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How do tenascins influence the birth and life of a malignant cell?

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Abstract

Tenascins are large glycoproteins found in embryonic and adult extracellular matrices. Of the four family members, two have been shown to be overexpressed in the microenvironment of solid tumours: tenascin-C and tenascin-W. The regular presence of these proteins in tumours suggests a role in tumourigenesis, which has been investigated intensively for tenascin-C and recently for tenascin-W as well. In this review, we follow a malignant cell starting from its birth through its potential metastatic journey and describe how tenascin-C and tenascin-W contribute to these successive steps of tumourigenesis. We consider the importance of the mechanical aspect in tenascin signalling. Furthermore, we discuss studies describing tenascin-C as an important component of stem cell niches and present examples reporting its role in cancer therapy resistance.

Keywords: tenascin • genomic instability • stem cells • primary tumour • EMT • angiogenesis • extracellular matrix • metastasis • mechanical forces • cancer therapy • microenvironment • stroma

Introduction

Tumourigenesis has traditionally been described as a cell-autonomous process when a cell, after accumulation of genetic alterations, loses its control over growth, undergoes aberrant proliferation and thus gives rise to a tumour. It is now accepted that the microenvironment surrounding a potential tumour cell plays a crucial role influencing its fate [1]. For instance, experiments exposing pre-neoplastic or tumour cells to various mesenchymal environments have underlined the critical role of the stroma in this context [2–4]. Among the proteins known to be overexpressed in tumour-associated stroma are tenascin-C and tenascin-W. Tenascins are large glycoproteins found in the extracellular compartment of various tissues. The tenascin family has four members: tenascin-C, tenascin-R, tenascin-X and tenascin-W. They all share a characteristic modular structure with an oligomerization domain followed by EGF-like repeats, fibronectin (FN) type III repeats and a fibrinogen globe (see Ref. [5] and Fig. 1). In the case of tenascin-C and tenascin-R, alternative splicing can lead to the generation of multiple isoforms that contain additional FN type III repeats. Only two of the tenascin members, the original tenascin-C [6, 7] and the more recently discovered tenascin-W [8], have been shown to be overexpressed in tumours compared to healthy tissues (for review, see Ref. [9]). In most cases, healthy tissues are not completely deprived of tenascin-C [10]. However, corresponding tumour tissues express much higher levels of tenascin-C or harbour different, larger isoforms [11]. Tenascin-W expression is much more restricted in adult healthy tissues [10], which makes it an excellent tumour marker: it is absent in healthy tissue but present in most breast [12], colon

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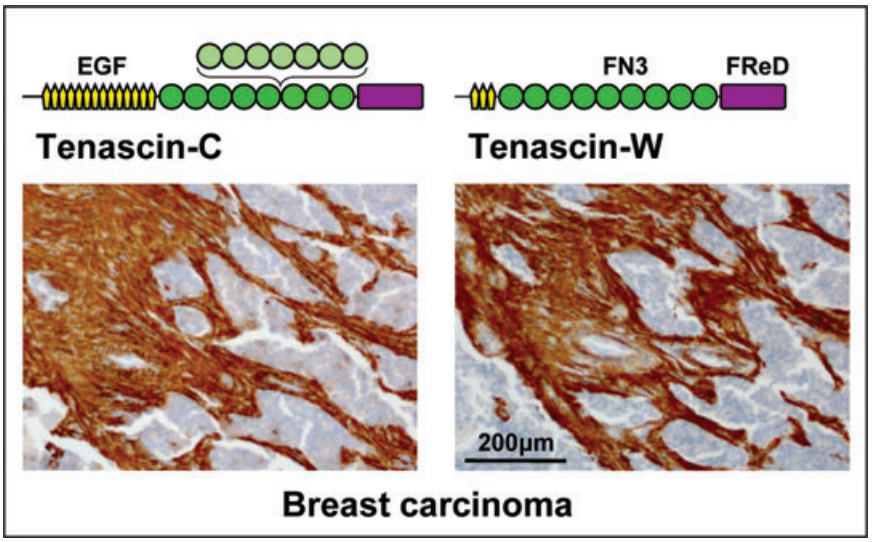

