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Invited Review

Tenascin in the developing and adult human intestine

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Summary. The tenascins are a family of multifunctional extracellular matrix glycoproteins subject to complex spatial and temporal patterns of expression in the course of various organogenetic processes, namely those involving epithelial-mesenchymal interactions. In the intestine, the tenascins, in particular tenascin-C, have been found to be differentially expressed in the developing and adult small intestinal and colonic mucosa as well as in neoplasm. While tenascin-C emerges as a key player likely to be involved in intestinal mucosa development, maintenance and disease, its exact role in the regulation of fundamental intestinal cell function(s) such as proliferation, migration and tissue-specific gene expression remains however to be established.

Key words: Extracellular matrix, Tenascin, Integrin, Small intestine, Colon

Introduction

The intestine, like many other organs, requires dynamic and reciprocal epithelial-mesenchymal interactions for its morphological and functional development as well as its maintenance at the adult stage (Haffen et al., 1989; Yasugi, 1993). One key element in these interactions is the extracellular matrix (ECM), in particular, the basement membrane (BM), which is located at the epithelial-mesenchymal interface (Leblond and Inoue, 1989; Rosman et al., 1993; Timpl and Brown, 1996). It is now recognized that ECM composition defines the necessary microenvironment for various cellular functions, such as adhesion, proliferation, migration, cell survival and tissue-specific gene expression, during development and at maturity (Adams and Watt, 1993; Lloyd Jones et al., 1993; Rosekelly et al., 1995). In the intestine, a detailed analysis of BM components such as laminins and type IV collagens as well as various BM associated molecules such as fibronectin has revealed that most of them are subject to particular spatial and temporal patterns of expression (Simon-Assmann et al., 1995; Beaulieu, 1997). However, one of the most striking examples of differential expression is tenascin-C. Although present constitutively in smooth muscle cells, this macromolecule was found to be expressed relatively late during embryonic development (i.e. only after short villi had formed) in the small intestine while being absent in the fetal colonic mesenchyme but, in contrast to most other organs, was detected in their adult counterparts, being expressed according to an increasing gradient along the crypt-villus axis and crypt-surface epithelium axis in the small intestine and colon, respectively.

In this review, we will present an overview of the structural features and functional properties of tenascin-C and other members of the family discovered more recently. We will then discuss what is currently known about the expression and distribution of these macromolecules as well as some of their cellular receptors in the intestine under the normal and pathological states.

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Structural features of tenascins

The tenascins are extracellular matrix glycoproteins characterized by their multi-branched structure. They were originally described as CSP (Cell Surface Protein; Yamada et al., 1975), restrictin (Carter and Hakomori, 1981), GMEM antigen (Bourdon et al., 1983) myotendinous antigen (Chiquet and Fambrough, 1984), hexabranchion (Erickson and Inglesias, 1984), J1 glycoprotein (Kruse et al., 1985), and cytotactin (Grumet et al., 1985) before obtaining their final designation as tenascin (Chiquet-Ehrismann et al., 1986).

While some tenascins are homotrimeric, tenascin-C is a hexamer (Fig. 1). The monomeric glycoprotein, varying from 180-320 kD, is made up of four distinct segments. At the most N-terminal portion is a 150 amino acid alpha helix. In this segment, four heptad repeats are flanked by cysteine residues which are responsible for the joining of three tenascin subunits into a triple stranded coil (Erickson and Bourdon, 1989) while a unique cysteine residue flanking this motif, Cys-64, is

required for the joining of two tenascin trimers (Luczak et al., 1998). Next to this is the thin proximal segment made up of EGF-like repeats, the number of which varies greatly (Nies et al., 1991; Matsumoto et al., 1992; Fuss et al., 1993). The next area, corresponding to the thick distal segment, is composed of FnIII-like repeats. Their number also varies greatly and depends upon alternative splicing. In human tenascin-C, it is those repeats located between FnIII 5 and 6 (identified as A1-A4, B, AD2, AD1, C and D; Fig. 1B) that can undergo alternative splicing (Erickson and Bourdon, 1989; Sriramarao et al., 1993; Mighell et al., 1997). Interestingly, both AD1 and AD2 have been identified only in tumor-derived cell lines (Sriramarao et al., 1993; Mighell et al., 1997). The sequence is terminated by a globular domain homologous to the globular domain of ß- and γ-fibrinogen (Erickson and Bourbon, 1989).

