

Tumor Biology

COL11A1/(pro)collagen 11A1 expression is a remarkable biomarker of human invasive carcinoma-associated stromal cells and carcinoma progression --Manuscript Draft--

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Abstract:	<p>The COL11A1 human gene codes for the $\alpha 1$ chain of procollagen 11A1 and mature collagen 11A1, an extracellular minor fibrillar collagen.</p> <p>Under regular conditions, this gene and its derived products are mainly expressed by chondrocytes and mesenchymal stem cells as well as osteoblasts. Normal epithelial cells and quiescent fibroblasts from diverse locations do not express them.</p> <p>Mesenchyme-derived tumours and related conditions such as scleroderma and keloids, are positive for COL11A1/(pro)collagen 11A1 expression, as well as high grade human gliomas/glioblastomas. This expression is almost absent in benign pathological processes such as breast hyperplasia, sclerosing adenosis, idiopathic pulmonary fibrosis, cirrhosis, pancreatitis, diverticulitis, and inflammatory bowel disease. By contrast, COL11A1/(pro)collagen 11A1 is highly expressed by activated stromal cells of the desmoplastic reaction of different human invasive carcinomas, and this expression is correlated with carcinoma aggressiveness and progression, and lymph node metastasis.</p> <p>COL11A1 up-regulation has been shown to be associated to TGF-β1, Wnt and Hh signalling pathways, which are especially active in cancer-associated stromal cells. At the front of invasive carcinomas, neoplastic epithelial cells, putatively undergoing epithelial-to-mesenchymal transition, and carcinoma-derived cells with highly</p>

	<p>metastatic capabilities, can express COL11A1. Thus, in established metastases, the expression of COL11A1/(pro)collagen 11A1 could rely on both the metastatic epithelial cells and/or the accompanying activated stromal cells.</p> <p>Conclusion: COL11A1/(pro)collagen 11A1 expression is a remarkable biomarker of human carcinoma-associated stromal cells and carcinoma progression.</p>
<p>Response to Reviewers:</p>	<p>Reviewer #1: The review titled "COL11A1/(pro)collagen 11A1 expression is a remarkable biomarker of human invasive carcinoma-associated stromal cells and carcinoma progression" details the current knowledge of this topic and reference 97 previous studies. The authors are have been involved with this research topic and therefore can contribute with their own experience. The topic is important in cancer biology and has clinical implications.</p> <p>Reply to comments</p> <p>The figures that are now included are suboptimal. Figure 1 and 2 should be reconsidered with the aim of generating one figure with the necessary information.</p> <p>We have now combined former Figures 1 and 2 in just one new Figure 1, which has been redrawn, keeping the information we have considered as most relevant.</p> <p>Figure 3 is unclear and does not convey the information provided in text that both stromal and epithelial cells within the tumor may express col11a1.</p> <p>We have partially redrawn former Figure 3 -now Figure 2- , making a reference to the putative expression of COL11A1/(pro)collagen11A1 in the epithelial front of invasive carcinomas.</p> <p>The example of immunohistochemistry (fig. 4) could be improved given the authors previous experience. The Authors could display examples of human primary tumors (and stroma) as well as cell lines.</p> <p>We have now added more photos of examples of the expression of procollagen 11A1 in different kind of human tumors and cell lines -new Figure 3-.</p> <p>Some data on different tumors could be shown with a table, which could facilitate comparative analysis.</p> <p>We have now tried to summarize in a new Table 1 the up-regulation of COL11A1/(pro)collagen 11A1 expression in different human tumours, according to the current literature.</p> <p>There are 9 authors listed: this is unusual for a review paper. What are the contributions of all these authors?</p> <p>This review was intended as an update of those aspects of the biology of COL11A1/(pro)collagen 11A1 in which our team has been involved for the last years. It is the result of experimental observations, clinical findings, literature revisions and discussions, in which all of us have participated and have been actively engaged. For these reasons, we think that all the members of our team are entitled to be acknowledged as authors.</p> <p>Reviewer #3 : The presented article is perfectly written, designed, explained and defended. For myself I find no more comments to add.</p>

Reply to comments

Perhaps slightly revise of the abstract since its reading becomes a little difficult because is so sketchy.

