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Review Article

Elucidating the Functional Role of Collagen X in Order to Explore a Novel Potential Target in Various Cancer Types

Malaney R O'Connell*

Departments of Biology and Human Health Sciences, McMurry University, United States

***Corresponding Author:** Malaney R O'Connell, Departments of Biology and Human Health Sciences, McMurry University, United States.

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Abstract

Short chain, network forming collagen X is a member of the collagen superfamily. The importance of collagen X in endochondral ossification has been well established. Recently multiple reports have suggested that collagen X may also be playing an important role in various cancers. The aim of this review is to present important findings regarding collagen X and cancer while highlighting areas in which further studies may provide important insight into the functional roles collagen X may be playing in both endochondral ossification and tumor progression.

Keywords: *Collagen X; Endochondral Ossification; Tumorigenesis; Tumor Microenvironment; Invasion; Metastasis; Biomarker*

Abbreviations

ECM: Extracellular Matrix; FACIT: Fibril Associated Collagen; SMCD: Schmid Metaphyseal Chondroplysia; SMD: Spondylometaphyseal Dysplasia; RT-PCR: Reverse Transcriptase Polymerase Chain Reaction; qRT-PCR: Quantitative Reverse Transcriptase Polymerase Chain Reaction; NHDFs; Normal Human Dermal Fibroblasts; DCIS: Ductal Carcinoma In Situ; IBC: Inasive Breast Carcinoma; TCGA: The Cancer Genome Atlas; EMT: Epithelial Mesenchymal Transition; Collagen X: Structure *And* Role *In* Endochondral Ossification

Introduction

Collagen X belongs to the collagen family of proteins. Collagens contain at least one triple helical repeat and are the most abundant protein in mammals. There have been 26 genetically unique collagens described and each differs in their structure, number of helical domains, assembly and function. Collagens are a major component of the extracellular matrix (ECM) and have a broad range of functions. Collagen proteins can be grouped into a number of classes or families based on their structure or the structures that they form. The classes are generally classified as fibril-forming collagens, fibril associated collagens (or FACIT collagens), network forming collagens, collagens that form anchoring fibrils, transmembrane collagens, collagens that form beaded filaments, and multiplexins.

Collagen X is considered to be a short chain, network forming collagen. The human COL10A1 gene is located on the long arm of chromosome 6 at position 22 (6q22) [1]. Mutations in the COL10A1 gene have been reported to result in skeletal disorders including Schmid metaphyseal chondroplaysia (SMCD) which is characterized by abnormalities in growth plates resulting in a short stature and a unique gait [2] and spondylometaphyseal dysplasia (SMD) which is characterized by modifications in the spine and tubular bones resulting a

short stature due to shortening of the trunk [3]. The COL10A1 transcript (NM_000493.4) is 3302 base pairs and contains 3 exons, as demonstrated in Figure 1A [1,4]. Bases 96-2138 of the COL10A1 transcript are translated into the 680 aa precursor polypeptide. The collagen alpha-1(x) chain precursor polypeptide is composed of an N-terminal signaling domain, two non-helical regions (NC2 and NC1), and a triple helical region as demonstrated in Figure 1B [1]. Collagen X is a homo-trimeric protein composed of three alpha-1(x) chains that forms a unique hexagonal network [5,6]. It has been well established that collagen X plays a pivotal role in endochondral ossification. Endochondral ossification describes the formation of long bone via conversion of cartilage to bone. Mesenchymal cells differentiate into chondrocytes which leads to the formation of cartilage templates. Chondrocytes undergo a stage of hypertrophy and increase their cell volume; these dramatically larger cells are termed hypertrophic chondrocytes. Hypertrophic chondrocytes then secrete collagen X and other ECM components into the extracellular matrix and this allows for calcification of the extracellular matrix surrounding the hypertrophic chondrocytes. Due to calcification of the hypertrophic chondrocyte extracellular matrix, nutrients are prevented from reaching the chondrocytes and apoptosis results. The apoptosis of the hypertrophic chondrocytes creates a void or hole allowing blood vessel formation to occur. Osteoclasts and osteoblasts are then recruited to assist in removal of cartilage and begin deposition of bone matrix. During the endochondral ossification process, collagen X appears to be restricted to the matrix of the hypertrophic chondrocytes in which collagen X constitutes ~45% of the total collagen produced [7,8]. The gene responsible for collagen X production is active only in mature hypertrophic chondrocytes and is under the control of multiple negative regulatory elements that act in an additive manner to restrict expression of the collagen x gene to only hypertrophic chondrocytes [8,9]. Although the role of collagen X has been well established in the endochondral ossification process, there are still unresolved questions on the exact functional role of collagen X especially in regards to how collagen X allows for calcification of the extracellular matrix of hypertrophic chondrocytes (is it a direct or indirect effect) and the possible role that collagen X may be playing in enhancing vascularization.

