were greatly increased in a sub-population of cells and induced myoblast differentiation. This study suggests a role of collagen XIX in the early stages of muscle differentiation (Myers et al., 1999). This essential role of collagen XIX in muscle differentiation and physiology was also confirmed by Sumiyoshi *et al* (Sumiyoshi et al., 2004).

In mouse, the double invalidation of collagen XIX gene leads to a greater mortality than 95% in the first three weeks after birth. Animals die mainly of malnutrition. The autopsy reveals the presence of a mega-esophagus. The animals present a motor dysfunction of smooth muscles associated with an alteration of the transformation of smooth muscle cells in striated muscle cells, particularly in the abdominal portion of the esophagus. In addition, mice also exhibit a hypertonicity of their cardia. These abnormalities are related to a disruption of extracellular matrix and a lack of activation of myogenic regulatory factors. Heterozygous mice are viable, fertile and with normal morphology. By consequence, collagen XIX seems to be heavily involved in the stomach sphincter functions and muscle development, mainly esophageal (Sumiyoshi et al., 2004).

More recently, a study showed overexpression of collagen XIX in muscles of patients with amyotrophic lateral sclerosis (Shtilbans et al., 2011).

4.2 Collagen XIX in central nervous system

Collagen XIX is found in specific populations of hippocampal interneurons such as the interneurons expressing neuropeptide Y, somatostatin and calbindin. In a mouse model mutated for collagen XIX gene, Su et al showed that the morphology of the neurons was normal but that the formation of inhibitory synapses was diminished at the hippocampal level and in the subiculum, two parts of the temporal lobe largely involved in memory. Therefore, the expression of collagen XIX in interneurons appears to be essential for synapse formation in hippocampal tissue (Su et al., 2010).

In 2010, Hilario et al showed that collagen XIX had a role in axon guidance in a zebrafish model. During the embryonic period, axons of the neurons of spinal cord are progressing toward their target organ. Herein, axon growth cone is guided by various molecules that determine specific relations between various structures of the developing organism. Moreover, collagen XIX acts as a relay to guide these axons. This new feature appears to be concentration-dependent, permitting a regulation of axonal migration (Hilario et al., 2010).

4.3 Other potential implications of collagen XIX.

Due to its localization in vascular basement membranes of many organs, some authors have suggested that collagen XIX may also be involved in the angiogenesis process (Myers et al., 1997).

Arya *et al* have found strong evidence for a gene on chromosome 6q that influences variation in birth weight in Mexican and American populations. The gene of collagen XIX is in this region and is consequently a candidate gene among other for its involvement in low birth weight (Arya et al., 2006).

5. Collagen XIX and tumor progression

5.1 Collagens of the basement membrane zone

Collagen XIX is associated to the basement membrane zone. Basement membranes are specialized extracellular matrix, present in all vertebrates and some invertebrates. They are located at the interface between epithelia or endothelia and connective tissue, at blood vessel wall, axons of peripheral nerves, adipocytes or muscle cells (Paulsson, 1992). They are amorphous and dense structures, which play a role of cell support (Kalluri, 2003). Apart

from this architectural role, basement membranes exert many important biological functions and play key roles in many physiological and pathological situations (Kruegel and Miosge, 2010). Basement membranes dynamically interact with cells *via* adapter proteins, mainly integrins, to determine the cell polarization, and regulate many biological functions such as adhesion, differentiation, proliferation, chemotaxis or cell migration (Aumailley and Timpl, 1986; Paulsson, 1992). More recently, associations between basement membrane and minor collagens such as type XVIII, XV and XIX were reported and it is better now to talk about basement membrane zone (Yurchenco, 2011; Yurchenco and O'Rear, 1994). Collagen XVIII and XV are the better known. They exert anti-tumor and anti-angiogenic properties, especially though their NC1 domains (Endostatin and Restin respectively) (O'Reilly et al., 1997; Ramchandran et al., 1999). We and others have previously shown that NC1 domains of collagen IV exhibit anti-tumor and / or anti-angiogenic activity (Thevenard et al., 2006; Thevenard et al., 2010). For example, tumstatin, the NC1 domain of α3(IV) chain, tetrastatin, the NC1 domain of α4(IV) chain, canstatin, the NC1 domain of α2(IV) chain and arresten, the NC1 domain of α3(IV) chain, have been described as having anti-tumor and / or anti-angiogenic properties in several experimental in vitro and in vivo models (Floquet et al., 2004; Pasco et al., 2005). More recently, our group demonstrated the antitumor properties of the NC1 domain of collagen XIX (Ramont et al., 2007; Toubal et al., 2010).