According to Reviewer #3 comment(s), we now provide a longer version of the Abstract to make it easier to read.

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***COL11A1*/(pro)collagen 11A1 expression is a remarkable biomarker of
human invasive carcinoma-associated stromal cells
and carcinoma progression**

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1 **Abstract**

2 The *COL11A1* human gene codes for the $\alpha 1$ chain of procollagen 11A1 and mature
3 collagen 11A1, an extracellular minor fibrillar collagen.

4 Under regular conditions, this gene and its derived products are mainly
5 expressed by chondrocytes and mesenchymal stem cells as well as osteoblasts. Normal
6 epithelial cells and quiescent fibroblasts from diverse locations do not express them.

7 Mesenchyme-derived tumours and related conditions such as scleroderma and
8 keloids, are positive for *COL11A1*/(pro)collagen 11A1 expression, as well as high grade
9 human gliomas/glioblastomas. This expression is almost absent in benign pathological
10 processes such as breast hyperplasia, sclerosing adenosis, idiopathic pulmonary fibrosis,
11 cirrhosis, pancreatitis, diverticulitis, and inflammatory bowel disease. By contrast,
12 *COL11A1*/(pro)collagen 11A1 is highly expressed by activated stromal cells of the
13 desmoplastic reaction of different human invasive carcinomas, and this expression is
14 correlated with carcinoma aggressiveness and progression, and lymph node metastasis.

15 *COL11A1* up-regulation has been shown to be associated to TGF- β 1, Wnt and
16 Hh signalling pathways, which are especially active in cancer-associated stromal cells.

17 At the front of invasive carcinomas, neoplastic epithelial cells, putatively
18 undergoing epithelial-to-mesenchymal transition, and carcinoma-derived cells with
19 highly metastatic capabilities, can express *COL11A1*. Thus, in established metastases,
20 the expression of *COL11A1*/(pro)collagen 11A1 could rely on both the metastatic
21 epithelial cells and/or the accompanying activated stromal cells.

22 **Conclusion:** *COL11A1*/(pro)collagen 11A1 expression is a remarkable biomarker of
23 human carcinoma-associated stromal cells and carcinoma progression.

24

25 **Keywords:** *COL11A1*; (pro)collagen 11A1; stromal cells; human invasive carcinoma

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1 (Pro)collagen 11A1 structure and normal tissue distribution

2
3 The *COL11A1* human gene codes for the $\alpha 1$ chain of procollagen and mature
4 collagen of type XI, which is an extracellular minor fibrillar collagen.

5 Each collagen protomer is usually made of three different polypeptides, designed
6 as $\alpha 1$, $\alpha 2$, and $\alpha 3$, and coded by specific gene sequences. In collagen of type XI, the $\alpha 1$
7 and $\alpha 2$ chains are coded by the *COL11A1* and *COL11A2* genes, respectively; however,
8 the $\alpha 3$ chain is identical to $\alpha 1(\text{II})$, coded by the *COL2A1* gene, which is the main
9 component of collagen of type II. These polypeptides are synthesized as procollagens,
10 which include the globular N- and C-propeptides flanking the prototypical collagen
11 triple helix. Upon secretion, the propeptides are excised by proteolytic cleavage; at the
12 ends of the triple-helical collagen molecule, short N- and C-telopeptides remain [1].
13 Then, the mature collagen molecules self-assemble into fibrils, on the cell surface
14 and/or in the extracellular matrix, through covalent cross-links between telopeptides and
15 specific Triple-helical Telopeptide-Binding Regions [2] (Fig. 1).

16 Minor fibrillar collagens of type V and XI are considered to act as nucleators,
17 controlling the assembly of collagen fibrils, in such a way that they become mostly
18 buried, under major fibrillar collagens I, II and III, in the core of the mature heterotypic
19 extracellular fibril [3]. Extracellular collagens are main components of the extracellular
20 matrix, along with proteoglycans and glycoproteins such as fibronectin and tenascin C,
21 among others.

22 In the adult, (pro)collagen 11A1 is present in the ocular vitreous, in the inner ear,
23 in hyaline cartilage, and in the *nucleus pulposus* of the intervertebral disc [4]. In the
24 latter, it is mainly produced by chondrocytes; in the case of the ocular vitreous, by
25 keratocytes (corneal fibroblasts). It is also expressed by mesenchymal stem cells and
26 osteoblasts [5-6] (Fig. 2).

