

Collagen XV: Exploring Its Structure and Role within the Tumor Microenvironment

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Abstract

The extracellular matrix (ECM) is a critical component of stroma-to-cell interactions that subsequently activate intracellular signaling cascades, many of which are associated with tumor invasion and metastasis. The ECM contains a wide range of proteins with multiple functions, including cytokines, cleaved cell-surface receptors, secreted epithelial cell proteins, and structural scaffolding. Fibrillar collagens, abundant in the normal ECM, surround cellular structures and provide structural integrity. However during the initial stages of invasive cancers, the ECM is among the first compartments to be compromised. Also present in the normal ECM is the nonfibrillar collagen XV, which is seen in the basement membrane zone but is lost prior to tumor metastasis in several organs. In contrast, the tumor microenvironment often exhibits increased synthesis of fibrillar collagen I and collagen IV, which are associated with fibrosis. The unique localization of collagen XV and its disappearance prior to tumor invasion suggests a fundamental role in maintaining basement membrane integrity and preventing the migration of tumor cells across this barrier. This review examines the structure of collagen XV, its functional domains, and its involvement in cell-surface receptor-mediated signaling pathways, thus providing further insight into its critical role in the suppression of malignancy. *Mol Cancer Res*; 11(12); 1481–6. ©2013 AACR.

Introduction

Collagen XV was first isolated from a human placental cDNA library in a clone that encoded a different sequence and structure from collagen I to XIV types, which were identified previously. The gene encoding human collagen XV (*COL15A1*) is located on chromosome 9q21 (1). The gene spans 145 kilobases of genomic DNA and contains 42 exons (2). The corresponding homologous mouse collagen XV gene is found on chromosome 4.

The hypothesis that collagen XV might be a tumor suppressor was first proposed in 2003 (3), on the basis of cytogenetic analysis of tumorigenic segregants of somatic cell hybrids in which malignancy was suppressed. Reversion of malignancy was accompanied by consistent loss of a small region of mouse chromosome 4 and disappearance of a collagenous extracellular matrix (ECM). The chromosome 4 fragment was subsequently shown to encompass the mouse *Col15a1* gene and to be syntenic with a region of human chromosome 9.

Collagens are divided into two subfamilies on the basis of structural features: fibrillar collagens form classic collagen

fibril bundles, whereas nonfibrillar collagens generate different structures. Collagen XV is a nonfibrillar collagen and has multiple interruptions within its collagenous domain, enabling more structural flexibility (4, 5). Collagen XV and collagen XVIII (multiplexins) show some N- and C-terminal sequence homology. Collagen XV is a secreted 1,388 amino acid residue protein that exists as 250 or 225 kDa polypeptides, which differ in their C-terminal domain (6, 7). The collagen XV protein has four main functional parts: a putative signaling peptide, a N-terminal noncollagenous domain, an interrupted collagenous domain, and a C-terminal noncollagenous domain (Fig. 1).

Collagen XV: structural considerations

Putative signaling peptide. The N-terminal region of collagen XV begins with a series of hydrophobic amino acids with homology to the signal peptides of other secreted ECM proteoglycans (8). These 25 amino acids within collagen XV contain a leucine at position 23 and an alanine at position 25, which indicate a signaling peptide (4, 9). Hence, although it has not been experimentally verified, it is probable that the N-terminal 25–27 amino acid residues of collagen XV direct the polypeptide to secretory pathways that take it to the ECM.

N-terminal noncollagenous domain. The N-terminal noncollagenous domain consists of 530 amino acids and contains two cysteines at residues 179 and 235 (4). This domain also contains eight sites for attachment of glycosaminoglycans (GAG), long unbranched disaccharide chains consisting of either N-acetylgalactosamine (GalNAc) or N-acetylglucosamine and glucuronic acid (GlcA; reviewed in ref. 10). Thus, collagen XV is a true proteoglycan. Native collagen XV contains chondroitin sulfate modifications on