Fig. 1. Tenascin structure and tumour stroma staining. A schematic model representing one subunit of the hexameric tenascin-C and tenascin-W proteins is shown above a breast carcinoma section stained with antibodies against the two proteins. Both tenascins are built from the following domains: a central, N-terminal oligomerization domain (black line), EGF-like repeats (EGF, yellow), FN type III repeats (FN3, green; FN3 repeats subject to alternative splicing in light green) and a C-terminal fibrinogen related domain (FReD, violet). Antibodies against tenascin-C and tenascin-W strongly stain the stroma of the breast carcinoma, although the epithelial tumour nests (nuclei in blue) are negative.

[13] and brain [14] tumours. In contrast, tenascin-R and tenascin-X expression is rather constant and is not modulated by tissues malignancy (for review, see Ref. [15]). In this review, we will follow the major steps in the life of a tumour cell and its descendants and describe how tenascin-C and tenascin-W influence their fate.

Birth of a malignant cell

The birth of a malignant cell is a multi-step process, which starts with the acquisition of alterations in genes encoding proteins implicated in cellular growth control. For this reason, the first defense of an organism against cancer relies on the prevention of genomic alterations. At the cellular level, many responses to mutagenic challenges have been developed. For instance, cell cycle arrest is triggered to enable proofreading of DNA and ensure genomic stability. Interestingly, the state of cell adhesion is suspected to alter pathways controlling genomic stability (for review, see Ref. [16]). Because tenascins modulate the adhesion status of cells [17], it is tempting to speculate that they may also influence genomic stability [9]. Consistent with this hypothesis, BRCA1-associated RING domain 1 (Brad1), H2A histone family member X (H2AX), as well as other molecules with assigned functions in genome stability were found to be down-regulated in the presence of tenascin-C in glioblastoma cells [18]. The hypothesis that tenascin-C could favour the birth of malignant cells implies its expression before the development of the tumour. Remarkably, transgenic mice expressing an autoactivating mutant of the matrix metalloproteinase stromelysin-1 in mammary epithelia show inappropriate expression of tenascin-C, starting by day 6 of pregnancy and followed by the development of mammary pre-neoplastic and neoplastic lesions [19]. Furthermore, fibroblasts expanded from Gorlin syndrome patients, which are prone to develop basal cell carcinomas, show phenotypic traits reminiscent of cancer-associated fibroblasts. In particular, tenascin-C is highly expressed by these cells [20]. It is believed that the dermis contributes to the predisposition of these patients to develop basal cell carcinomas, which is compatible with the hypothesis that a tenascin-C rich environment favours the birth of malignant cells.

Niches for self-renewable cells

The capacity to proliferate indefinitely and to self-renew is a shared hallmark of stem cells and tumour cells. Hence, major signalling pathways initially aimed at regulating normal stem cells, such as Wnt, Shh and Notch signalling are re-utilized by the tumour cell machinery (for review, see Ref. [21]). It is interesting to note that tenascin-C was shown to be associated with stem cell niches in the neural, haematopoietic and epidermal system (for review, see Ref. [22]). Tenascin-C in brain is highly expressed in specialized germinal zones such as the subventricular zone where it may play an important role in the regulation of stem cells because transgenic mice lacking tenascin-C show altered numbers of central nervous system stem cells [23]. Specific carbohydrate side chains of tenascin-C could play a key role in the regulation of embryonic neural stem cell proliferation. For instance, human natural killer 1 (HNK1) carbohydrate epitopes carried by large splice variants of tenascin-C are involved in the proliferation of neural stem cells *via* modulation of the expression level of epidermal growth factor (EGF) receptor [24]. In parallel, tenascin-C was identified as a cell surface glycoprotein marker for glioblastoma-derived stem-like cells [25]. Tenascin-C is also present in

the haematopoietic stem cell microenvironment [26], where it may regulate stem cell recruitment because mice lacking tenascin-C show reduced haematopoiesis [27]. In addition, tenascin-C could be part of the specialized extracellular matrix characterizing the niche of epidermal stem cells because it is strongly up-regulated in the hair follicle bulge, which is known to be a stem cell reservoir [28].