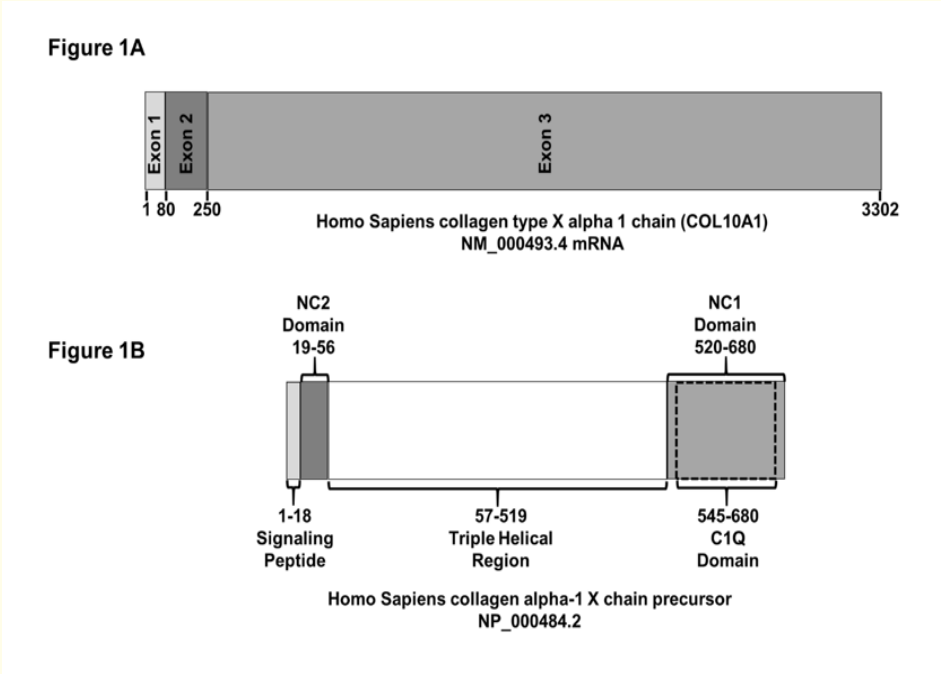

Figure 1: *FCOL10A1 Transcript and Collagen X Protein Maps.*

Collagen X and cancer

To further add to the potential functional roles of collagen X, recent reports suggest that collagen x may also be involved in tumorigenesis and invasion/metastasis. COL10A1 has been shown to be overexpressed in breast, lung, stomach, testis, pancreas, bladder, esophagus, ovary and colorectal tumors [10]. Overexpression of COL10A1 has been detected via high throughput sequencing or microarray analysis and then confirmed via RT-PCR, wester blot, and immunofluorescence assays. Chapmen., *et al.* evaluated COL10A1 upregulation in various tumor tissues as compared to normal tissue using microarray analysis. Authors evaluated 128 tumor samples representing over 20 tumor types and reported that COL10A1 was upregulated greater than 2 fold in breast, stomach, lung, pancreas, testis, bladder and ovary tumor types when compared with their normal tissue of origin. Results of the microarray analysis was confirmed by qRT-PCR. Of interest, COL10A1 was undetected in all tumor cell lines evaluated by Chapmen., *et al.* (n = 68) demonstrating the importance of the tumor microenvironment on tumor formation and progression. Authors concluded that collagen X may play an important role in tumor progression across multiple tumor types and that COL10A1 may serve as an important biomarker in detecting tumor vs. normal tissue [10].