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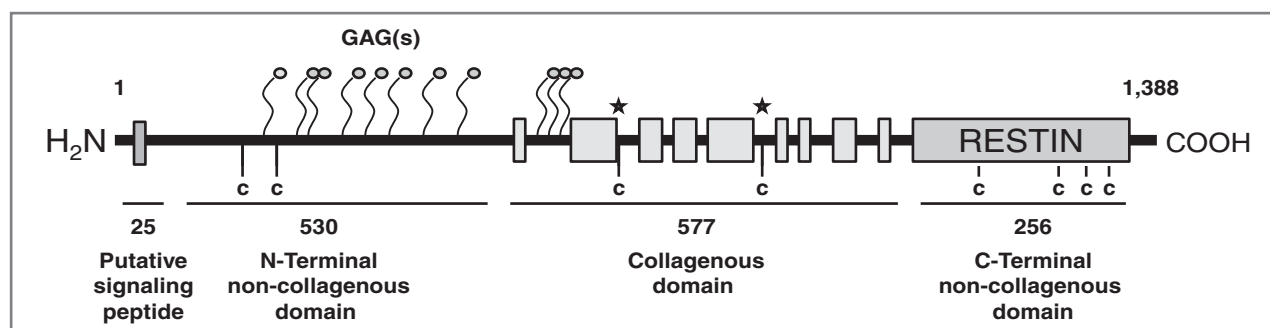


Figure 1. Schematic representation of the structure of collagen XV. Collagen XV is a secreted 1,388 amino acid protein with a putative signaling domain, N-terminal noncollagenous domain with glycosaminoglycan chains (GAGs), a central interrupted collagenous domain, and the C-terminal restin domain. Cysteine residues (C) are shown and those critical for intermolecular disulfide interactions marked (*). Modified from Amenta and colleagues, 2005 (25).

its glycosaminoglycan chains, with differential sulfation specifically on unbranched GalNAc and/or GlcA residues (7). These sites may interact with other components of the ECM and with cell-surface receptors, cytokines, and growth factors. These types of interactions are critical to stromal and cellular cross-talk within the tumor microenvironment (11). In comparison with other collagens, the N-terminal domain of collagen XV and collagen XVIII are similar, demonstrating 45% homology. This domain has extensive sequence homology with thrombospondin, suggesting a role in mediating cell-to-cell and cell-to-matrix interactions (4, 12). Consistent with this prediction, overexpression of a collagen XV cDNA (COLXV) inhibits invasion of BxPC-3 pancreatic adenocarcinoma cells through a collagen I gel (13). Moreover, removal of all the GAG residues from COLXV, by site-directed mutagenesis, partially restored the ability of these cells to invade (Clementz and colleagues; unpublished data).

Collagenous domain. The discontinuous collagenous domain encompasses 577 amino acids and contains nine collagenous domains with eight noncollagenous interruptions (4). This region contains two critical cysteine residues involved in intermolecular disulfide bonding. These cysteines are separated by 231 amino acids and are found in the interruptions of the collagenous domain: one at the start of a 31 amino acid interruption, and the other in the center of a 34-amino acid interruption (7). Mutagenesis of just one of the cysteine residues is sufficient to abolish the active conformation of COLXV as measured by its ability to enhance the adherence of D98 AP2 cervical cancer cells to a collagen I substrate (6, 14). The collagenous regions contain the classic Gly-X-Y motif, where X and Y are commonly hydroxylated prolyl and lysyl residues, forming a homotrimeric α -helical chain (4). The discontinuity between the collagenous regions is thought to be a result of intron splicing of flanking ancestral Gly-X-Y motifs (15). The collagenous domain also contains asparagine residues that are putative sites for N-linked glycosylation modifications (6). Comparing the multiplexins, the collagenous domain of collagen XV and collagen XVIII is similar, sharing 32% sequence homology (16).

C-terminal noncollagenous domain. The C-terminal noncollagenous domain consists of 256 amino acids, which is cleaved to form a short polypeptide (181 amino acids)

known as an endostatin-related fragment or restin domain (4, 17, 18). Restin has 61% sequence homology with endostatin but the two peptides have divergent properties, as endostatin, unlike restin, has been implicated as an antiangiogenic polypeptide fragment (17, 19). Moreover, in experiments to test the hypothesis that collagen XV is a dose-dependent suppressor of tumorigenicity, the restin domain alone was shown not to be involved, whereas the N-terminal portion of the protein (without the C-terminal restin peptide) retained the function (14, 20). Specifically, the ability of COLXV to enhance adherence of D98 AP2 cells to a collagen I substrate and to suppress the growth of tumors arising from the same cells in nude mice was not dependent on the restin fragment (14). The C-terminal domain contains four cysteine residues at positions C1237, C1339, C1369, and C1377 (21). Two intramolecular disulfide bridges can form between C1237, C1369, and between C1339 and C1377, which are similar to the two disulfide bonds within endostatin (21, 22). However, despite some homology with the C-terminal peptide of collagen XVIII, the restin domain seems to have different functional properties from endostatin.

