

## Expression and Roles of Lumican, a Small Leucine-rich Proteoglycan, in Gastrointestinal and Other Cancers

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### INTRODUCTION

Microenvironments including extracellular matrices (ECMs) and tumor vessels are important in tumor growth, migration, invasion, and metastasis. Tumor cells and a variety of nonmalignant stromal cells including fibroblasts, endothelial cells, macrophages, lymphocytes, pericytes, and mesenchymal stem cells synthesize the ECM. Proteoglycans are a major component of the ECM, consisting of a core protein and one or more glycosaminoglycan side chains. They regulate the water balance of the ECM; influence tissue biomechanics; facilitate cellular adhesion, proliferation, and migration; and modulate growth factor and cytokine activities<sup>[1-3]</sup>. In tumor stromal tissues, proteoglycans are widely distributed and perform multiple functions in cancer and angiogenesis by their ability to interact with both ligands and receptors that regulate neoplastic growth and neovascularization<sup>[4,5]</sup>.

Members of the small leucine-rich proteoglycan (SLRP) family have relatively small molecular sizes with core proteins of approximately 40 kDa, and possesses 6-10 leucine-rich repeating units between the flanking cysteine-rich disulfide-bonded domains at the N and C termini of the core protein<sup>[6-8]</sup>. The SLRPs have 12 or more structurally related members, and they are classified into classes I, II, and III<sup>[9]</sup>. Class II of the SLRP family consists of fibromodulin, keratocan, proline/arginine-rich end leucine-rich repeat protein (known as PRELP), osteoadherin, and lumican. Molecular modeling analysis has suggested that all members of the SLRP superfamily possibly build horseshoe-shaped structures<sup>[10]</sup>. These SLRPs interact with the surface of collagen fibrils in the ECM and may be involved in maintaining the tissue stromal structure<sup>[11]</sup>. Furthermore, SLRPs are considered important in facilitating protein-protein interactions with different cell and matrix components<sup>[12]</sup>. Knockout animals for each member of the SLRP family have abnormal connective tissue phenotypes<sup>[13]</sup>. In tumor tissues, SLRPs are reported to have important roles in tumor cell migration and

### ABSTRACT

Lumican is a member of class II of the small leucine-rich proteoglycan family, and it was first reported as a keratin sulfate-containing proteoglycan in the cornea. Lumican plays an important role in collagen fibrillogenesis in cornea and other various human tissues. Recently, a number of reports have shown that lumican is expressed in several types of malignant tumors including gastrointestinal cancers; however, the roles of lumican in the tumors are quite variable. In neuroendocrine tumors of the colon, for example, lumican expression in the cytoplasm is negatively correlated with tumor malignancy grade. Increased expression of lumican in malignant melanoma and osteosarcoma inhibits their aggressive growth. In contrast, the localization of lumican in the stromal tissue correlates with advanced cancer stage and retroperitoneal and duodenal invasion and residual tumor presence of pancreatic cancer, and increased secretion of lumican in the extracellular space induces growth of the cancer. Lumican in colorectal cancer has tended to correlate with the spread of lymph node metastasis and the depth of tumor invasion. The reason for these opposing effects of lumican in different cancers is not well understood, but different glycosylation patterns of lumican or receptors and in the cell signaling pathways involved may play pivotal roles in its cancer cell behaviors. This review article summarizes the expression and roles of lumican in malignant tumors, including gastrointestinal cancers, and discusses the possibility of using lumican as a diagnostic marker and novel therapeutic target for cancers.

proliferation, in addition to their ECM functions in tissue hydration and collagen fibrillogenesis.