Some of the most significant findings regarding COL10A1 expression have been demonstrated in breast cancers. Chapmen., *et al.* reported an increase of COL10A1 by a fold change of 5.5 when comparing breast tumor tissue to normal breast tissue (from microarray data described above). Authors also utilized immunofluorescence to visualize the localization of collagen X and found that collagen X was primarily detected in the vasculature of breast tumors and was undetectable in normal breast tissues. These findings highlight the importance of COL10A1 during vascularization in breast cancer [10].

Chang., *et al.* utilized microarray analysis to analyze gene expression changes in tumor tissues (infiltrating lobular carcinoma, metaplastic carcinoma, and infiltrating ductal carcinoma) as compared to adjacent tumor free tissue. COL10A1 was found to be 1 of 371 genes with 2-fold or higher expression levels. Authors validated the microarray data with RT-PCR and found that COL10A1 was significantly higher in 12/15 breast tumors evaluated as compared to normal adjacent tissue [11].

Desmendt., *et al.* evaluated gene expression profiles from CD10+ tumor associated cells and CD10+ cells from normal breast tissue in order to develop a 12 gene CD10+ stroma gene expression signature (including COL10A1). Authors demonstrated that the described gene expression signature was able to discriminate between tumors that progress from in situ to invasive breast cancer. Desmendt., *et al.* also stated that COL10A1 expression did not show any variation between different cells line (using a data set generated by Neve et al.) again suggesting that overexpression of COL10A1 in breast tumors was most likely not due to the epithelial cells found within tumors and reinforcing the importance of the tumor microenvironment in tumorigenesis [12].

Brodsky., *et al,* investigated the role of the tumor microenvironment in treatment response of ER+/HER-2 positive breast cancers. Authors evaluated differences in expression of genes in tumors of responding vs nonresponding (to treatment) patients via microarray analysis. COL10A1 was one of 30 identified genes shown to be differentially expressed when comparing patients who achieved a good response vs patient that did not achieve a good response. Differential expression was confirmed by qRT-PCR and immunohistochemistry analysis was conducted to define collagen X expression pattern. Normal breast tissue was shown to be negative for collagen X staining while tumor samples demonstrated intense staining. Authors concluded that increased collagen X staining strongly correlated with a poor response to treatment and suggested that collagen X may play a causative role in how a tumor responds to therapy [13].

From a cohort of Lebanese women with invasive breast cancer, Makoukji., *et al.* subjected invasive breast cancer tissue and non-tumor adjacent tissue to microarray analysis. COL10A1 was shown to be significantly upregulated in invasive breast tumor samples as compared to non-tumor tissue across all grades (I, II, III) and 4 out of 5 molecular subtypes (Luminal A, Luminal B, HER2, and Basal) and COL10A1 overexpression was validated via qRT-PCR. Authors suggested that COL10A1 may play a role in breast cancer cell invasion [14].

Giussani., *et al.* reported that COL10A1 was significantly upregulated when comparing tumor and normal specimens in publicly available datasets. COL10A1 was also found to be one of the most significantly upregulated genes when the gene expression profiles of normal human dermal fibroblasts (NHDFs) compared to NHDFs conditioned with epithelial breast cancer cells and these results were confirmed by western blot analysis. Using RT-PCR, expression levels of COL10A1 were found to be higher in patient derived cancer associated fibroblasts when compared to matched normal fibroblasts. In an attempt to identify serum-based tumor markers for breast cancer development, authors used ELISA to evaluate collagen X levels in human plasma from breast cancer patients and determined COL10A1 may represent a potential diagnostic marker in discriminating breast cancer patients from patients with benign tumors [15-17].

In order to identify a prognostic biomarker that can potentially predict tumors that have the propensity to advance to invasive breast cancer, Schultz., *et al.* used microarray analysis to identify differentially expressed transcripts between DCIS (ductal carcinoma in situ) and IBC (invasive breast carcinoma) tissue samples. COL10A1 was shown to be significantly upregulated in IBC as compared to DCIS and was validated via qRTPCR. Of importance COL10A1 was found to have low expression in pure DCIS (with low risk of developing into IBC), intermediate expression in DCIS (with high risk of developing into IBC), and high expression in IBC and authors concluded that COL10A1 may serve as a potential indicator of high risk DCIS [18].