Aside from tenascin-C (Tn-C), four other members of the tenascin family have been identified and characterized: tenascin-R (Tn-R), tenascin-X (Tn-X), tenascin-Y (Tn-Y) and tenascin-W (Tn-W). Tn-C and Tn-R are very similar in structure and can form hexabranchion structure (Erickson, 1993a). On the other hand, it seems that Tn-X and Tn-Y, lacking the cysteine residue in their C-terminal heptad region essential for the union of two tenascin trimers, are only synthesized in a trimeric form (Erickson, 1993a). Tn-R (restrictin, J1 160/180) was recently identified and mapped in man (Leprini et al., 1996). Mostly restricted to the central nervous system, this molecule is characterized by a short EGF-like segment and a unique FnIII domain (called R1 or A) both of which are subject to alternative splicing, therefore creating only two different splicing variants (Pesheva et al., 1989; Nörenberg et al., 1995; Carnemolla et al., 1996). Tn-X is quite different from the other tenascins. Encoded by the XB gene located on chromosome 6, the human Tn-X has a EGF-like segment comparable to Tn-C, but a very long FnIII-like repeat segment (Matsumoto et al., 1992; Bristow et al., 1993). With a subunit of ~500 kD, Tn-X is the largest member of the family. Tn-Y has only been identified in the chicken and exhibits unique structural features (Hagios et al., 1996) while Tn-W could be the zebrafish equivalent of tenascin-C (Weber et al., 1998).

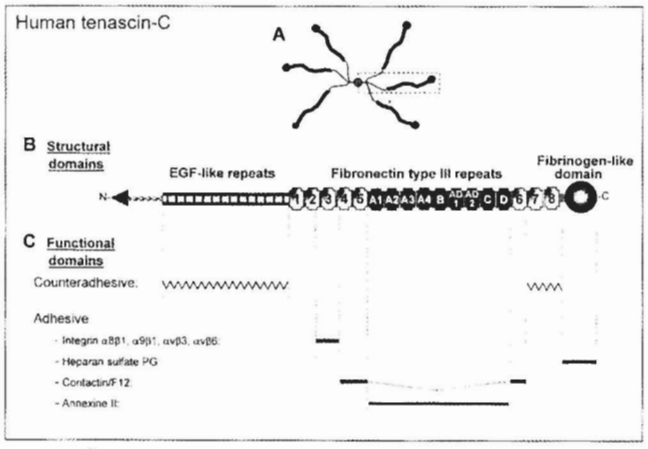

Fig. 1. Schematic representation of Tn-C whole molecule and domains. Tn-C is a hexameric molecule. Each arm comprises a proximal thin segment, a distal thick segment and a terminal globular domain **(A)** which correspond to the EGF-like repeats domain, the fibronectin type III repeats domain and the fibrinogen-like domain, respectively **(B)**. These various domains have been mapped for adhesive and counteradhesive activities **(C)**.

Functional properties of tenascins

Based on its unique distribution during organogenesis, wound healing and in various pathological conditions including cancer (Chiquet-Ehrismann et al., 1986), tenascin has generated great interest as a potential key element in tissue remodeling. While extensive experimental data convincingly demonstrate the bioactivity of tenascin in vitro (Chiquet-Ehrismann, 1995a; Crossin, 1996; Mackie, 1997; see also below), the finding that a total Tn-C knockout mouse can develop normally (Saga et al., 1992) was received with deep disappointment. Indeed, although mechanisms of compensation for the loss of Tn-C have been proposed (Erickson, 1993b; Chiquet-Ehrismann et al., 1994; Tucker et al., 1994; Sakakura and Kusakabe, 1994) while the lack of phenotype itself has been challenged (Crossin, 1996), this finding has stressed the fact that until significant developments are made, the identification of the possible functions of tenascin-C has to continue to rely on the analysis of their tissue distribution and from in vitro experiments.