Lumican was first reported as one of the major keratan sulfate (KS)-containing proteoglycans in the chicken cornea<sup>[14]</sup>. Lumican colocalizes with fibrillar collagens in the corneal stroma and regulates the assembly and diameter of collagen fibers and interfibrillar spacing<sup>[6,15]</sup>. Lumican-null mice show corneal opacity and skin fragility resulting from disorganized and loosely packed collagen fibers<sup>[16-21]</sup>. In addition to the cornea, lumican is reported to be expressed in various human tissues or organs, including skin, artery, lung, intervertebral disc, kidney, bone, aorta, articular cartilage, colorectum, and pancreas<sup>[22-33]</sup>. Lumican expression has been studied in several pathological conditions closely related to collagen fibrillogenesis. We previously reported that lumican is predominantly localized in areas of pathological fibrosis, including the thickened intima of coronary arteries, ischemic and reperfused hearts, and acute pancreatitis<sup>[34-36]</sup>. In ischemic lesions of rat heart, lumican is expressed at high levels in many capillary endothelial cells, and a few myocardial cells close to an ischemic lesion express lumican mRNA<sup>[34]</sup>. In atherosclerosis, lumican is overexpressed in most of the vascular smooth muscle cells that migrate into the thickened intima<sup>[35]</sup>. Lumican is transiently synthesized by acinar cells and fibroblasts in acute pancreatitis and may contribute to an immature and transient fibrosis in acute pancreatitis<sup>[36]</sup>.

Recently, expression of lumican has been reported in various types of cancer, but the roles of lumican in the tumors are varied. In this review article, we summarize the expression and functions of lumican in various types of cancers, including gastrointestinal cancers.

## STRUCTURE OF LUMICAN

The human lumican gene is located on chromosome 12q21.3–q22 and consists of three exons separated by introns of 2.2 and 3.5 kilobase pairs<sup>[27,37]</sup>. The lumican protein has 338 amino acids in four domains: (1) a putative signal peptide of 18 amino acids; (2) a negatively charged N-terminal domain; (3) tandem leucine-rich repeats; and (4) a carboxy terminal domain of 50 amino acids<sup>[38]</sup>. The central region of the molecule possesses four asparagine residues capable of participating in N-linked glycosylation<sup>[39]</sup>. Because of the different glycosylation patterns, lumican includes a core protein, glycoprotein, and proteoglycan forms<sup>[40]</sup>. The

molecular weight of the core protein of lumican is reported at 37 kDa, and the glycoprotein form and proteoglycan forms are considered to be about 55-57 and 50-100 kDa, respectively. The proteoglycan form of lumican is mainly found in corneal tissues, whereas the glycoprotein form occurs mainly in other tissues<sup>[41,42]</sup>. In the adult cornea, lumican exists as a proteoglycan; however, in the embryonic cornea, lumican has been reported to localize as a glycoprotein, suggesting that the glycosylation pattern of lumican correlates with corneal transparency<sup>[42]</sup>. The lumican gene does not contain a conventional TATA box; rather, a unique TATCA box is present upstream of the transcription initiation site<sup>[37]</sup>. A GC box upstream of the transcription start site is also essential for transcription initiation of human lumican<sup>[43]</sup>. Sp3 has been identified as the transcriptional activator binding to the GC box, and a repressor element has been identified upstream of the transcription start site. A GAGA-binding site located between -386 to -391 is necessary for repression of transcription of human lumican<sup>[43]</sup>. Lumican is ectopically expressed in cancer developments and transiently expressed in wound healing processes, and these findings suggest that expression levels are controlled by genetic and/or epigenetic regulations. However, the regulatory mechanisms are not well clarified.

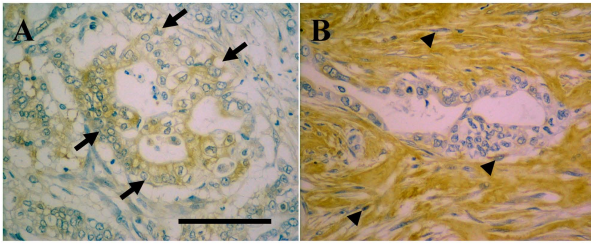
## LUMICAN IN PANCREATIC CANCER

Lumican expression has been reported in pancreatic and colorectal cancers, and recently in esophageal cancer and early hepatocellular carcinomas (HCC); to our knowledge, there are no reports of it in gastric or biliary tract cancers<sup>[25,26,44-47]</sup>. In normal pancreas, lumican is mainly localized in alpha cells of islets, which express glucagon, and in stromal tissues<sup>[26]</sup>. However, lumican is not localized in exocrine components of the pancreas, including acinar and ductal cells. In pancreatic cancer tissues, lumican and its mRNA are strongly expressed in cancer cells and chronic pancreatitis-like (CP-like) lesions adjacent to the cancer cells<sup>[26]</sup>. Lumican expression in the cytoplasm of pancreatic cancer cells does not correlate with clinicopathological factors, whereas lumican expression in stromal tissues correlates with advanced stage, retroperitoneal and duodenal invasion, and residual tumor<sup>[44]</sup> (Figure 1). Furthermore, lumican expression in stromal tissues tends to correlate with shorter survival in patients with pancreatic cancer. These findings suggest that lumican in stromal tissues is influential in the aggressiveness of pancreatic cancer (Table 1).