Using a bioinformatic approach, Zhang., *et al*. evaluated mRNA expression of COL10A1 in breast cancer patients via the Oncomine database and collagen X protein expression in breast cancer tissue using the UALCAN database. Authors demonstrated that elevated COL10A1 transcript levels were correlated to worse overall survival and relapse-free, distant metastasis-free and disease-free survival. Authors concluded that COL10A1 may be a prognostic biomarker of breast cancer [19].

Other key findings in regards to collagen X and cancer have been reported in colon cancer, bladder cancer, and gastric cancer. Using microarray analysis, Sole., *et al.* identified COL10A1 as a potential biomarker capable of differentiating between colon cancer cases and non-tumor samples. Authors also demonstrated that collagen X was detected in high concentrations by ELISA in serum of patients with colon cancer and adenomas and was statistically different between non-cancer controls and stage II-IV colon cancer. Authors concluded that COL10A1 represented a promising serum based biomarker for identifying adenomas and invasive cancer [20].

Using bioinformatic analysis, Liu., *et al.* mined TCGA (The Cancer Genomome Atlas) to evaluate gene expression of bladder cancer samples. Authors found that COL10A1 overexpression was shown to be associated with a high-risk score and was negatively correlated to overall survival. These results demonstrate that COL10A1 may function as a strong prognostic indicator for bladder cancer [21].

In one of the first functional studies involving collagen X and it's role in cancer, Huang., *et al.* evaluated the molecular mechanisms in which COL10A1 may be involved in colorectal cancer progression. Authors used paired colorectal cancer and adjacent normal tissue to determine COL10A1 transcript and protein status. COL10A1 expression was shown to be upregulated in 62.5% (by qRT-PCR) of colorectal cancer tissue as compared to their corresponding normal tissue. Collagen X protein was shown to be upregulated in 82.5% (by western blot analysis) of colorectal cancer tissue when compared to normal. Western blot data was confirmed by IHC. COL10A1 was overexpressed in HCT116 and LoVo colorectal cancer cell lines (both displayed expression of collagen X) and was knocked down in SW480 and SW620 colorectal cancer cell lines (both displayed an upregulation of collagen X). Overexpressing/knockdown cell lines were subjected to wound healing assays to evaluate changes in rates of migration. When COL10A1 was overexpressed, cells demonstrated an increase in their migration ability and rate while knockdown of COL10A1 had an inverse effect demonstrating that COL10A1 was playing an important role in a colorectal cancer cell's ability to migrate and invade. COL10A1 overexpression was also shown to promote tumor growth (using an in vivo tumor growth model) and was shown to promote the epithelial-mesenchymal transition of colorectal cancer cells (when measuring EMT markers: E-cadherin, N-cadherin, Beta-catenin, Slug and Snail). Authors also reported that COL10A1 expression in colorectal cancer was correlated to poor prognosis and overall survival. Results from this study provide important insight into the role collagen X may be playing in colorectal cancer [22].

Recently, Tingting., *et al.* used RNAseq of tissue samples of normal gastric tissues and tissues from early and late stage gastric cancers to identify genes differentially expressed genes between normal vs cancer gastric tissue. COL10A1 was found to be significantly overexpressed in cancer tissues as compared to their corresponding normal tissue and these results were validated by qRT-PCR. Authors found that COL10A1 was significantly associated with tumor size, lymph node metastasis, invasion and reoccurrence risk. Elevated COL10A1 expression levels were correlated with a negative prognostic impact and shorter overall and recurrence-free survival. Protein expression of collagen X was confirmed to be increased in gastric cancer tissues as compared to matched non-cancerous gastric mucosa. To evaluate collagen X and it's functional role in gastric cancer, authors knocked down COL10A1 in a MKN45 gastric cancer cell line and overexpressed COL10A1 in a SGC7901 cell line. Overexpression resulted in a change in cell morphology from round, flat, or mixed morphology to spindle-shaped (taking on a fibroblastic-like phenotype) while knockdown achieved the opposite effect. Using wound healing assays to evaluate migration ability and transwell assays to evaluate invasive capability, authors demonstrated that COL10A1 overexpression in gastric cancer cells enhanced migration and invasion while knockdown resulted in the opposite effect. In an in vivo metastatic assay, authors demonstrated that knockdown of COL10A1 in gastric cancer cells dramatically decreased the number and size of metastatic lung growths. These results highly suggests that collagen X plays an important role in enhancing the metastatic potential of gastric cancer cells. Lie., *et al.* also demonstrated that transcriptional activator, SOX9, binds directly to the COL10A1 promoter to activation COL10A1 transcription, providing important insight into the mechanisms mediating regulation of COL10A1 gene expression [23].