The functional properties reported for tenascins are numerous and, in several instances, contradictory, largely depending on the cell type and experimental conditions used. While it would be beyond the scope of this review to analyze them in detail (see Chiquet-Ehrismann, 1993, 1995a; Crossin, 1996; Mackie, 1997 for recent and more in depth reviews), the adhesive and antiadhesive properties attributed to tenascins, which are of particular interest in the context of epithelial-mesenchymal interactions, are becoming better understood. Indeed, it appears more and more evident that some domains within the tenascin molecule can support cell adhesion while others can inhibit cell attachment to matrix components such as fibronectin and are thus referred to as being counteradhesive (Erickson, 1993a; Chiquet-Ehrismann, 1995b). As recently reviewed by Chiquet-Ehrismann (1995a,b) and Crossin (1996), the counteradhesive activity has been mapped to two distinct sites, the EGF-like repeats domain and the fibronectin type III repeats 7 and 8, while important adhesion sites appears to be located in the fibronectin type III repeat 3 and in the C-terminal fibrinogen domain (Fig. 1C). A number of cell surface binding molecules have been identified including the heparan sulfate side chain of some proteoglycans (C-terminal fibrinogen domain), annexin II (alternatively spliced fibronectin type III repeats A through D), contactin/F11 and fibronectin (boundary of the alternatively spliced region

of the fibronectin type III repeat domain) and integrins (third fibronectin type III repeat). Integrins identified to bind tenascin at its third fibronectin type III repeat include α8b1, α9ß1, αvß3 and αvß6 (Joshi et al., 1993; Prieto et al., 1993; Yokosaki et al., 1994; Schnapp et al., 1995; Denda et al., 1998). Interestingly, in contrast to the other tenascin-binding integrins, α9ß1 recognizes an IDG containing sequence instead of the classical RGD motif (Yokosaki et al., 1998).

In such a context where tenascin activity appears to be the product of the summation of its adhesive and counteradhesive domains, in conjunction with the

