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TENASCIN EXPRESSION IN NORMAL AND PATHOLOGICAL CONDITIONS OF THE MUSCULOSKELETAL SYSTEM

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SUMMARY

• *Tenascin is an extracellular matrix protein with highly regulated expression and uncertain functions. It is prominently expressed during musculoskeletal embryogenesis. The pattern of distribution of tenascin in healthy adult musculoskeletal tissues is spatially and temporally restricted. It can be only detected in a small amount in the muscle-tendon junctions, tendons, perichondrium, periosteum, endosteum, the superficial layer of articular cartilage and the subintimal connective tissue ofsynovium. Elevated tenascin expression is found in inflammatory, degenerative and neoplastic lesions of the musculoskeletal system. The peculiar pattern of tenascin expression suggests it may play a role in the regulation of cell behavior at the interfaces bet\veen different elements of the musculoskeletal system and in various pathological processes, in particular those involving attachment and/or detachment of cells from the extracellular matrix and their proliferation and collagenase secretion.*

INTRODUCTION

• Tenascin (Tn) has also been referred to as hexabrachion (1), cytotactin (2), glioma mesenchymal extracellular matrix protein (3), Jl glycoprotein (4) and nryotendinous antigen (5). Tn is a large oligomeric, modular glycoprotein of extracellular matrix consisting of six similar subunits joined together at N-terminal domain by disulphide bonds in the central region. This six-armed structure, called hexabrachion, is probably subjected to a rapid intracellular assembly. The N-terminal domain is connected to two coiled triplexes, each of which is formed by a combination of heptad repeats of three subunits. The extended subunit is completed by a series of epidermal growth factor (EGF)-like repeats, a series of fibronectin type III repeats and a carboxyl (C)-terminal fibrinogen-like domain (6, 7) (Fig. 1,2).

Different Tn isoforms are generated by alternative splicing of pre-mRNA. In normal cells this alternative splicing is controlled by extracellular pH (8). These isoforms have different numbers of fibronectin type III repeats and thus different molecular sizes (9-11). There are two main isoforms of human Tn: the small Tn isoform has eight fibronectin type III repeats and a molecular weight of approximately 190 kD, whereas the large Tn isoform contains seven extra fibronectin type III repeats inserted between domains five and six and has a molecular weight of approximately 290 kD (Fig.3). They may have differential functions, with the small variant being preferentially bound to purified fibronectin and to fibronectin-containing extracellular matrix in fibroblast cultures (Fig.3). The major fibronectin binding site is located in the third fibronectin type III repeat and with an additional weak binding site, probably

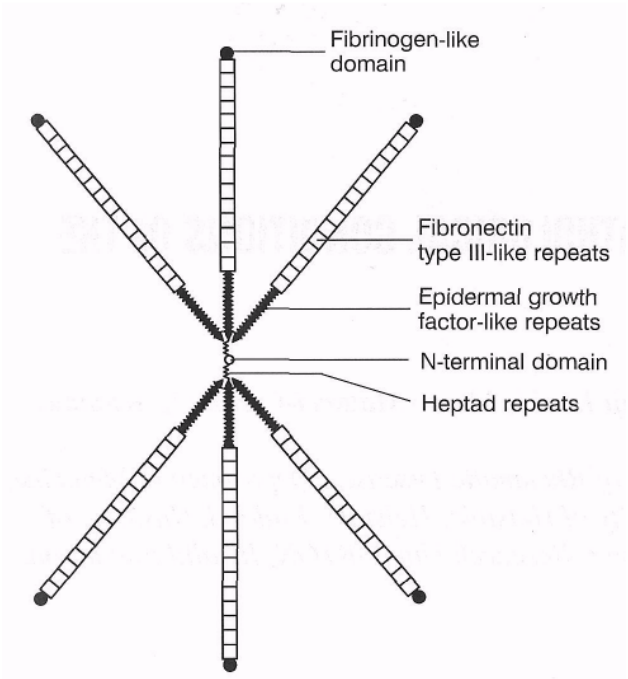


Figure 1. The large 290kDtcnascin-C isnform is shown schematically with all the six arms of the hexabrachion being extended away from the N-terminal central domain.