5.2 Collagen XIX in breast cancer

Amenta *et al* studied the expression of collagens XV and XIX in breast cancer. During tumor invasion, an early reduction of collagen XIX in the epithelial basement membrane zone was found. This reduction occurred in the early stages of tumor progression, even before the complete destruction of the basement membrane. Therefore, this decrease may be involved in the extracellular matrix remodeling processes that precede tumor invasion (Amenta et al., 2003). Various hypotheses have been proposed to explain this decrease, such as an increased susceptibility of collagen XIX to proteolysis or a decrease in its synthesis.

5.3 The NC1 domain of collagen XIX

The partial and controlled proteolysis of macromolecules of the extracellular matrix, results in the release of peptides with biological activities, called "matrikines" (Maquart et al., 1999) or “matricryptins” (Davis et al., 2000). Some matrikines derived from the proteolysis of basement membrane zone collagens, particularly the NC1 domains of collagens IV, XV, XVIII and XIX, exert anti-tumor and/or anti-angiogenic activities (Monboisse et al., 2014). Various enzymatic systems are involved in the proteolysis of extracellular matrix components, such as matrix metalloproteinases (MMPs) or the plasminogen-plasmin system.

The NC1 (XIX) domain located at the C-terminus of the α1(XIX) chain is very short. It consists of 19 amino acid residues with the following sequence: NPEDCLYPVSHAHQRTGGN. Collagen XIX and its fragments can be detected in cell culture supernatants, in tissue extracts and in many biological fluids such as amniotic fluid, cord blood or human serum (Oudart et al., 2013).

The physiological mechanisms leading to the release of this matrikine remained unknown for a long time. Therefore, the study of collagen XIX proteolysis appeared as a key step to understand its antitumor mechanisms. Our laboratory investigated the potential cleavage of collagen XIX by different enzymes specifically involved in tumor invasion. Initially, experiments conducted with MMP-2, MMP-9 and MMP-14 showed no release of this peptide. However, the presence of an arginine in the junction area between NC1 and Col1 domains, led us to study the proteolysis of collagen XIX by plasmin. We demonstrated that plasmin was able to cleave collagen XIX. We identified by mass spectrometry the cleavage products and demonstrated the release of a peptide comprising the first 15 amino acids of the NC1 domain, preceded by a cysteine and minus the 4 amino-acids residues (TGGN). This peptide reproduce the anti-tumor activity of the complete NC1(XIX) domain. In cell culture, we demonstrated that melanoma cells transfected with an anti-tPA siRNA (one of the plasminogen activators) became unable to activate plasmin and to cleave collagen XIX for liberating the active anti-tumor peptide (Oudart et al., 2015).

5.4 NC1(XIX) domain and melanoma

Studies in our laboratory enabled us to demonstrate that NC1(XIX) significantly inhibits tumor growth in a mouse melanoma model and in a xenograft model of human melanoma in nude mice. Histological study of the tumors showed a decrease in the size and number of vessels in the tumors treated with the NC1(XIX) peptide associated with a significant decrease in VEGF expression, suggesting an anti-angiogenic effect (Ramont et al., 2007). In vitro, NC1(XIX) inhibited cell migration in artificial wound models and video-microscopy analysis showed slow and uncontrolled movements of cells. Furthermore, NC1(XIX) inhibited Matrigel® invasion by tumor cells in modified Boyden chambers. New vessel formation by endothelial cells seeded on a Matrigel® layer was also inhibited in the presence of NC1(XIX) (Toubal et al., 2010). The NC1(XIX) domain must be considered as a new anti-tumor and anti-angiogenic matrikine.