**Table 1** Expression and roles of lumican in cancer.

	Localization of lumican	Functions	Molecular weights
Pancreatic cancer	Cytoplasm in cancer cells <sup>[44]</sup>	Not correlated	37-100 kDa <sup>[26,45]</sup>
	Stromal tissue adjacent to cancer cells <sup>[44]</sup>	Increased growth, advanced stages	70 kDa <sup>[45]</sup>
	Culture medium <sup>[49]</sup>	Increased <i>in vitro</i> cell growth Suppressed <i>in vivo</i> tumor growth and angiogenesis	46 kDa
Colorectal cancer <sup>[46]</sup>	Cytoplasm in cancer cells	LN metastasis, tumor invasion, poor prognosis	37-100 kDa
Neuroendocrine tumor <sup>[51]</sup>	Cytoplasm in carcinoid > NEC	Inhibition of growth	ND
Esophageal cancer <sup>[47]</sup>	Cytoplasm cancer cells and/or stromal tissues adjacent to cancer cells	ND	ND
Hepatocellular carcinoma <sup>[52]</sup>	ND	ND	ND
Breast cancer <sup>[60]</sup>	Fibroblasts adjacent to cancer cells	High pathological tumor grade, low estrogen receptor levels in the cancer tissue, and young age	65-150 kDa
Cervical cancer <sup>[30]</sup>	Cytoplasm in cancer cells at the peripheral lesion of cancer cell nest	ND	40-200kDa
Malignant melanoma <sup>[61,62,67]</sup>	Stromal tissue adjacent to tumor cells	Suppressed growth of tumors, inhibited tumor metastasis	57kDa
Osteosarcoma <sup>[63]</sup>	Culture medium	Inhibited growth, induced migration, and chemotactic response	60-90kDa
Lung cancer <sup>[64]</sup>	Cytoplasm of ADC	Pleural invasion, larger tumor size	37-100 kDa
	Stromal tissue adjacent to ADC	Not correlated	37-100 kDa
	Cytoplasm of SCC	Keratinization	37-100 kDa
	Stromal tissue adjacent to SCC	Vascular invasion	37-100 kDa
Prostate cancer <sup>[59]</sup>	Stromal cells (decreased expression)	Metastatic niche formation	ND
Ovarian cancer <sup>[57]</sup>	ND (decreased expression)	Genetic variants in lumican not associated with cancer risk	ND

NEC: Neuroendocrine cell carcinoma; ADC: Adenocarcinoma; SCC: Squamous cell carcinoma; LN: Lymph node; ND: Not determined.



**Figure 1** Immunohistochemical images of lumican in pancreatic cancer. Lumican is strongly expressed in pancreatic cancer cells (A, arrows). In other cases, tumor stroma and spindle-shaped fibroblast-like cells (B, arrowheads) also express lumican. The light brown color indicates that lumican is present in the area. Bar, 100  $\mu$ m.

Lumican and its mRNA are expressed in most pancreatic cancer cell lines examined, and the protein from the pancreatic cancer cells weighs from 37 to 100 kDa<sup>[26]</sup>. This variability suggests that lumican proteins in pancreatic cancer cells possess many different types of glycosylated side chains and include core protein, glycoprotein, and proteoglycan types. An enzymatic digestion study has revealed that the lumican from pancreatic cancers has non/ poorly sulfated polyglucosamine side chains rather than highly sulfated KS chains<sup>[26]</sup>. Recently, we found that the lumican proteins from cancer cells and secreted lumican protein from cancer cells to the extracellular space have different molecular weights<sup>[45]</sup>. Surprisingly, secreted lumican from pancreatic cancer cells to the extracellular space presents only one band with a molecular weight of 70 kDa. This 70-kDa lumican protein was mainly modified by N-glycan and partially possesses non-sulfated KS or polyglucosamine side chains, but does not have highly sulfated KS chains. Forced expression and secretion of the 70-kDa lumican protein into the extracellular space using gene expression techniques indicates that increased extracellular lumican stimulates pancreatic cancer cell growth through activation of the ERK pathway. Together with immunohistochemical analysis using human pancreatic cancer patients, these results suggest that secreted 70-kDa lumican protein from cancer cells may be important in cancer cell proliferation.