1 Under regular conditions, normal epithelial cells and quiescent fibroblasts, from
2 diverse locations, do not express *COL1A1*/(pro)collagen 11A1.

3 4 **Regulation of the expression of *COL1A1*/(pro)collagen 11A1**

5 So far, two transcription factors have been reported to interact with the
6 *COL1A1* promoter. Matsuo et al. [7] and Hida et al. [8] have shown that the
7 transcription factor NF-Y regulates the proximal promoter activity of the *COL1A1*
8 gene in both cartilage and non-cartilage cells. Lymphocyte enhancer-binding factor 1
9 (Lef1), which participates in the Wnt signalling pathway, and is important in osteoblast
10 maturation [9], indirectly activates *COL1A1*.

11 Human mesenchymal stem cells (HMSCs), upon exposure to TGF- β 1,
12 differentiate to carcinoma-associated fibroblast-like cells and up-regulate their
13 *COL1A1* expression [10-12]. In line with this, in fibroblasts, it was confirmed that
14 TGF- β signalling activates the transcription of the *COL1A1* gene [13]. Recently, TGF-
15 β 1 has been shown to up-regulate NF-Y and to modulate its binding to the *COL1A1*
16 promoter, resulting in induction of *COL1A1* mRNA [14]. Similarly, the activation of
17 the Hedgehog (Hh) pathway increases the expression of *COL1A1* [15].

18 In cancer-associated stromal cells, several signalling activation pathways, such
19 as the TGF- β 1, Wnt or Hh, have been identified to be active [16-19]. The expression of
20 *WISP-1*, a downstream mediator of the Wnt signalling pathway has been found to be
21 correlated with the expression of *COL1A1* in sporadic colorectal carcinomas [20].
22 Activation of Wnt signalling in stroma from pancreatic cancer is also associated to high
23 *COL1A1* expression [21]. Hh signalling promotes desmoplasia and is restricted to the
24 stromal compartment in pancreatic cancer [22-23].

1 The *COL11A1* gene is expressed in mesenchymal-type/soft tissue human
2 tumours such as rhabdomyosarcoma, chondrosarcoma, fibrosarcoma, osteosarcoma or
3 Ewing's sarcoma [24-25], as well as in solitary fibrous tumours [26].

4 A high expression of *COL11A1* has been found as well in at least some human
5 gliomas/glioblastomas, especially of high-grade; these tumours are thought to be
6 derived from mesenchymal stem cells [27-30].

7 Keloids are benign dermal fibroproliferative tumours, characterised by dense
8 nodules of collagens and fibroblasts; the TGF β /Smad pathway is paramount in this
9 disease. *COL11A1* has been found to be overexpressed in human keloid fibroblasts
10 related to normal skin fibroblasts [31-33].

11 In scleroderma skin, another condition with extensive fibroblast activation and
12 up-regulation of the TGF β and Wnt signalling pathways, the overexpression of
13 *COL11A1* has also been reported [34].

14 Thus, according to all these observations, *COL11A1* expression is mainly
15 restricted to mesenchyme-derived cells (Fig. 2).

16

17 ***COL11A1*/(pro)collagen 11A1 expression as biomarker of carcinoma-** 18 **associated stromal cells and carcinoma progression**

19 Classically, fibroblasts are described as spindle-shaped stromal cells, which
20 express mesenchymal biomarkers such as *VIM*/vimentin.

21 They can be activated under a number of conditions, and they express some
22 additional biomarkers, such as fibroblast activation protein (FAP).

23 One of the conditions which leads to the activation of fibroblasts is their
24 association to malignant tumours. They are then generically called cancer-associated
25 fibroblasts (CAFs), a heterogeneous group which includes various stromal cell types,

1 which along with the accompanying extracellular matrix components, build up the
2 desmoplastic reaction. According to Togo et al., 2013 [35], “Several different markers,
3 such as α -SMA, tenascin-C (TN-C), periostin (POSTN), neuron-gial antigen2 (NG2),
4 PDGFR α/β , fibroblast activated protein (FAP), palladin and podoplanin are reported to
5 be useful for detecting activated stromal fibroblast populations in CAFs”; however, *in*
6 *strictu sensu*, some of these markers are not exclusive to CAFs.