Expression patterns in tumours

In tumours arising from epithelial organs (*i.e.* in most carcinomas), the source of tenascins is restricted to the structural, mesenchymal compartment: very strong tenascin immunostaining is found in the tumour stroma surrounding tenascin-free tumour nests (see Fig. 1, for a typical staining pattern). The stromal tenascin-C expression can be influenced by cancer cells because co-culture of fibroblasts with tumour cells has been shown to stimulate tenascin-C expression in the fibroblasts [29, 30]. In other tumours such as melanoma and brain tumours, the tumour cells themselves are the source of tenascin-C [31, 32]. Interestingly, brain tumours arise from structural cells (oligodendrocytes for oligodendrogliomas, astrocytes for astrocytomas and glioblastomas), which may explain that these cells have common features with mesenchymal cells rather than with carcinoma cells. Melanomas develop from neural crest-derived melanocytes. Neural crest cells express high levels of tenascin-C [33], which is required for their migration in embryogenesis [34]. In contrast to tenascin-C, tenascin-W expression has yet to be described in tumour cells themselves. However, tenascin-W is also expressed in the stroma of breast and colon carcinomas. In brain tumours, both tenascin-C and tenascin-W can be observed around blood vessels. However, their pattern of expression is distinct because tenascin-C surrounds both endothelial and pericyte cell layers, whereas tenascin-W is restricted to the endothelial cell layer [14].

Promotion of tumour cell proliferation

An important feature of tumour cells is their ability to overproliferate. Interestingly, tenascin-C has been shown to promote tumour cell proliferation in standard cell culture conditions [7, 35]. More specifically, culturing glioblastoma and breast carcinoma cells on mixed fibronectin/tenascin-C substrata not only attenuated their adhesion but also increased their proliferation rate compared to a pure fibronectin substratum [35]. Similar observations were recently made in three-dimensional mammary epithelial tissues reconstructed *in vitro* [36]. In this model, human mammary epithelial cells form polarized multicellular acini characterized by the presence of a hollow lumen. In the presence of tenascin-C, acini fail to generate a continuous basement membrane and cell proliferation is increased. Similarly, growth of melanoma spheres (known to be enriched for stem-like cells) is significantly reduced in absence of tenascin-C [37]. *In vivo*, expression of tenascin-C at the invasion border of early breast cancers was associated with a higher proliferation rate assessed by Ki-67 staining and the analysis of the fraction of cells in S-phase [38].

Promotion of tumour cell migration

Tenascin-C was initially described as an anti-adhesive molecule that antagonizes the capacity of tumour cells to adhere and spread on fibronectin [39]. Experiments performed in three-dimensional fibrin matrices containing fibronectin have revealed that tenascin-C suppresses actin stress fibre formation *via* inhibition of RhoA activation, and instead induces actin-rich filopodia [40]. A very specific adhesion state is required for the cell to be able to migrate (for review, see Ref. [41]). When a cell adheres too strongly, the links between the cytoskeleton and the extracellular matrix are difficult to break and the cell remains fixed to the substratum. Conversely, when a cell adheres weakly, contractile forces required for motility are missing [42]. Adhesion to a tenascin-C–rich matrix can be defined as intermediate, and thus favours both cell motility [43, 44] and invasion [45]. Interestingly, the site of tenascin-C responsible for focal adhesion disassembly has been located in the alternatively spliced FN type III repeats [46]. In agreement, large tenascin-C variants containing FN type III extra-repeats expressed by co-cultured fibroblasts elicit increased cell migration and invasion in breast cancer models [47]. In contrast to tenascin-C, tenascin-W does not interfere with the cell adhesive function of fibronectin [12]. However, it also stimulates migration of mammary cancer cells [12, 48].

Contribution to epithelial–mesenchymal transition (EMT)

Epithelial-mesenchymal transition is a developmental process re-utilized by cancer cells and characterized by loss of cell adhesion, repression of E-cadherin expression as well as increased cell mobility. Tenascin-C has been shown to be associated with EMT because tumour cell lines undergoing transforming growth factor-β (TGF-β) induced EMT secrete tenascin-C [49]. Furthermore, vimentin (an EMT marker) and tenascin-C expressions are associated in cancer cells [50], and cancer cell lines with clear epithelial morphology secrete far less tenascin-C than cancer cell lines characterized by a fibroblastic morphology (our observations). Finally, a recent study reports that tenascin-C is required for injury-induced EMT of lens epithelium [51]. However, except for the last example, it remains to be determined whether tenascin-C expression is only a consequence of EMT or may also be required for this process.