In pancreatic cancer tissues, lumican has also been detected in CP-like lesions close to cancer cells<sup>[26]</sup>. Acinar cells, islet cells, and fibroblasts express lumican protein in CP-like lesions. Other work has shown that lumican is expressed from pancreatic stellate cells in patients with pancreatic cancer<sup>[48]</sup>. That study found increased expression of lumican mRNA in pancreatic cancer tissues relative to pancreatic tissue from organ donors and focused on pancreatic stellate cells as a major source of lumican in the cancer. The expression levels of lumican mRNA from the stellate cells was influenced by the conditioned medium from pancreatic cancer cell lines. The authors suggested that pancreatic cancer affects the composition of the ECM through TGF- $\beta$ 1 and other soluble factors and alters the tumor microenvironment in a tumor-favorable way.

A recent report showed that lumican-overexpressing pancreatic cancer cells present opposing effects on tumor growth *in vitro* versus *in vivo*<sup>[49]</sup>. Lumican-overexpressing cells secreted a 46-kDa lumican protein into the medium and increased proliferation *in vitro*, whereas they formed smaller tumors and showed reduced vascular density and enhanced Fas-mediated endothelial cell apoptosis *in vivo* (Table 1). These findings suggest that reduction in tumor size is specifically the result of apoptosis of endothelial cells. The main source of lumican in pancreatic cancer has not been well clarified, but cross-talk between cancer and stromal cells may play an important role in cancer cell growth through expression of extracellular lumican protein. Further studies using *in vivo* models are needed to clarify whether lumican is a novel therapeutic target for pancreatic cancer.

## LUMICAN IN COLORECTAL CANCER

In human colorectal tissues, lumican localizes in stromal fibroblasts and neural cells but not in normal epithelial cells<sup>[25]</sup>. Lumican is expressed in the cytoplasm of approximately 60% of colorectal cancer cells from patients and tends to correlate with lymph node metastasis and depth of tumor invasion<sup>[46]</sup>. Furthermore, the survival rate is significantly lower in patients with a high lumican expression level compared to those with a low lumican expression level (Table 1). Lumican is expressed in five of the five colorectal cancer cell lines examined, and the lumican in cytoplasm has non-sulfated or poorly sulfated polyglucosamine side chains, similar to lumican in pancreatic cancer<sup>[25]</sup>. These findings suggest that lumican in the cytoplasm of colorectal cancer is involved in the aggressiveness of the cancer. Relative to radiotherapy, lumican is more expressed in responders with rectal cancer in response to preoperative radiotherapy than in non-responders, based on DNA microarray analysis<sup>[50]</sup>.

In the analysis of neuroendocrine cell tumors, including carcinoid tumors and neuroendocrine cell carcinomas in the colorectum, lumican is significantly more expressed in the cytoplasm of carcinoid tumors than in neuroendocrine cell carcinomas<sup>[51]</sup>. In contrast, expression levels of lumican in stromal tissues do not differ in carcinoid tumor and neuroendocrine cell carcinomas. These findings may indicate that lumican in cytoplasm correlates with the growth inhibitory effects on carcinoid cells; thus, further studies are required, including analysis of glycosylation of these diseases using cell culture techniques.

## LUMICAN IN OTHER GI CANCERS

Lumican is highly expressed in esophageal squamous cell carcinoma (ESCC), based on DNA microarray analysis, and additional work from the same group showed that lumican protein was increased in 100% of 137 different ESCC samples on tissue microarray<sup>[47]</sup>. A recent study using cross-species comparative genomic analysis showed significant expression of lumican in both early rat and human HCC<sup>[52]</sup>. However, the roles of lumican in ESCC and HCC have not been identified. The role of lumican in GI cancers is controversial for each GI cancer type; thus, clarifying its functions in these cancers is important.