7 α -SMA is a general biomarker of myofibroblasts, either resting or activated.
8 Tenascin C is highly expressed in tissue repair and chronic inflammation [36]. Periostin
9 is expressed by airway epithelial cells [37]. In the adult intestine, NG-2/CSPG4/MCSP
10 expression is observed within myofibroblasts and pericytes [38]. PDGFR α/β is also
11 expressed by fibroblasts of the idiopathic pulmonary fibrosis [39]. FAP has increased
12 expression during tissue damage, wound healing, fibrosis and inflammation [40].
13 According to Rönty [41], palladin is widely expressed in both epithelial and
14 mesenchymal tissues, in muscle cells and in non-muscle cells. As shown by Schacht et
15 al. [42], the lymphatic marker podoplanin is expressed by different cell types, and by
16 alveolar epithelial type I cells in lung. Some other markers, such as Fibroblast Surface
17 Protein and fibroblast specific protein-1 (FSP-1)/S100A4, are not either specific of CAFs.

18 Under normal regular conditions, COL1A1/(pro)collagen 11A1 is not expressed
19 in stromal cells of head and neck, breast, lung, stomach, liver, pancreas and colon; and it
20 is almost absent in benign pathological processes such as breast hyperplasia, sclerosing
21 adenosis [43], idiopathic pulmonary fibrosis, cirrhosis [44], pancreatitis [45],
22 diverticulitis, and inflammatory bowel disease [46].

23 In invasive carcinomas, the extracellular collagens are key players of tumour
24 behaviour and are subjected to continuous remodelling in such a way that they both

1 inhibit and promote tumour progression depending on the stage of tumour development
2 [47].

3 *COL11A1*/(pro)collagen 11A1 is highly expressed by activated stromal cells of
4 the desmoplastic reaction of human invasive carcinomas of oral cavity/pharynx [48],
5 head and neck [49-50], breast [43, 51-55], lung [56-60], esophagus [61], stomach [62-
6 63], pancreas [44, 64-66], colon [20, 67-71], and ovary [14, 72] (Fig. 2). In these
7 scenarios, the expression of *COL11A1*/(pro)collagen 11A1 is correlated with carcinoma
8 aggressiveness and progression, and lymph node metastasis (Table 1).

9 According to Vecci et al. [62], *COL11A1* was the gene with the overall highest
10 fold-change in advanced gastric cancer in comparison with early gastric cancer. These
11 observations were confirmed later on by Zhao et al. [63] as *COL11A1* was found to be
12 expressed by stromal cells in the vicinity of developing carcinoma *in situ* in stomach,
13 increasingly with the progression of the carcinoma, allowing to distinguish between
14 premalignant and malignant lesions.

15 Similarly, Freire et al. [55], Stuetz et al. [73], Ma et al. [74], Lee et al. [75],
16 Castellana et al. [76], and Vargas et al. [77], reported that *COL11A1*/(pro)collagen 11A1
17 is overexpressed in invasive ductal carcinoma (IDC) of the breast relative to ductal
18 carcinoma *in situ* (DCIS).

19 The expression of *COL11A1* has also been shown to be associated with
20 progression and poor survival of ovarian cancer patients [14, 72]. Based on *in vitro*
21 observations, Sok et al. [50] reached similar conclusions regarding head and neck
22 squamous cell cancer growth and invasion. More recently, Galván et al. [71] found that
23 the immunodetection of procollagen 11A1 is associated with the development of distant
24 metastases and advanced Dukes staging of human colon adenocarcinoma.

1 Through co-immunostainings on pancreatic ductal adenocarcinoma samples,
2 procollagen 11A1+ cells have been shown to express some other mesenchymal markers,
3 such as vimentin, α -SMA or desmin in different proportions [66], which confers to them
4 an “activated myofibroblast-like” phenotype. In all the different types of human
5 invasive carcinomas so far studied, these cells are mainly peritumoral, located around
6 the tumor foci. It is intriguing that only a fraction of the peritumoral spindle-shaped α -
7 SMA+ myofibroblast-like cells are procollagen 11A1+. Since it has been shown that
8 some carcinoma-associated stromal cells are in part derived from mesenchymal
9 progenitors and some of these progenitors express *COL1A1*/(pro)collagen 11A1, it has
10 been suggested that peritumoral procollagen 11A1+ cells could be a more specialized
11 subpopulation of activated myofibroblasts [71].