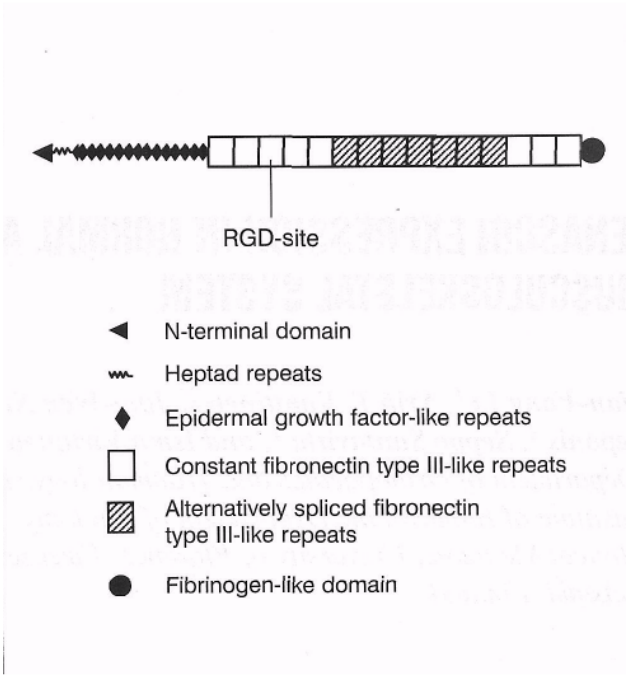
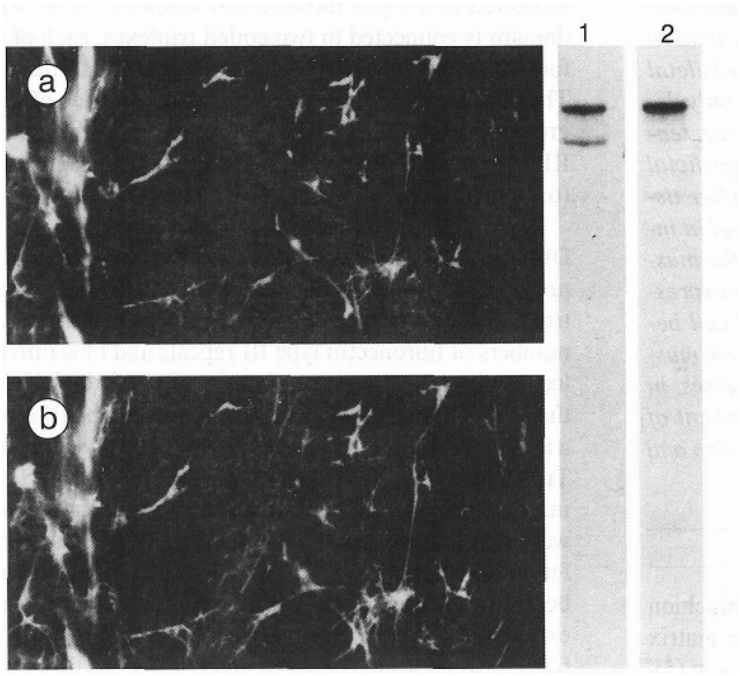


Figure *2. A schematic presentation of the extended arm of the large 290 kD ten ascin-C isoform. The heptad repeat region depicted consists of a triplex formed by the heptad domain of three subunits.*



FigureS. *Panel A. Double indirect immunofluorescence staining of cultured human embryonal fibroblasts for tenascin with a monoclonal antibody (a) and for fibronectin with a rabbit antiserum (b). Note that tenascin immunoreactivity only partially codistributes with that of fibronectin fibers, being particularly abundant in more coarse fibers. Panel B^ Fluorography ofS^{3i}-methionine metabolically labelled cultured human embryonal skin fibroblasts analysed for production of tenascin polypeptides into the culture medium. Immunoprecipitation with monoclonal antibodies to tenascin shows in one of the human skin fibroblast strains both the 190 kD and 290 kD tenascin polypeptide* ***(lane*** *1), while in another strain only the 190 kD tenascin polypeptide is precipitated* ***(lane*** *2). Both cell strains assemble tenascin into extracellular matrix similarly.*

in domains 6-8 being exposed in the small 190 kD Tn isoform. The structural and functional sites of human Tn are as follows (12)