## LUMICAN EXPRESSION IN INFLAMMATORY GI DISEASES

Expression and activity of lumican in inflammatory diseases have been reported in pancreatitis, colitis, nonalcoholic liver disease, chronic hepatitis B, and autoimmune liver diseases<sup>[36, 53-56]</sup>. In a rat necrotizing pancreatitis model, several sizes of bands corresponding to lumican, including 42, 57, and 92 kDa bands, were detected in addition to 50 kDa bands observed in normal pancreas<sup>[36]</sup>. In pancreatitis, lumican has been localized in immature fibrosis and lumican mRNA strongly detected in acinar and islet cells and fibroblasts. Also in pancreatitis, lumican, which was synthesized by these cells, may play roles in immature and transient fibrosis. A mouse model of chronic colitis using administration of 2-4-5, trinitrobenzene sulfonic acid (TNBS) via weekly enemas showed Crohn's colitis-like transmural and tissue damage. TNBS-treated lumican knockout mice (Lum<sup>-/-</sup>) had a lesser increase in levels of CXCL1, tumor necrosis factor alpha, neutrophil infiltration, and nuclear factor kappa B<sup>[55]</sup>. These findings suggest that lumican promotes homeostasis of the colon by aiding innate immune and inflammatory responses in the early stages of colitis. DNA microarray analysis using human liver biopsy specimens showed that lumican was more abundantly present in the advanced stages of chronic hepatitis B compared with the other liver diseases such as autoimmune

hepatitis, primary biliary cirrhosis, and drug-induced liver disease<sup>[54]</sup>. Furthermore, histologically progressive nonalcoholic fatty liver disease has been associated with overexpression of lumican in liver tissues<sup>[53]</sup>. The roles of lumican in these liver diseases are not well examined, but lumican may correlate with fibrosis and lipotoxicity.

## LUMICAN EXPRESSION IN NON-GI CANCERS

Lumican expression has been reported in non-GI cancers including breast cancer, cervical cancer, malignant melanoma, osteosarcoma, lung cancer, prostate cancer, and ovarian cancer<sup>[57-59]</sup> (Table 1). In these non-GI cancers, lumican-expressing cells and the roles of lumican are markedly different. In breast cancer and malignant melanoma, lumican is mainly synthesized by stromal cells, and the expression of lumican correlates with aggressiveness of the tumors<sup>[60-62]</sup>. On the other hand, cervical cancer and osteosarcoma cells produce lumican by themselves, and lumican inhibits growth and invasion of osteosarcoma<sup>[30, 63]</sup>. In lung cancer, lumican expression occurs in cancer cells and stromal cells in adenocarcinoma and squamous cell carcinoma, and the expression of lumican in these cells differentially correlates with their clinicopathological findings<sup>[64]</sup>. Lumican synthesized by stromal cells decreases in prostate cancer tissues and is suggested to play a role in the formation of a metastatic niche via crosstalk with the cancer cells<sup>[59]</sup>. In serous epithelial ovarian cancer, lumican and decorine show reduced stromal expression, whereas an inherited variation of lumican and decorine are not directly associated with risk of cancer<sup>[57]</sup>.

### Breast cancer

In breast cancer tissues, expression levels of lumican are highest among the SLRP samples, and lumican is expressed more in breast tumor tissues than in normal breast tissues from the same patients<sup>[65]</sup>. There are six single nucleotide polymorphisms in the lumican gene, and one of these, LUM rs2268578, is associated with breast cancer, particularly estrogen receptor-positive breast cancer<sup>[66]</sup>. In breast cancer tissues, lumican mRNA is overexpressed in fibroblasts adjacent to cancer cells but not in cancer cells. Furthermore, the high expression level of lumican is associated with a high pathological tumor grade, low estrogen receptor levels in the cancer tissue, and young age of patients<sup>[60]</sup> (Table 1).