12 Although *COL1A1* has been reported to be expressed to some extent by
13 vascular smooth muscle cells [78] and tumour endothelial cells [79], immunostaining
14 of blood vessel walls has never been observed with the highly specific anti-human
15 procollagen 11A1 DTMX1/1E8.33 mAb [54, 55, 66, 71].

16

17 ***COL1A1*/(pro)collagen 11A1 in epithelial-to-mesenchymal transition**

18 **(EMT) and metastases**

19 At present, there are three recognized subtypes of “Epithelial-to-Mesenchymal
20 Transition” (EMT) [80-81]. Type 3 EMT occurs at the invasive front of carcinomas in
21 such a way that carcinoma cells lose adhesiveness and acquire motility and migration
22 capabilities. Traits associated with a Type 3 EMT are the acquisition of a spindle shape,
23 the up-regulation of vimentin (*VIM*), and the “cadherin switching”, consisting in the
24 progressive loss of E-cadherin (*CDH1*) and the increase in N-cadherin and OB-cadherin
25 or cadherin-11 (*CDH11*) expression. Together with this, EMT is also associated with

1 the up-regulation of some transcription regulators such as *SNAI1* (Snail), *SNAI2* (Slug),
2 *TWIST1* (Twist) and *ZEB2* (SIP1) [81-82].

3 It remains a matter of speculation and controversy what the origin and/or nature
4 of the cells is which, at the front of human invasive carcinomas, express
5 *COL11A1*/(pro)collagen 11A1 [72, 77, 83-84]; this aspect warrants further detailed
6 study (see more below).

7 The development of distant metastases is the major cause of death from some
8 carcinomas. These metastases originate from small tumour emboli, which separate from
9 the primary tumour mass, and, through bloodstream or lymphatics, reach and nestle into
10 another body location. These emboli are not usually accompanied by stromal
11 components but, in the establishment of pancreatic cancer metastasis, the co-migration
12 of pancreatic stellate cells and tumour cells has been demonstrated [85].

13 Transcription profiling observations from circulating breast and prostate cancer
14 cells indicate that these cells do not express *COL11A1*; in contrast, expression of
15 cadherin-11 – a surface adhesion molecule, which establishes interactions with stromal
16 cells for anchorage and nesting of distant metastases – has been shown to be associated
17 with a circulating and metastasizing phenotype of these cancer cells [86-89].

18 The overexpression of *COL11A1* has been correlated with a multi-cancer
19 metastasis-associated gene expression signature [90], and with lymph node metastasis
20 of non-small lung cancer [57]. Reports on the differential expression of *COL11A1*
21 between primary breast tumours and lymph node metastases have pointed toward a
22 higher expression in the primary tumours [91-93].

23 These studies did not pay especial attention to the kind of cells which express
24 *COL11A1* in metastases; and moreover, so far there have not been detailed reports on

1 the immunodetection of (pro)collagen 11A1 in metastases of human invasive
2 carcinomas.

3 While *COL11A1*/(pro)collagen 11A1+ cells seem to be predominantly activated
4 stromal cells in the primary tumour, some observations indicate that carcinoma-derived
5 cells, with high metastatic capabilities, can express *COL11A1* [94-95]. The SNU182
6 poorly differentiated hepatocellular carcinoma cell line expresses high levels of
7 *COL11A1*, along with cadherin-1 and mesenchymal markers such as vimentin, *SNAI1*
8 (Snail), *SNAI2* (Slug), *TWIST1* (Twist) and *TWIST2* [94]. Lung-metastatic LM2 cells,
9 originally derived from the clear cell renal (RCC) carcinoma SN12C cell line, have been
10 shown to have a highly up-regulated *COL11A1* gene [95].

11 According to the Gene Expression Atlas, ArrayExpress E-MTAB-37 [96], one
12 of the human cancer cell lines with the highest *COL11A1*-specific mRNA expression is
13 the large cell lung carcinoma NCI-H661. This cell line was derived from the lymph
14 node of a patient with large cell cancer of the lung. We have assessed by
15 immunocytochemistry that this cell line expresses high levels of procollagen 11A1 as
16 well (Fig. 3).