Finally, we identified for the first time the αvβ3 integrin as a receptor of NC1(XIX). Moreover, we demonstrated that NC1(XIX) inhibits the FAK (Focal Adhesion Kinase) / PI3K (PhosphoInositide 3-Kinase) / Akt (protein kinase B) / mTOR (mammalian Target Of Rapamycin) pathway, by decreasing the phosphorylation and activity of the major proteins

involved in this pathway (Oudart et al., in press). On the other hand, NC1(XIX) induced an increase of GSK3β activity by decreasing its degree of phosphorylation (Oudart et al., in press). Treatments targeting this central signaling pathway in the development of melanoma are promising and new molecules should be developed. NC1(XIX) appears to have the potential for the design of new anti-cancer drugs.

6. Conclusion

Collagen XIX is a minor collagen associated with basement membranes zone. It was isolated for the first time in a human cDNA library from rhabdomyosarcoma cells and belongs to the FACIT (Fibril Associated Collagens with Interrupted Triple Helices) family. It is located in the basement membranes of vascular, neural and epithelial tissue. The α1(XIX) chain is composed of 5 collagenous domains interrupted by 6 non-collagenous domains with a short C-terminal 19 amino-acid residues non-collagenous domain (NC1). This collagen is involved in muscle cells differentiation, central nervous system development and esophagus formation but other biological functions cannot be excluded. We have shown that the short NC1(XIX) C-terminal domain inhibits migration and invasion capacities of melanoma cells. It also exerts a strong inhibition of *in vivo* tumor growth in murine or human melanoma models. In addition to its anti-tumor properties, NC1(XIX) domain shows potent anti-angiogenic effects though an intense inhibition of MMP-14 and VEGF expression. Collagen XIX is expressed in human cell cultures, tissue extracts and various biological fluids, such as serum, amniotic fluid, cord blood and many other body fluids (Oudart et al., 2013). Finally, we identified the αvβ3 integrin as a receptor of NC1(XIX) and demonstrated that NC1(XIX) inhibits the FAK/PI3K/Akt/mTOR pathway. The discovery of natural anti-tumor and anti-angiogenic peptides such as NC1(XIX) and their development as anti-angiogenic drugs might open new therapeutic opportunities with fewer deleterious side effects compared to synthetic drugs.

Table 1: Main locations of transcripts from the COL19A1 gene during the embryonic period and in adult mice (Sumiyoshi et al., 1997).

Organ	Embryonic period	Adult
Muscle (esophagus, stomach, tongue)	+	-
Heart	+	-
Aorta	Not determined	Traces
Skin	+	-
Intestine	+	-
Central nervous system	+	++
Eyes	Not determined	+
Tail	+	-
Lung	+	Traces
Liver	-	-
Kidney	+	Traces
Testis	Not determined	+

Table 2: Main locations of collagen XIX in human adults (Amenta et al., 2003; Myers et al., 2003; Myers et al., 1997; Su et al., 2010).

Location	Immuno-localization
Brain	Hippocampus
Skeletal muscle	Vascular basement membrane zone Peripheral nerve fiber Endomysium
Spleen	Vascular basement membrane zone Splenics sinusoids and Billroth's cords Splenic Capsule
Prostate	Vascular basement membrane zone
Kidney	Mesangium Bowman's capsule Vascular and tubular basement membrane zone
Liver	Vascular basement membrane zone Hepatic sinusoids (low level)
Colon	Vascular and epithelial, basement membrane zone
Placenta	Vascular basement membrane zone
Umbilical cord	Vascular basement membrane zone
Skin	Vascular, smooth muscle and neural basement membrane zone Dermo-epidermal basement membrane zone