Localization of collagen XV

Collagen XV is expressed within the heart, in skeletal and smooth muscle tissue, and is abundant in the placenta. It is also found in kidney and in interstitial tissues in the pancreas where it is produced in part by fibroblasts within the ECM (23). Subsequently, collagen XV expression was also detected in testis, ovaries, small intestine, and colon (24). Ultrastructural studies have shown collagen XV to be localized to the distal side of basement membrane zones (the outermost lamina densa) in association with banded collagen fibers in the interstitial tissue (24, 25). Collagen XV may be synthesized in many cell types including myoblasts, fibroblasts, endothelial, and some epithelial cells, although the majority of collagen production stems from mesenchymal cells (23, 26). Studies in *Col15a1*^{-/-} null mice have demonstrated general alterations in cardiovascular and skeletal matrix biology and molecular signaling events (27). An analysis of these mice also demonstrated a role for collagen XV in peripheral nerve maturation and heart muscle, where its loss caused cardiomyopathy (28, 29). Of particular

interest in the context of this review is that loss of collagen XV from the basement membrane zone of many tissues has been observed before metastasis of tumors (30–32). Later in tumor progression, low levels of aberrant stromal staining may be associated with invasive tumors (31). The mechanism that underlies the disappearance of collagen XV from the basement membrane zone is of interest, but is currently unknown. It might involve transcriptional downregulation of the gene and/or degradation of collagen XV protein, perhaps by matrix metalloproteinases (MMP) or other proteases.

The basement membrane is a type of ECM that supports and promotes the growth of epithelial cell layers. It contains a multitude of highly conserved networks consisting of structural proteins, cytokines, and growth factors. Many of these proteins are highly glycosylated and commonly harbor glycosaminoglycan, heparin, and/or chondroitin sulfate modifications giving the ECM a largely anionic charge (33, 34). Four major components of the basement membrane are laminin, nidogen, perlecan and collagen IV, which have the capacity to self-assemble (35). The ECM has been implicated in cell differentiation, proliferation and polarity, and it also plays a role in cell survival and migration (34, 36, 37). The scaffold of proteins comprising the ECM has both opposing and complementary functions. In comparison with many other ECM proteoglycans, the functional role of collagen XV as a nonfibrillar collagen contributing to the ECM is poorly studied. However, it is known to have a knot-like tertiary structure that could enable intermolecular interactions (38). Moreover, because collagen XV increases the adhesion of cultured cells to collagen I and is associated with fibrillar collagens at the distal side of the basement membrane zone in the ECM (14, 24–25), its unique placement may determine and facilitate its specific function and influence its interacting proteins.

Collagen XV and interactions within the ECM

The multiple signaling proteins, proteases, and stromal proteins in the extracellular environment play critical roles in normal epithelial cell biology and many are misused in cancer. For example, during epithelial-to-mesenchymal transition (EMT), tumor progression involves many stromal, cellular, and environmental responses including hepatocyte growth factor (HGF), TGF- β , MMPs, and other signaling proteins in the ECM (reviewed in ref. 39). Moreover, tethered transmembrane proteins are also in contact with the ECM, where they not only signal to nearby cells but also signal intracellularly as a response to the ECM. These proteins include, among others, cadherins, integrins, and receptor tyrosine kinases (RTK).

E-cadherin is an important transmembrane protein, which normally mediates cell–cell adhesion in a calcium-dependent manner. It is also implicated in pathways of tumor suppression, as it is lost and/or relocalizes during tumor progression. The majority of E-cadherin protein is localized to adherens junctions, although it is also present at low levels throughout the cytoplasm of polarized cells in both apical and basolateral zones (reviewed in ref. 40). It is well established that during EMT, E-cadherin expression is either greatly reduced, or the

protein relocalizes and becomes internalized by endocytosis to early endosomes (41). In contrast, mesenchymal markers including N-cadherin, Snail, Slug, Twist1, and SIP1 are upregulated during EMT (reviewed in ref. 42). Loss of E-cadherin affects cellular polarity and migration.

We recently showed by coimmunoprecipitation that COLXV directly interacts with E-cadherin (13) at the surface of BxPC-3 pancreatic adenocarcinoma cells. Moreover, overexpression of COLXV, which is secreted from these cells and interacts with them from the outside, stabilized E-cadherin at the cell surface, preventing its internalization. Concurrently, we observed inhibition of the scatter response that BxPC-3 cells exhibit when grown on a collagen I substrate (13, 43). The scatter response is an *in vitro* recapitulation of EMT.