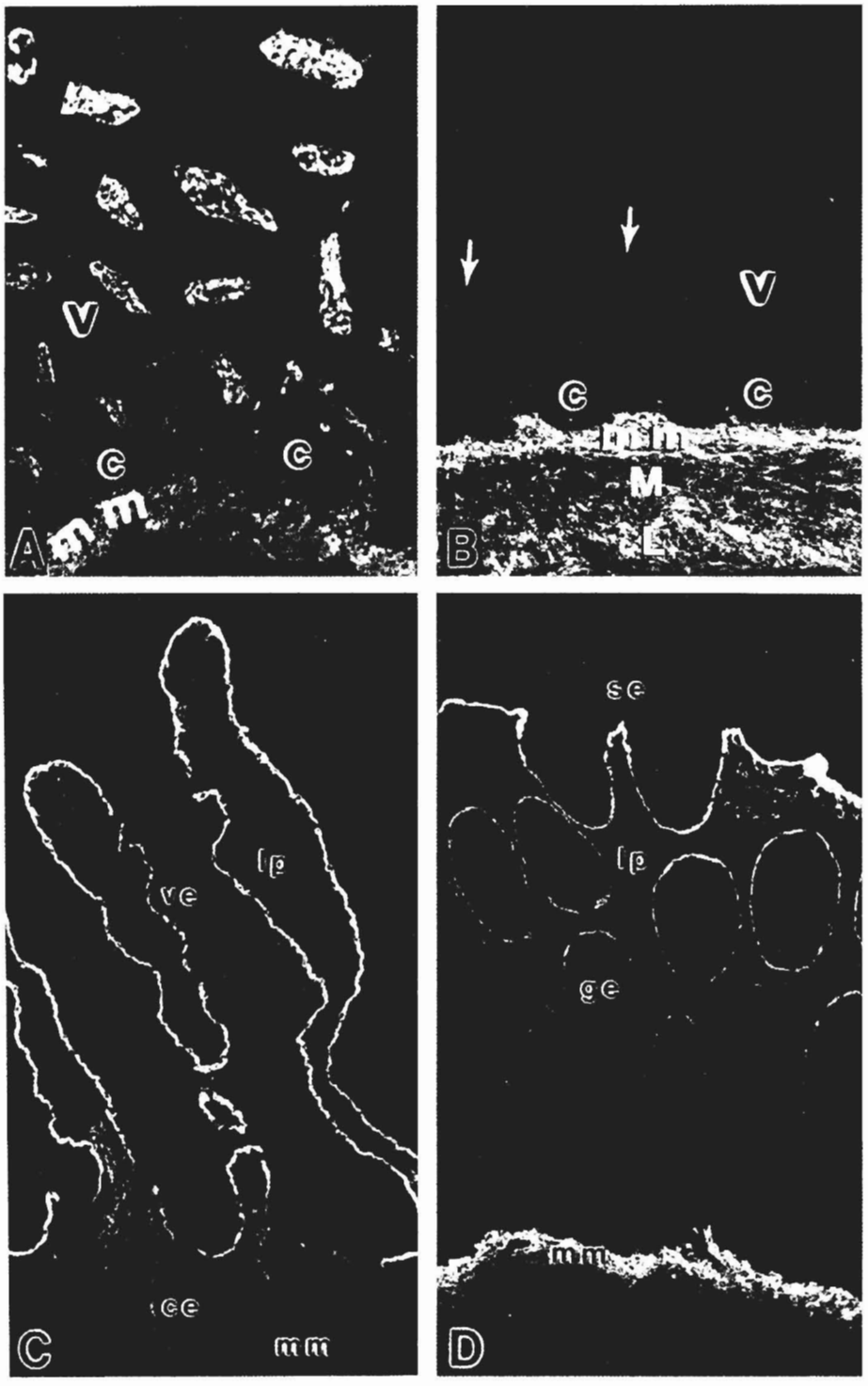

Fig. 2. Expression of Tn-C in the human intestinal mucosa. Immunolocalization of Tn-C in the developing small intestine **(A)** and colon **(B)** reveals a similar pattern of expression in the muscularis mucosa (mm) while villus cores, which show intense staining in the small intestine (A), appear mostly negative in the colon (B). In the adult small intestine **(C)** and colon **(D)**, Tn-C is found predominantly in the muscularis mucosa (mm) as well as in the epithelial-stromal interface according to a typical increasing gradient from the crypt (ce) to the villus (ve) (C) and the gland (ge) to the surface (se) (D). (A, B: from Desloges et al., 1994; C, D: from Beaulieu, 1997, with permission).

repertoire of binding proteins and extracellular matrix proteins expressed by particular types of cells, it is becoming obvious why, in vitro, cell-tenascin interactions can modulate in one way or another basic cell functions such as adhesion, migration, growth and differentiation.