Figure captions

Fig. 1. Molecular organization of the α1(XIX) chain. Collagen XIX belongs to the FACIT (Fibril Associated Collagens with Interrupted Triple Helices) family. Collagen XIX is composed of three α1(XIX) chains, forming a homotrimer of 400 kDa. Each α chain consists of 1142 amino acids with a molecular mass of 112 kDa. Each chain contains 5 collagenous domains (Col1 to Col5) interrupted by 6 noncollagenous domains (NC1 to NC6), composed of 19, 44, 23, 31, 20, 291 amino acid residues, respectively. NC6 α1(XIX) domain also shares very strong homologies with the N-terminal module of thrombospondin. The NC2 α1(XIX) domain has a stable helix structure and is probably responsible for the trimerization of the α1(XIX) chains. The α1(XIX) chain also presents 14 cysteine residues. The presence of cysteine promotes the formation of inter-chain and intra-chain disulfide bonds, allowing the formation and the stabilization of the triple helix. The NC1(XIX) domain located at the C-terminus of the α1(XIX) chain is very short. Plasmin was able to cleave collagen XIX and release a peptide comprising the first 15 amino acids of the anti-tumor NC1 domain.

Fig. 2. NC1(XIX) binding to αvβ3 integrin receptor interfere with the FAK (Focal Adhesion Kinase) / PI3K (PhosphoInositide 3-Kinase) / Akt (protein Kinase B) / mTOR (Mammalian Target Of Rapamycin) pathway by decreasing the phosphorylation and activity of the major proteins involved in this pathway. On the other hand, NC1(XIX) induces an increase in GSK3β activity by decreasing its phosphorylation level. The inhibition of this pathway could explain anti-tumor properties of the NC1(XIX) domain.