17 Therefore, in established metastases, the expression of *COL11A1*/(pro)collagen
18 11A1 could originate from both the metastatic epithelial cells and/or the accompanying
19 activated stromal cells.

20

21 **Conclusion**

22 In summary, under the influence of various growth factors and signalling pathways
23 which are known to be active in carcinomas and promote *COL11A1* expression, we may
24 conclude that *COL11A1*/(pro)collagen 11A1 expression is a remarkable biomarker of
25 human carcinoma-associated stromal cells and carcinoma progression.

1 In agreement with this, a very recent review by Raglow and Thomas [97]
2 highlights the role of *COL11A1*/(pro)collagen 11A1 in cancer.

3
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10
11 **Conflicts of interest** The authors declare no conflicts of interest.

12 13 **References**

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3 Fig. 1 Schematic drawing of collagen fibrillogenesis by fibroblasts (adapted from [1]

4 and [2], with permission of *BiomedCentral* and *The Company of Biologists Ltd*).

5 Procollagen polypeptides are individually synthesized and then they form trimers in the

6 cytoplasm. Once secreted, the terminal propeptides are excised, remaining the typical

7 triple helix of the mature collagen molecule which conserves short telopeptides at both

8 ends. Covalent cross-links through these telopeptides and specific Triple-helical

9 Telopeptide-Binding Regions allow mature collagen molecules self-assemble into

10 fibrils.

11

12 Fig. 2 Summarized representation of *COL11A1*/(pro)collagen 11A1 expression in

13 normal healthy tissues and in tumours. A reference to the putative expression of

14 *COL11A1*/(pro)collagen 11A1 in neoplastic epithelial cells at the front of invasive

15 carcinomas has been made.

16

17 Fig. 3 Representative immunostainings with the anti-human procollagen 11A1

18 DTMX1/1E8.33 mAb (cell line cultures, original magnification x400; tissue samples,

19 original magnification x200). A) Negative staining of a human pancreatic

20 adenocarcinoma CAPAN-1 cell line culture; B) Pancreatic ductal adenocarcinoma; C)

21 Negative staining of a human alveolar lung carcinoma A549 cell line culture; D) Lung

22 adenocarcinoma; E) Very positive staining of a human large cell lung carcinoma NCI-

23 H661 cell line culture; F) Head and neck squamous cell carcinoma. In tissue samples,

24 only peritumoral stromal cells show a strong intracytoplasmatic staining.

Table 1 Up-regulated *COL11A1*/(pro)collagen 11A1 expression in human tumours

Cancer type	Cell type	Study	Up-regulation associated to	References
Soft tissue (rhabdomyosarcoma, chondrosarcoma, fibrosarcoma, osteosarcoma, Ewing's sarcoma, solitary fibrous tumours)	Mesenchymal-type, fibroblastic	cDNA microarray, protein	Malignancy	[24-26, 54, 84]
Glioma/ glioblastoma	Mesenchymal-type	cDNA microarray, protein	High grade	[27-30]
Keloid	Mesenchymal-type, fibroblastic	cDNA microarray	Excessive extracellular matrix	[31-33]
Carcinoma				
Oral cavity/pharynx	Not determined	cDNA microarray	Lymph node metastasis	[48]
Head and neck	Tumour –derived fibroblasts, transformed epithelial cell lines	cDNA microarray, RT-PCR, siRNA	Tumour proliferation, migration and invasion	[49-50]
Breast	Stromal cells, invading neoplastic epithelial cells	cDNA microarray, Q-RT-PCR, protein	Progression from ductal carcinoma <i>in situ</i> (DCIS) to invasive ductal carcinoma (IDC)	[43, 51-55, 73-77, 90-93]
Lung	Carcinoma-associated fibroblasts	cDNA microarray, Q-RT-PCR, protein	Tumour size, stage, invasion, lymph node metastasis, poor prognosis	[56-60]
Esophagus	Not determined	cDNA microarray	Malignancy	[61]