Tissue distribution of tenascins: the gut paradigm

Analyzing the tissue distribution of tenascins represents a complementary approach to in vitro experiments for the identification of possible functions of this family of extracellular matrix molecules. The expression of Tn-C, Tn-R and Tn-X in mammals has been analyzed in detail in a number of tissues and organs (reviewed in: Chiquet-Ehrismann, 1993, 1995a; Erickson, 1993a; Sakakura and Kusakabe, 1994; Crossin, 1996; Mackie, 1997). While Tn-R is exclusively detected in the central nervous system, more particularly in oligodendrocytes and neurons, Tn-C and Tn-X appear to be more widely distributed. Tn-X has been reported to be predominantly expressed in the extracellular matrix of muscle cells and blood vessels while Tn-C appears to be transiently expressed during embryonic development at various sites, including the epithelial-mesenchymal interface of most organs studied such as the mammary gland and the skin, and is upregulated in the stroma of tumors and in wound healing. One clear exception to this general pattern of expression is the gut, where Tn-C remains strongly expressed at maturity in both human (Beaulieu, 1992; Riedl et al., 1992) and laboratory animals (Aufderheide and Ekblom, 1988; Probstmeier et al., 1990). Indeed, as summarized in Figure 2, for the human, Tn-C is strongly expressed in most mesodermal derivatives at mid-gestation (Fig. 2A,B) while in the adult, its distribution is mainly restricted to smooth muscles and the stromal-epithelial interface (Fig. 2C, D). As expected from previous studies in various organs (see above), a number of Tn-C spliced variants were detected in both fetal small intestine and colon by RT-PCR (Fig. 3) indicating that multiple Tn-C isoforms are simultaneously expressed in the gut.

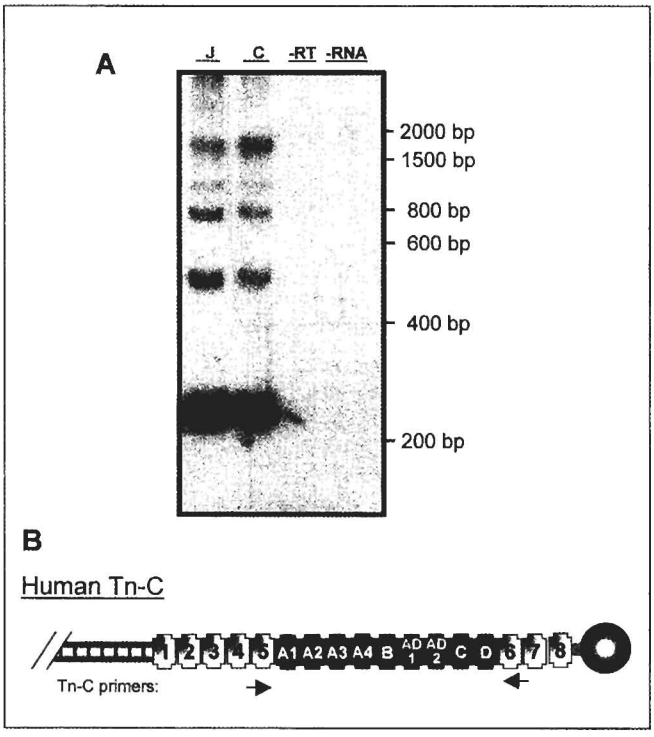

Fig. 3. Tn-C splicing variants of the human small and large intestine. Reverse transcriptase-polymerase chain reaction amplification of the tenascin-C splice variants **(A)** using primers flanking the alternative splicing region **(B)**, from RNA isolated from 18 week-old fetal jejunum and colon. The results show 6 distinct products at 230, 530, 790, 150, 1700 and 1900 pb, approximately, for both segments. Negative controls include omission of reverse transcriptase (-RT) and omission of RNA (-RNA).