### Cervical cancer

Lumican and its mRNA have not been detected in squamous epithelial cells of a normal uterine cervix. In cervical squamous cell carcinomas, lumican is synthesized by cancer cells and is predominantly localized in cancer cells at the periphery of the nests of cancer cells<sup>[30]</sup>. The accumulated lumican protein in cancer cells at the periphery of cancer nests may be involved in the growth or invasion of human cervical cancer cells (Table 1).

### Malignant melanoma

Immunohistochemical analysis using human melanoma tissues has shown that lumican localizes in the peritumoral stroma of melanoma but is not found in melanoma cells<sup>[61]</sup>. The expression level of lumican in peritumoral stroma significantly decreases with increasing Clark levels, which describes the level of anatomical invasion of the melanoma in the skin. After subcutaneous injections of lumican-transfected mouse melanoma B16F1 cells into syngeneic mice, lumican expression significantly suppresses subcutaneous tumor formation *in vivo*, with a concomitant decrease in cyclin D1 expression levels<sup>[62]</sup> and enhanced apoptosis of B16F1 cells with increased Bax expression

levels. Lumican-transfected B16F1 cells form smaller and fewer lung metastases than mock cells *in vivo*<sup>[67]</sup> (Table 1). In metastatic nodules, lumican-transfected cells show increased apoptosis, decreased vascular endothelial growth factor and blood vessel formation, and unchanged cell proliferation abilities.

### Osteosarcoma

Human osteosarcoma cell lines express and secrete lumican partly substituted with KS glycosaminoglycans. Lumican inhibits Saos2 osteosarcoma cell growth but promotes their migration and chemotactic response to fibronectin (Table 1). When secreted to the ECM, lumican is an endogenous inhibitor of TGF- $\beta$ 2 activity, resulting in downstream effector modulation including pSmad2, integrin  $\beta$ 1, and pFAK to regulate osteosarcoma adhesion<sup>[63]</sup>.

### Lung cancer

In human lungs, lumican is a major component of the proteoglycan matrix<sup>[32]</sup>. In lung adenocarcinoma, the expression level of lumican in cancer cells correlates with pleural invasion and larger tumor size but in stromal tissues does not correlate with clinicopathological factors<sup>[64]</sup> (Table 1). In squamous cell carcinoma, the expression level of lumican in cancer cells correlates with formation of a keratinized pattern, and stromal expression correlates with vascular invasion. These findings suggest that the expression patterns and the glycosylation type of lumican in cells and stromal tissues correlate with the aggressiveness of adenocarcinoma and squamous cell carcinoma.

## POTENTIAL DIAGNOSTIC AND THERAPEUTIC BENEFITS FOR CANCER

Selected reaction monitoring-based targeted proteomics analysis using plasma from patients with pancreatic cancer has revealed lumican as one of five candidate proteins for pancreatic cancer biomarker<sup>[68]</sup>. Proteomic profiles have also revealed that 364 protein spots corresponding to 58 gene products, including lumican, are highly expressed in serum from lung cancer patients<sup>[69]</sup>. These findings suggest that lumican is a candidate biomarker for cancer. Furthermore, we found using immunohistochemical analysis that the expression level of lumican is different in several types of skin tumors, including Bowen's disease and actinic keratosis (unpublished data). This finding may indicate the usefulness of lumican in the histological differential diagnosis of some cancer types.

A peptide from a leucine-rich repeat 9 domain of the lumican core protein, designated as lumcorin, has been reported to inhibit the migration of malignant melanoma cells *in vitro*<sup>[70]</sup>. A small interfering RNA targeting lumican mRNA inhibits growth of pancreatic cancer<sup>[45]</sup>. These findings indicate that lumican is a possible therapeutic target for malignant tumors, including GI cancer. However, several lines of evidence suggest that different glycosylation patterns of lumican exert different actions on cancer cells. Further studies are needed to develop a molecular target therapy involving lumican.

## CONCLUDING REMARKS

Expression patterns and roles for lumican are variable in several kinds of cancers, possibly in part because of different glycosylation modifications, receptors, and intracellular signaling pathways involved. Regulation of expression levels of lumican in cancer cells and stromal cells may yield effective therapy for some cancers, including gastrointestinal cancer. Further studies are expected for developing diagnostic and treatment methods using lumican in cancers.

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