To explore the functional role collagen X plays in lung cancers, Liang., *et al.* first evaluated 40 paired lung tumors and normal tissues and determined that COL10A1 was upregulated in tumor tissues as compared to their adjacent normal tissues and that this upregulation was shown to be significantly correlated with poor prognosis and tightly associated with tumor metastasis. COL10A1 was then overexpressed in A549 cells and migration and invasion capabilities were shown to be increased while apoptosis was shown to be suppressed. When COL10A1 was also knocked down in H1299 cells, inverse effects on migration, invasion and apoptosis were observed and when evaluated in vivo, knocked down cells demonstrated decreased tumor growth and fewer metastasis when injected into nude mice as compared to control H1299 cells. Authors also demonstrated that collagen X may be playing an important role in the FAK signaling pathway which promotes both cell migration and invasion. Authors conclude that collagen X is a critical biomarker that promotes migration and invasion of lung adenocarcinoma cells [24].

Conclusion

Recent reports on collagen X and the potential role in tumorigenesis and invasion/metastasis represents an opportunity to further explore the functional role of Collagen X. As in the endochondral ossification process, many questions remain unanswered as to what functional role collagen X may be playing in tumor progression and in the propensity of tumors to invade and metastasize to distant locations. One of the most interesting observations is the localization of collagen X to the tumor vasculature in breast tumors. COL10A1 was shown to be localized to endothelial cells in breast tumor vasculature (as demonstrated by co-staining experiments0. The presence of collagen X in the vasculature formed within the tumors suggests that there may be an unidentified role of collagen X in the process of vascularization (as discussed by Chapmen., *et al.* [10]). Exploring the link between collagen X and vascularization may provide insight into the role collagen X plays in the process of vascularization in both tumor progression and the endochondral ossification process. Findings demonstrating that COL10A1 expression is undetectable in breast cancer cell lines stresses the importance of the microenvironment in tumorigenesis. The microenvironment has been shown to play pivotal roles in formation and progression of tumors, the ability of a tumor to invade and metastasize and the chemotherapeutic response of a tumor (as discussed by Brodsky., *et al.* [13]). Understanding the role collagen X is playing in the tumor microenvironment represents an important area that needs to be explored further. Cellular origins of COL10A1 and mechanisms meditating overexpression are not yet clear, as discussed by Liang., *et al* [24]. As to date, studies evaluating COL10A1 expression have been restricted to epithelial cell types. Additional studies are necessary to provide insight into the cellular origins of COL10A1 in heterogenous tumors. Of importance, questions also remain on the structural role collagen X may be playing in

mediating invasion and metastasis of cancer cells. For example, is the role collagen plays in calcification during the ossification process similar to the role collagen X may be playing in the tumor microenvironment in regards to enhancing metastasis of tumor cells? The molecular mechanisms mediating COL10A1 remain largely unknown as does the molecular pathways COL10A1 overexpression may be mediating. Elucidating these functions and mechanisms remain important to understanding the functional role collagen X plays. Recent reports highlighted here stress just how critical COL10A1 may be in serving as a prognostic/diagnostic biomarker. Functional studies suggest COL10A1 may serve as a therapeutic target in targeting and treating various types of cancers. Collagen X represents an exciting target in the fields of cancer biology and pharmacology/toxicology.

Conflict of Interest

The author has no financial interest of conflict to declare.

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