In contrast with E-cadherin, N-cadherin is known to be upregulated and aberrantly expressed in invasive tumors (44). N-cadherin is expressed in mesenchymal cells and correlates with an invasive phenotype. Moreover, its exogenous expression causes increased migration and metastasis (45). It is known that coordinated signaling via collagen I through integrins and receptor tyrosine kinases can cause EMT and upregulation of N-cadherin (43). This raised the possibility that an important role for collagen XV in the normal basement membrane zone might be to interfere with the interaction of collagen I with its cell-surface receptors, as this would effectively compromise pathways of N-cadherin upregulation associated with cell migration. In contrast, when collagen XV is lost before metastasis, this could facilitate the binding of these receptors to collagen I with subsequent upregulation of N-cadherin.

In this context, there is already substantial evidence for cross-talk between RTKs, integrins, and E-cadherin (43, 46), and the vital role of these interactions in the stimulation/inhibition of classic cancer-associated signaling cascades. These pathways are activated by the EGF receptor (EGFR), TGF- β , Wntless-related integration site 1 (Wnt1), and downstream effectors such as mitogen-activated protein kinases (MAPK) and phosphatidylinositol 3-kinases. E-cadherin does not have any enzymatic activity, but does rely on RTK(s), integrins, and other signaling molecules to mediate its downstream effects. Because the integrity of the microenvironment is critical to intracellular homeostasis and the establishment of a proper niche for normal cellular growth (47), disruption of this equilibrium by gain or loss of growth factors, cytokines, and perhaps loss of collagen XV could provide a permissive microenvironment for malignant transformation.

We recently showed that collagen XV has the capacity to disrupt the normal balance of coordinated signaling through integrins and RTKs that is associated with collagen I-mediated EMT and upregulation of N-cadherin (13, 43; Fig. 2C). The integrin receptors are well-characterized examples of transmembrane proteins that interact with the ECM. Specifically, they interact with collagens, including collagen I and IV, and with fibronectin and laminin among other proteins. Integrin receptors are vital transmembrane proteins tethering the cells to the

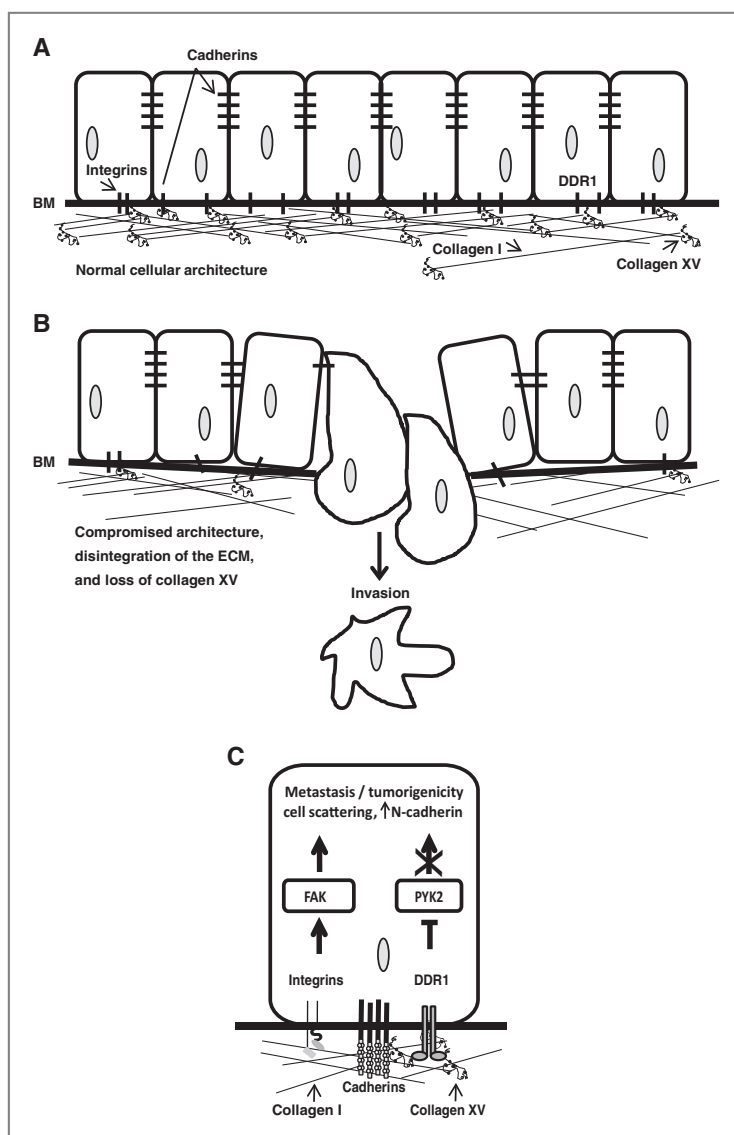


Figure 2. Tumor progression and loss of collagen XV. A, normal epithelial architecture supported by polarization, cadherins, RTKs, and integrins. The microenvironment is established around a network of fibronectin, laminin, fibrillar collagens other structural proteins, and collagens including the nonfibrillar collagen XV (structure based on ref. 38). BM, basement membrane. B, absence of collagen XV creates a compromised fenestrated space facilitating tumor invasion. C, schematic to show interactions of collagen XV with collagen I, E-cadherin, DDR1, and the downstream signaling pathways of $\alpha 2\beta 1$ integrin and DDR1 (modified from ref. 13).