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Stomach	Stromal cells	cDNA microarray, Q-RT-PCR	Progression from premalignant to malignant lesions	[62-63]
Pancreas	Carcinoma-associated fibroblasts, pancreatic stellate cells	cDNA microarray, Q-RT-PCR, protein	Progression to ductal adenocarcinoma	[44, 64-66]
Colon	Carcinoma-associated stromal cells	cDNA microarray, Q-RT-PCR, protein	Stage, lymph node metastasis	[20, 67-71]
Ovary	Tumour epithelial cell lines, carcinoma-associated stromal cells, rare foci of tumour epithelial cells	cDNA microarray, Q-RT-PCR, protein	Tumour progression, lymph node metastasis, poor prognosis	[14, 72]

Figure 1
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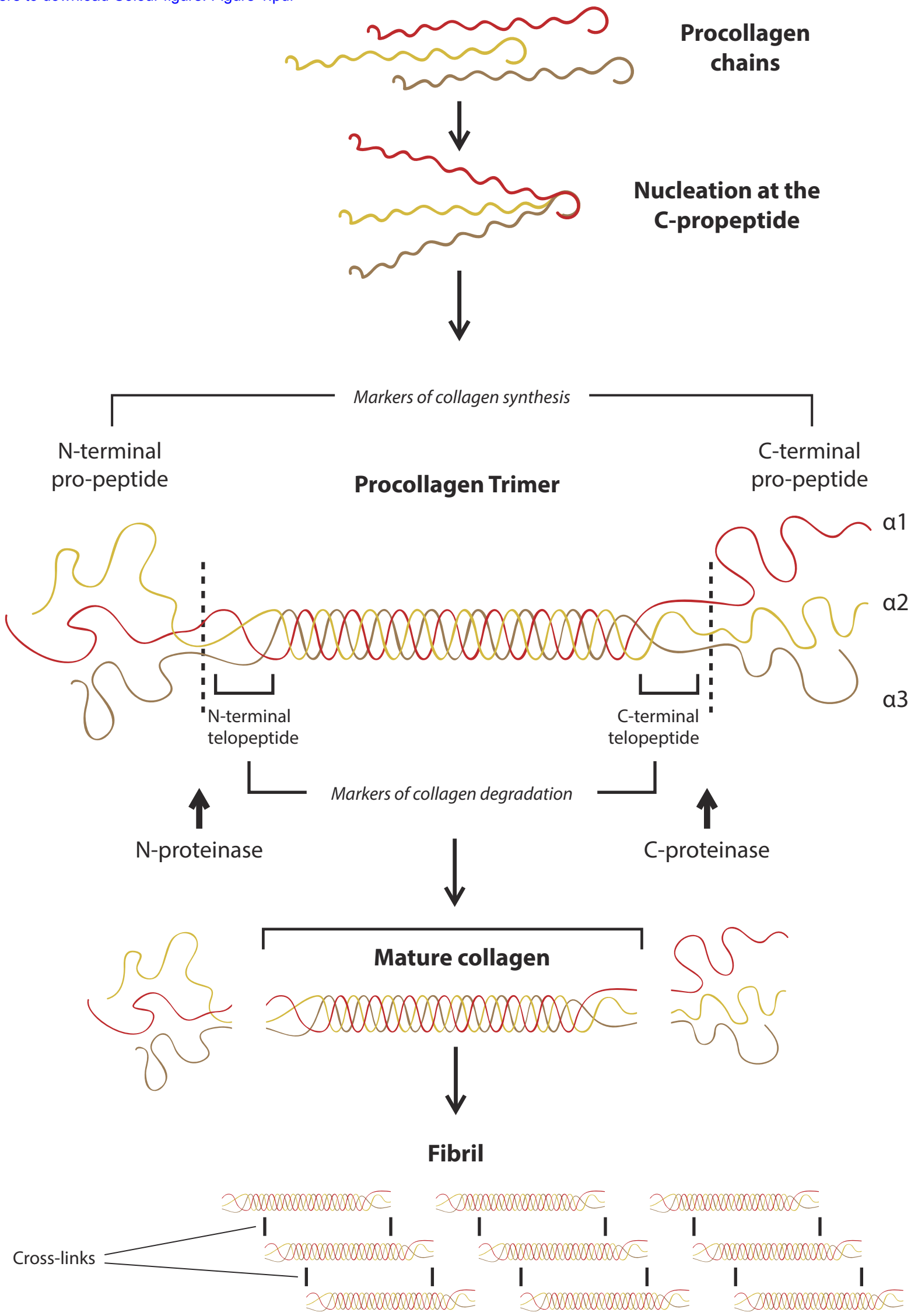