Tn-C in the enteric smooth musculature

Concerning the smooth musculature, the presence of tenascin-C as a normal extracellular matrix component of intestinal differentiated muscle cells is well documented (Aufderheide and Ekblom, 1988; Probstmeier et al., 1990; Beaulieu et al., 1991; Natali et al., 1991; Beaulieu, 1992; Crossin, 1996). As shown in Fig. 2, tenascin-C is a prominent component of circular and longitudinal muscle layers of the muscularis propria as well as of the muscularis mucosa of both the immature and adult small intestine and colon. As deduced by Western blotting analysis, the intestinal musculature contains both the 220 kD and the 320 kD forms of tenascin-C (Beaulieu et al., 1993a; Desloges et al., 1994; unpublished data). The function of tenascin-C at this site is still unknown. Given the adhesive/anti-adhesive property of the molecule, one may speculate that its presence in the extracellular matrix of smooth muscle cells contributes to the plasticity of the tissue (for instance, see Oberhauser et al., 1998). Besides such mechanical function, there are a number of observations suggesting that tenascin could be involved in the regulation of smooth muscle cell growth and/or differentiation (Hedin et al., 1991; Mackie et al., 1992; Majesky, 1994) and survival (Lloyd Jones et al., 1997). In the developing human small intestine, the analysis of the distribution of tenascin-C in conjunction with α-smooth muscle actin, as determined by means of double immunofluorescent staining, has revealed the existence of a potential relationship between tenascin expression and smooth muscle cell differentiation (Beaulieu et al., 1993b). It was shown that the early appearance of α-smooth muscle actin-reactive cells at various sites in the intestine was almost exclusively observed in regions already rich in tenascin-C, a process particularly evident during formation of the muscularis mucosa, a thin layer of smooth muscle cells delimiting the lower margin of the mucosa (see Fig. 2). However, direct proof supporting a role for tenascin-C on intestinal smooth muscle cell differentiation is still lacking.

The crypt-villus gradient of Tn-C in the mature small intestine

The epithelial-stromal interface is the second major site of tenascin-C expression in the gut. In the human adult small intestine, the molecule has been detected at the base of epithelial cells according to an increasing gradient along the crypt to villus axis (Fig. 2C). It is pertinent to note that such a preferential expression of tenascin-C in the upper part of villi has also been observed in the mouse small intestine (Aufderheide and Ekblom, 1988; Probstmeier et al., 1990). Interestingly, in their work, Probstmeier et al. (1990) used immuno-electron microscopy to reveal that the macromolecule was present at the basal lamina of villus epithelial cells. They also provide evidence that human colon adeno-carcinoma HT-29 cells fail to adhere to tenascin used as substrate, suggesting that tenascin-C plays a role in epithelial cell shedding. Conversely, Tn-C may simply be involved in epithelial cell movement toward the tip of the villus as suggested more recently (Hashimoto and Kusakabe, 1997).

Tn-C in the human colon and adenocarcinomas

In the mature normal colon, tenascin-C was found to be expressed according to a similar gradient of expression, the molecule being more abundant at the base of the surface epithelium than around the lower part of the gland (Fig. 2D). Its expression in adeno-carcinomas has been well documented over the last 10 years. Overall, there is a good consensus that tenascin-C is substantially upregulated in the stroma surrounding the neoplasic cells. Tn-C expression was not found to be altered in adenomas while it appears to be increased in most carcinomas studied (Natali et al., 1991; Riedl et al., 1992, 1997; Sakai et al., 1993; Hauptmann et al., 1995; Hanamura et al., 1997) according to two distinct patterns of staining: a subglandular staining pattern predominant in well-differentiated tumors and a diffuse interstitial stromal staining pattern predominant in moderate and poorly differentiated tumors (Sugawara et al., 1991; Iskaros et al., 1997; Kressner et al., 1997). Interestingly, it was shown that patients with more Tn-C expression and those showing the subglandular staining pattern had better long term survival than those with the diffuse interstitial staining pattern and/or low Tn-C expression (Iskaros et al., 1997; Kressner et al., 1997). These data are consistent with the fact that reduced Tn-C expression in human colon carcinoma correlates with DNA aneuploidy and may suggest that Tn-C fulfils a protective function in preventing tumor invasion (Sakakura and Kusakabe, 1994), a function likely to be dependent on the levels of proteolytic activity produced by the tumor cells (Imai et al., 1994; Siri et al., 1995).