Promotion of angiogenesis

When a tumour reaches a critical size, it has to attract the formation of new vessels to supply nutrients and oxygen to the proliferating cells. Both tenascin-C and tenascin-W can been classified as pro-angiogenic factors because they trigger endothelial cells to acquire a sprouting phenotype and increase their migration capacities [14, 52, 53]. Tenascin-C is known to play a crucial role in postnatal cardiac angiogenesis because tenascin-C–deficient mice fail to vascularize cardiac allografts in an established cardiac transplantation model [54]. Furthermore, in a xenograft tumour model it was shown that tumours were much more vascularized when they were grown in wild-type mice than in tenascin-C–deficient mice, suggesting that the presence of tenascin-C in the microenvironment is very important for tumour angiogenesis [55]. Tenascin-C was also associated with lymphangiogenesis [56], particularly in the context of podoplanin induction [57]. The proximity of tenascin-C and tenascin-W around blood vessels prompts the question whether or not these proteins can extravasate from the tumour site and circulate in the bloodstream. Indeed, tenascin-C was found in the serum of cancer patients, but because its presence in serum was also associated with conditions other than cancer (*e.g.* as a consequence of infection and inflammation, see Ref. [10]), tenascin-C turned out to be a questionable serum tumour marker [58]. In contrast, tenascin-W function in adults seems to be restricted to tumourigenesis and osteogenesis (for review, see Ref. [59]), so it may become a better tumour biomarker. Indeed, tenascin-W levels in serum from breast and colorectal cancer patients were found to be elevated compared to serum collected from healthy, control individuals [13]. However, individual tenascin-W levels were heterogeneous, with some patients exhibiting highly elevated values and others with values within the range of controls. Interestingly, most patients that developed early tumour recurrence were initially characterized by high serum tenascin-W levels. A follow-up study including a large cohort of patients should therefore be performed to confirm this tendency. Together, these results suggest that tenascin-W is a promising tumour biomarker, but should be used in combination with other markers to avoid generation of false negative distortions.

Promotion of metastasis

After escape from the primary tumour and circulation in the blood or lymphatic vessels cancer cells will, as an ultimate step, colonize other organs of the body. The nature of the 'soil' influences greatly the choice of host tissues in which the tumour cells will 'seed' [60]. Interestingly, extracellular matrix proteins produced by tumour cells themselves also determine their metastatic capacity. For instance, knocking-down tenascin-C in melanoma cells significantly decreased their capacity to form pulmonary metastases [37]. This holds true as well for certain breast tumour xenograft models [61, 62]. In support for a human relevance of these observations, high tenascin-C expression was associated with an 8-fold increased risk of metastasis in classic giant cell tumours of bone [63]. A recent study suggests that the fibrillar organization of tenascin-C, requiring stromal fibroblasts and active MMP2, is associated with metastatic pancreatic cancers [64]. However, activation of tenascin-C–dependent metastatic pathways may depend on the initial oncogenic alterations because tenascin-C is not found in all metastatic transcript profiles [65]. To our knowledge, tenascin-W expression has not been associated with metastasis in human beings. However, this possibility is difficult to investigate in experimental models because cancer cell lines expanded from any type of tumours were negative for tenascin-W expression (our own observation).

How do tenascins signal to cells?