Figure 2

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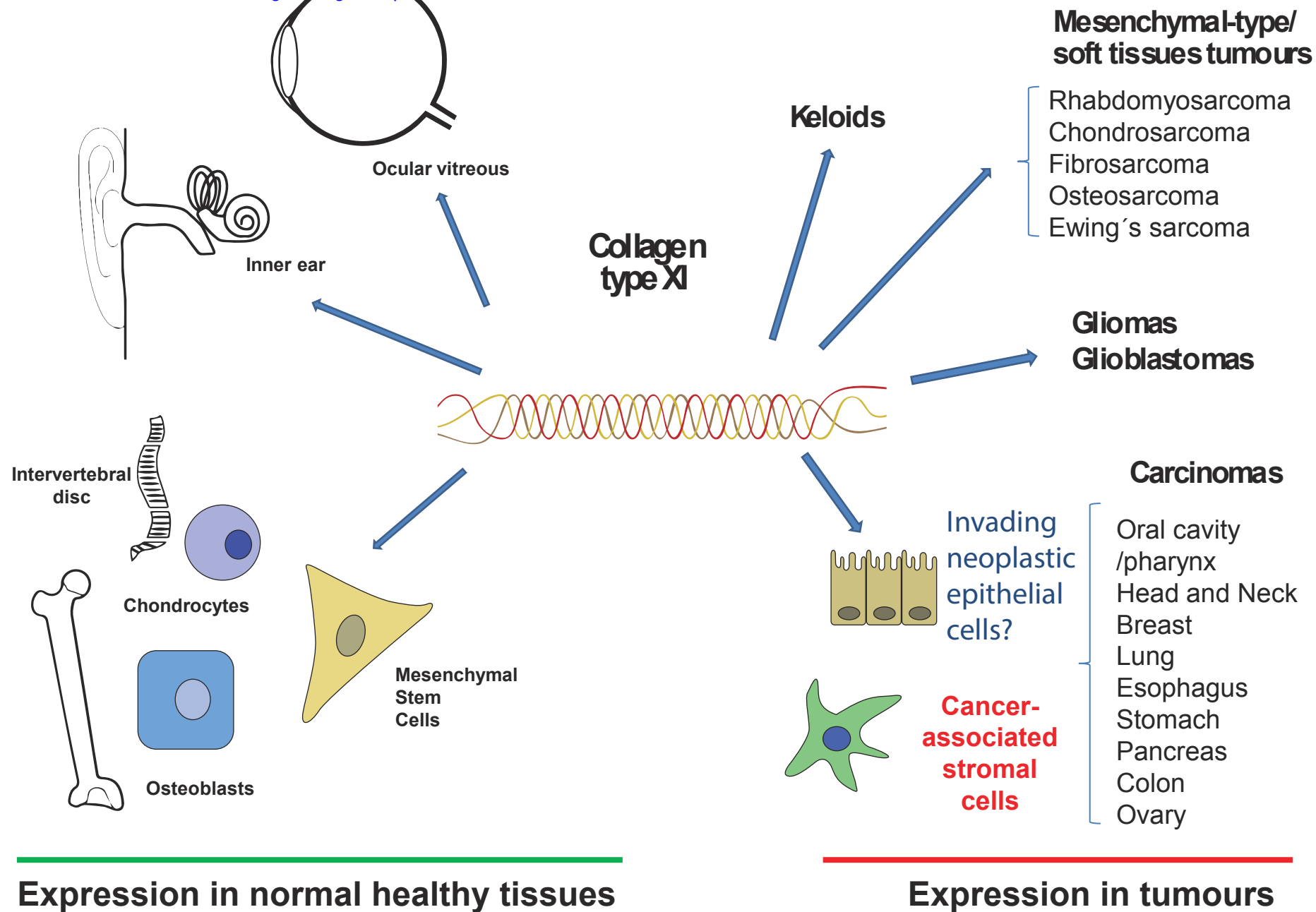


Figure 3
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