basement membrane and stabilizing cell–cell contacts. These heterodimeric complexes, depending on their constituent partners, can activate several internal signaling events including the p38 MAPK pathways and can induce proliferation and differentiation (48, 49). We showed that collagen XV influences signaling pathways downstream of $\alpha 2\beta 1$ integrin and the discoidin domain receptor tyrosine kinase 1 (DDR1) in BxPC-3 pancreatic adenocarcinoma cells (13), an observation which sheds light on the mechanism whereby it may function as a tumor suppressor. COLXV overexpressed in BxPC-3 cells is secreted into the extracellular milieu where it can interact with the cancer cells from the outside as it would *in vivo*. COLXV caused a slight increase (that did not reach statistical significance) in phosphorylation of Focal Adhesion Kinase (FAK), the immediate downstream signaling partner of $\alpha 2\beta 1$ integrin, indicating potential enhancement of this pathway. Concurrently, a direct interaction between COLXV and

DDR1 was observed by coimmunoprecipitation. Moreover, Pyk2, the immediate downstream signaling partner of DDR1, showed a significant decrease in phosphorylation in the presence of COLXV, demonstrating inhibition of this signaling pathway. Moreover, we showed that chronic exposure of BxPC-3 cells to high levels of COLXV caused downregulation of N-Cadherin in these cells, consistent with its interference with the EMT pathway.

Collagen XV in cancer: a tumor suppressor?

After proper glycosylation and complex structural assembly, collagen XV is secreted into the ECM and localizes within the outermost lamina densa in basement membrane zones (6). In an *in vivo* model system, using cervical carcinoma cells, engineered to overexpress the protein, collagen XV acted as a dose-dependent suppressor of tumorigenicity. Interestingly, although the growth rate of these cells was not changed, collagen XV expression did alter their morphology (20). The

observations that loss of collagen XV from the basement membrane zone precedes invasion and metastasis of aggressive breast tumors, colon carcinomas, and melanomas suggest that this protein may have a critical role in stabilizing the ECM and so preventing distal metastasis (Fig. 2A and B; refs. 30–32). Particularly relevant to this proposed function of collagen XV are data showing that cells overexpressing the protein demonstrate increased adhesion to collagen I in comparison with cells carrying vector alone (14). Thus, its loss could directly compromise the structural integrity of the ECM. The flexible structure of collagen XV and its unique location may explain how it tethers cells to the basement membrane. This model would be consistent with loss of collagen XV associated with the occurrence of tumorigenic segregants of somatic cell hybrids and tumor suppression associated with collagen XV expression in the hybrids (3, 20). Moreover, in collagen XV null mice (*Col15a1*^{-/-}), the local matrix biology is altered in multiple organs. Collagen XV probably plays a vital role in normal tissue remodeling events; not only is it involved in stromal/cellular protein interactions, but also its architecture within the stroma leads to matrix reorganization with subsequent molecular signaling events (28, 29). In this context, collagen XV null mice would not be expected to be cancer-prone but rather their response to spontaneous tumor growth and metastasis might be altered. Moreover, this phenotype might not be evident when foreign tumor cells were injected at heterotopic sites in the animal.

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Conclusions

Collagen XV is unusual among collagens in its location in the basement membrane zone, an area that is first compromised during tumor extravagation. This, together with its flexible nonfibrillar structure, may explain why the lack of this protein can impact signaling events that are mediated by cell integrity proteins such as cadherins, integrins, and RTKs. Moreover, its loss may cause fenestrations in the basement membrane microenvironment and lead to structural weakness (Fig. 2B). If collagen XV functions to maintain this stable physical and molecular barrier, its loss could facilitate tumor cell invasion and motility resulting in distal metastases. Hence, absence of collagen XV in tissues before metastasis may be a useful early marker of tumor invasion.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors' Contributions

Writing, review and/or revision of the manuscript: A.G. Clementz, A. Harris
Study Supervision: A. Harris

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