Tn-C in the developing intestine

In the developing human intestine, as well as in that of other species (Crossin et al., 1986; Aufderheide and Ekblom,1988; Probstmeier et al., 1990), tenascin-C is expressed in the mesenchyme according to a unique spatio-temporal pattern (Beaulieu et al., 1991, 1993a,b; Desloges et al., 1994). Indeed, in the human small intestine, tenascin was first detected at 11 weeks of gestation, which is 1-2 weeks after the formation of villus rudiments, and was restricted to the connective tissue at the tip of the villus. At later stages, a widespread distribution of tenascin-C in the mesenchyme from the tip of the villus to the base of the crypts (Fig. 2A) was observed according to a typical gradient of intensity (Beaulieu et al., 1991, 1993b). Analysis of the oligomeric forms of intestinal tenascin-C by Western blotting revealed the presence of three major components at 320 kD, 220 kD and 200 kD. The two larger ones were also found in cultured fibroblasts and most likely correspond to the previously described variants (Erickson and Bourdon, 1989). The identification of distinct forms of tenascin in the human intestine is consistent with the well-known complexity of expression of this macromolecule in different cell types as demonstrated at both the molecular and histological levels (Gulcher et al., 1989; Taylor et al., 1989, Chiquet-Ehrismann et al., 1991; Siri et al., 1991). Interestingly, while both the 320 kD and the 220 kD forms were detected uniformly at all stages of intestinal development studied (9-20 weeks), the 200 kD component, barely expressed at 9 weeks, increased by a factor of ~10 over the same period (Beaulieu et al., 1993a). This observation is not without precedent since the expression of molecular forms of tenascin-C variants was also reported during the development of the mouse and chick digestive tract (Crossin, 1996; Aufderheide and Ekblom, 1988; Saga et al., 1991). In the chick gizzard, there is clear evidence that the expression of tenascin-C variants is tissue-specific suggesting that the developmental appearance of a particular variant in this organ is related to the differentiation of a new type of tissue (Matsuoka et al., 1990). It is noteworthy that in the developing human small intestine, the expression of the 200 kD form correlates well with the accumulation of immunoreactive tenascin-C in the mesenchyme of the growing villi during the same period. Analysis of small intestinal fractions specifically enriched in villi by Western blot revealed that the 200 kD component is the major Tn-C form present in the villus (Beaulieu et al., 1993c). The role of such a villus-predominant form of tenascin-C, which is also developmentally expressed, remains to be determined. The expression of this molecule was also analyzed in the developing human fetal colon (Desloges et al., 1994). Indeed, a well-known particularity of the colonic segment is the transient presence of villi between 10 and 25 weeks of gestation, which, from a functional and morphological point of view, are similar to those present in the small intestine (Ménard and Beaulieu, 1994). Surprisingly, colonic villi were found to be devoid of immunoreactive tenascin-C at all stages studied (Fig. 2B) although the smooth

musculature was immunostained and the 220 kD and 320 kD forms of tenascin were detected in villus-depleted colonic extracts. Tenascin-C may thus not be required for intestinal villus development. However, its role in the intestinal mucosa may be more subtle. Although tenascin was not detected in the transient colonic villi at the protein level, Tn-C mRNA was found to be expressed at levels comparable to those found in small intestinal villi (Bélanger et al., unpublished data). Furthermore, it was strongly detected at the epithelial-mesenchymal interface in both developing and adult small intestinal mucosa and in the mature colonic glandular mucosa (see above). The mechanism of mucosal remodeling during late development in the fetal colon is still unknown (Ménard and Beaulieu, 1994) but in light of these latter observations, tenascin-C in its 200 kD form could be important in the maintenance of intestinal mucosa integrity, most likely by contributing to functional epithelial-stromal interactions.

Tn-X in the human intestine

Much less is known about the other members of the tenascin gene family in the intestine. The fetal human gut was first identified as a relatively strong expresser of Tn-X at the mRNA level (Bristow et al., 1993). By using specific antibodies against murine tenascin-X fusion proteins, Matsumoto et al. (1994) demonstrated reciprocal staining patterns for tenascin-C and X in the developing gut. Tenascin-X was found most prominently expressed in the mesenchyme surrounding smooth muscle and, in contrast to tenascin-C, was not detected in villus cores.