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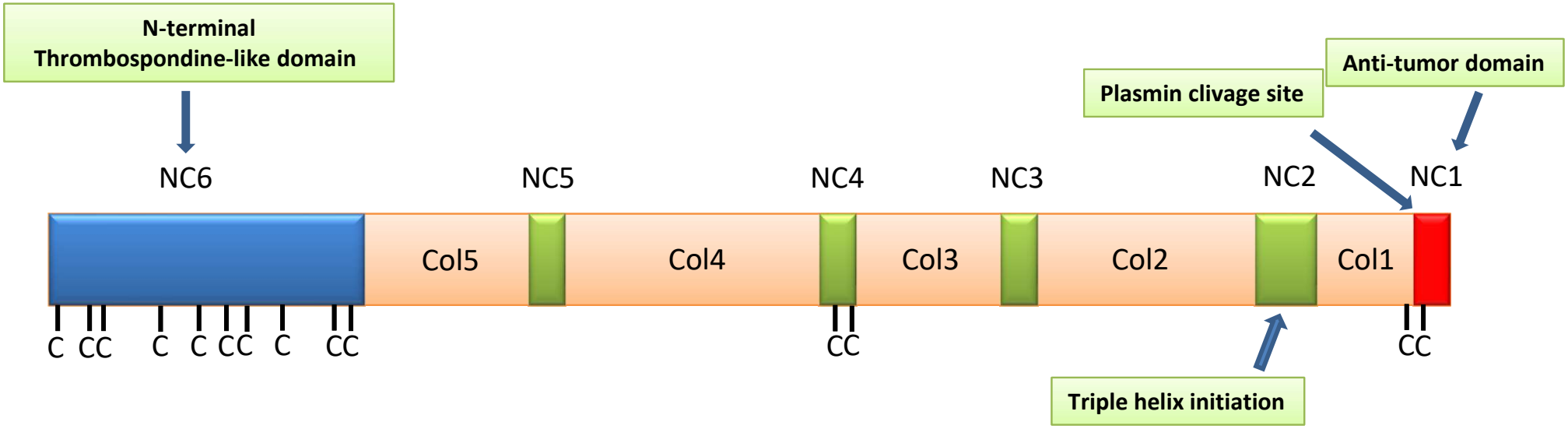
N-terminal
Thrombospondine-like domain
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Anti-tumor domain
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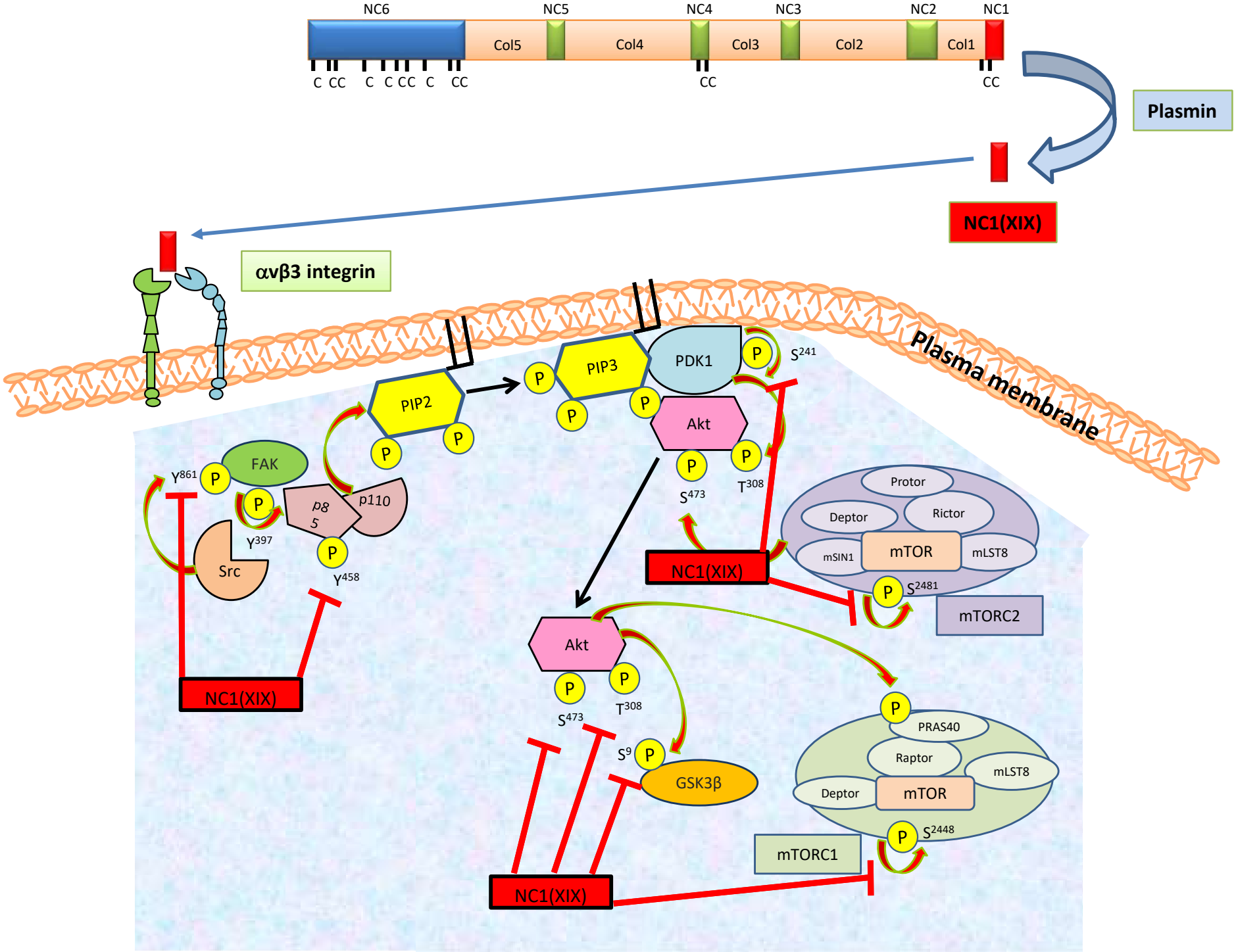
Triple helix initiation

Figure 1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NC1(XIX)
αvβ3 integrin
Plasma membrane
PIP2
PIP3
PDK1
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Akt
S473
T308
FAK
Y861
Y397
Y458
Src
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NC1(XIX)
Protor
Deptor
Rictor
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GSK3β
PRAS40
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mLST8
Deptor
mTOR
S2448
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NC1(XIX)

Figure 2