We described above the effects triggered by tenascins on tumour and endothelial cells, but a main question remains: how do the signals activated by tenascins reach the nucleus where regulation of gene expression takes place? There are many possibilities how this can occur. For example, the signal can be transmitted by an indirect, 'classical' pathway, that is through activation of transmembrane receptor proteins, cascades of kinases, and activation of transcription factors, which finally act in the nucleus to modulate gene expression. Alternatively, the signal could also be transduced mechanically in a more direct manner through the cell cytoskeleton, which physically connects the outside of a cell with the chromatin [66]. Concerning indirect ways of activation, there is no consensus for a given signalling pathway, suggesting that tenascin-C can activate many parallel context- and tissue-specific signalling cascades. On the level of cell surface receptors, a large set of integrins have been identified as receptors for tenascin-C ($\alpha2\beta1$, $\alpha v\beta3$, $\alpha7\beta1$, $\alpha8\beta1$, $\alpha9\beta1$, $\alpha5\beta3$, $\alpha5\beta6$ [67–71]) and for tenascin-W ($\alpha8\beta1$, $\alpha v\beta1$, $\alpha4\beta1$ [12, 48]). The fact that tenascins contain epidermal growth factor motifs suggests that they could bind to EGF receptors. Tenascin-C was classified as an 'insoluble growth factor ligand' [72] because it can bind to EGFR with a low affinity insufficient to trigger ligand internalization. This binding favours activation of motility rather than proliferation responses [73]. Interestingly, tenascin-C–enriched medium can stimulate invasion of colon cancer cells only in presence of a functional EGFR [45]. The receptor of hepatocyte growth factor, c-Met, was shown to work in close association with tenascin-C both in colon and mammary culture models [36, 45]. Tenascin-C triggers *in vitro* the formation of filled (instead of hollow) acini surrounded by an abnormal basement membrane. This effect is abrogated in the absence of functional c-Met receptors [36]. Another transmembrane protein, TLR4, a member of the toll-like receptor family which is known to contribute to infection and inflammation responses, is activated by tenascin-C [74]. Whether tenascin-C binds as a ligand to TLR4 remains to be investigated.

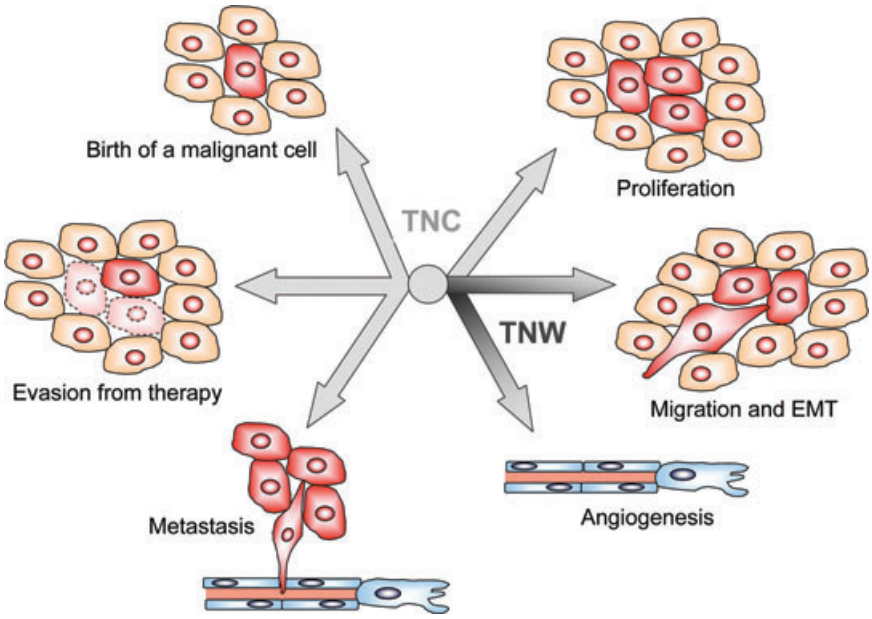

Fig. 2. Summary of the actions of tenascins in cancer. The centre of the figure depicts the six-armed tenascin-C (TNC) and tenascin-W (TNW) protein pointing with their arms towards processes that are influenced by their presence. Although TNC is involved in all processes depicted, including the birth of a malignant cell, proliferation, migration and EMT, angiogenesis, metastasis and evasion from therapy, TNW was shown to affect cell migration and angiogenesis (indicated by the darker arms).