Regulation of Tn expression

The fact that temporal and spatial patterns of expression for Tn-C and, to some extent, Tn-X, are highly variable during normal gut development as well as under abnormal conditions indicates that the expression of tenascins is highly controlled. This phenomenon has been particularly well exemplified in tissue culture where different types of cells can be modulated in their production of Tn-C, including epithelial cell lines derived from tissues which do not normally express this molecule in vivo. Among growth factors and cytokines known to upregulate Tn-C expression are transforming growth factor-ß, several members of the fibroblast growth factor family, interleukin-1 and 4, tumor necrosis factor-α and angiotensin II (Mackie et al., 1992, 1998; Sharifi et al., 1992; Hahn et al., 1993; Tucker et al., 1993; Chammas et al., 1994; Rettig et al., 1994; Sakai et al., 1994, 1995; Hakinen et al., 1995). In contrast, glucocorticoids seem to repress the expression of Tn-C (Ekblom et al., 1993) and Tn-X (Sakai et al., 1996). While the regulation of tenascin expression by these factors remains to be better documented in the gut, it is likely that altered expression of Tn-C as reported in cancer (see above) as well as in various other pathological conditions of the digestive tract (Riedl et al., 1992, 1998; Parikh et al., 1994; Aigner et al., 1997) is mediated at least to some extent by growth factors and cytokines (Fiocchi, 1997).

Tn receptors in intestinal cells

Intestinal cell-tenascin interactions remain to be poorly understood. Receptors for Tn-C have not yet been identified in mature villus cells of the small intestine or in surface cells of the colon, raising the possibility that mature intestinal cells may not express specific tenascin-binding proteins and that the molecule acts as a counter-adhesive component. However, considering the relatively low amounts of fibronectin in the adult intestine (Beaulieu, 1992), it seems unlikely that Tn-C works in conjunction with fibronectin to reduce adhesion along the villus migratory pathway. One interesting possibility is that the high level of Tn-C expression at the epithelial-stromal interface may compete with the strong adhesive cell-matrix interactions provided by other molecules such as laminin-1 and -5 and the α3ß1 and α6ß4 integrins, also expressed at high level in regions rich in Tn-C (see Beaulieu 1999 for a recent review). Clearly, more work will be needed to elucidate the phenomenon of intestinal cell migration in the adult intestine.

Recent observations from our laboratory indicate that the integrin α9ß1 could be involved in intestinal cell-tenascin interactions under certain circumstances, namely during development and in cancer progression. While absent from the normal adult colonic epithelium (Palmer et al., 1993), the integrin α9ß1 has been detected in a significant proportion of colon carcinomas (Basora et al., 1998). Furthermore, the integrin was also detected in the epithelium of both human fetal small intestine and colon, predominantly confined to cells located in the crypts (Basora et al., 1998; Desloges et al., 1998), which represent the proliferative compartment. Interestingly, integrin α9ß1 analysis in intestinal cell lines supported the relation between its expression and the proliferative status of these cells (Desloges et al., 1998). These observations are consistent with the finding that forced expression of the α9 integrin subunit in the SW480 colon carcinoma cell line stimulates, in a ligand-dependent manner, cell proliferation and concomitant phosphorylation of the mitogen-activated kinase, Erk2 (Yokosaki et al., 1996).

Conclusion

Several questions remain concerning tenascins in the human gut. Based on the determination of the expression patterns for the various members of this family of complex extracellular matrix molecules, Tn-C emerges as a key player likely to be involved in intestinal mucosa development, maintenance and disease. Its exact role on the regulation of fundamental intestinal cell function(s) such as proliferation, migration and tissue-specific gene expression remains however to be established.

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