In addition to EGFR, HGF/c-Met and TLR4, two other well-described pathways are suspected to be modulated by tenascin-C: these are TGF-β and Wnt signalling pathways. Gene expression profile analysis of T98G glioblastoma cells cultured in presence of tenascin-C revealed, for instance, down-regulation of follistatin, a known inhibitor of activins [18]. This study also led to the identification of Dkk1, a known inhibitor of the Wnt pathway, as a gene down-regulated by tenascin-C. Interestingly, a potential mechanism of cell cycle regulation by tenascin-C could also be through induction of 14-3-3τ [75] followed by p21 down-regulation [76].

Another more direct way for tenascin-C–triggered signals to reach the nucleus has been recently investigated in our laboratory [77]. These studies show that disruption of the physical link connecting the cell membrane to the nucleus perturbs the mechanical control of cell differentiation in the context of myogenesis. This suggests that tenascin-C–induced cytoskeletal rearrangements could also affect mechanotransduction in the frame of tumourigenesis.

It is important to note that transduction of tenascin signals to cells could also be influenced by other extracellular matrix molecules. For instance, through their binding to tenascin-C, fibronectin [78], perlecan [79], lectican [80], heparin [81], contactin [82, 83] and SMOC1 [84] have been shown to modify its effects.

Importance of the mechanical aspect

An important parameter for the progression of a tumour is the stiffness of the tissue in which it develops. Reconstruction of mammary epithelial acini *in vitro* has shown that the rigidity of the matrix strongly influences epithelial morphogenesis [85]. Consistently, *in vivo* reduction of lysyl oxidase-mediated collagen crosslinking to weaken the collagen fibres and to reduce their tensile strength decreases mammary tumour incidence [86]. Interestingly, mechanical strain not only influences features of the epithelial tumour cells themselves but may also stimulate neovascularization through activation of angiogenic genes [87]. It is therefore interesting to note that tenascin-C is induced by mechanical strain, requiring integrin-linked kinase [88] followed by RhoA/ROCK-dependent actin contractility [89]. Interestingly, fibroblasts cultured on attached, restrained collagen gels express much more tenascin-C than those cultured on floating, unrestrained collagen gels [90]. This may be an explanation why cancer-associated fibroblasts, which are exposed to high mechanical strain, overexpress tenascin-C compared to 'normal' fibroblasts populating healthy tissues. Stiffness of the tumour environment may also favour transdifferentiation of cancer-associated fibroblasts into myofibroblasts as myodifferentiation of primary lung fibroblasts has been recently reported to be influenced by cyclic mechanical stress [91].

Evasion of tumour cells from conventional therapy

Interestingly, tenascin-C has been shown to mediate chemotherapy-resistance in various contexts. Knocking-down tenascin-C

sensitizes melanoma cells to doxorubicin [37]. This happens most likely *via* down-regulation of ATP-binding cassette B5, a transporter known to provide the cells high efflux capacity for anti-mitotic drugs. In the frame of breast cancer, tenascin-C could be associated with resistance to tamoxifen therapy [92] and doxorubicin therapy [76]. Interestingly, the doxorubicin-induced G_1/S arrest could be abrogated through p21 regulation, which in turn is known to be affected by the presence of tenascin-C. It was also shown that large isoforms of tenascin-C and cell surface protein annexin A2 can interact and together decrease gemcitabine-induced cytotoxicity in pancreatic cancer cells [93]. Tenascin-C might also be linked to resistance to anti-angiogenesis treatment because hypoxic tumour cells adapt by inducing new sets of genes, among which tenascin-C is found [94].

Conclusions

We have summarized how tenascins have been shown to influence the successive steps of tumourigenesis (summarized in Fig. 2). Based on our knowledge of the properties of tenascin-C, two types of anti-cancer therapies have been established. The first type aims at the neutralization of its pro-tumourigenic effects by down-regulating its expression using anti-sense oligonucleotides or aptamers (for review, see Refs. [95, 96], respectively). The second type takes advantage of the specific overexpression of tenascin-C isoforms at tumour sites to selectively target tumour cells, using anti–tenascin-C antibody fragments coupled to a variety of anti-cancer molecules (for review, see Ref. [97]). Further studies will be necessary to extend our knowledge to tenascin-W, but studies reported so far suggest a promising future for tenascin-W as a tumour marker and potential drug target.

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Conflict of interest

The authors confirm that there are no conflicts of interest.

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