Signal peptide: 1-22
Heptad repeats: 119-125, 126-132, 133-139
EGF-Iike repeats: 174-185, 186-216, 217-247, 248-279, 280-310, 311-341, 342-372, 373-403, 404-434, 435-465, 466-496, 497-527, 528-558, 559-589, 590-620
Fibronectintype IH-like repeats: 621-708,709-801,802-891, 892-983, 984-1071, 1072-1162, 1163-1253, 1254-1344, 1345-1435, 1436-1526, 1527-1617, 1618-1708, 1709-1797, 1798-1885, 1886-1973
Alternatively spliced repeats: 1072-1162, 1163-1253, 1254-1344,1345-1435, 1436-1526, 1527-1617, 1618-1708
Fibrinogen-like domain: 1981-2190
Potential N-linked glycosylation sites: 38, 166, 184, 327, 788,1018,1034, 1079,1093,1119,1184, 1210,1261,1275, 1301, 1366, 1392, 1445, 1455, 1485, 1534, 1809, 2161
Arginine-aspartic acid-glycine (RGD) site: 877-879

Among Tn polypeptide family, Tn-C is the prototype discovered, with C indicating cytotactin. Three analogues to Tn-C have been recently discovered. First, Tn-R was originally described under the name of restrictin (13). It consists of the same type of domains as Tn-C while its preparations contain trimers, dimers and monomers instead of hexamers (14). Tn-R is found exclusively in the nervous system and is most prominent during development (15,16). Second, Tn-X was originally reported as a partial sequence encoded by gene XB in human major histocompatibility complex class III region. Its mRNA expression is most prominent in fetal muscle and testis (IT-19). Tn-Y is a newly described member of Tn family and is produced by differentiated fibroblasts of muscle connective tissue (20). The emphasis of this review is on Tn-C, and the name Tn, without a letter, will be used only for Tn-C.

DISTRIBUTION, FUNCTION, PRODUCTION AND REGULATION OF TENASCIN

• During embryonic development, Tn has a spatially and temporally restricted expression. It is strikingly expressed at the sites of epithelial-mesenchymal interactions, indicating that Tn may be induced by actively growing epithelium (21-23). Tn expression is also conspicuous in developing dense connective tissue (24).

In adult tissues, Tn is absent or much more restricted than in developing tissues. It can be detected at the epithelial-stromal interfaces, although sometimes faint stromal Tn is noted too (25-30). Tn is absent in some adult connective tissues but is prominently detected during certain stages of differentiation and tissue remodeling associated with developmental and pathological changes (31). In healing wound, Tn is present in the matrix of the granulation tissue and under the proliferating and migrating epidermal layer. After healing, the expression of Tn returns to normal (32, 33).

Because of its extensive expression in some malignant lesions and its absence in many normal adult tissues, Tn expression was once thought to be a marker of malignant growth. However, recent research has shown that Tn is also present in some normal tissues and benign lesions even though significant Tn enhancement has been shown in most carcinomas (26, 34). Although elevated serum Tn levels were found in most carcinoma patients (35), the highest level of serum Tn have been noted in patients with acute sepsis. It has been postulated that Tn is induced as an acute phase protein in liver through the action of inflammatory mediators (36).

The functions of tenascin are still largely unknown. Originally tenascin has been described to be a potent hemagglutinin (1). Tn possesses both adhesive cell-binding and matrix protein-binding domains and anti-adhesive domains. Tn has an anti-adhesive or adhesion-modulating effect through interference with the action of fibronectin (Fig. 3) and Tn may thus play a role in reducing cell adhesion to fibronectin (37, 38). The capacity of Tn to inhibit fibronectin dependent adhesion and migration may depend on its ability to block the binding sites on fibronectin or its receptors. However, recent studies show that interaction of Tn with its cell surface receptor, annexin II, is able to induce loss of focal adhesion (39). Together with fibronectin, Tn induces fibroblasts to secrete collagenase, which suggests that Tn has an effect on morphogenetic events and tissue remodeling involving proteolysis (40). Tn appears to stimulate cell growth and thus exhibits a mitogenic property on fibroblasts (41). Tn is thought to be a modulator of matrix-cell interactions and to be involved in various differentiation events (42). Tn has also been shown to have an immunosuppressive activity by inhibiting T-cell activation (43, 44).

RDG sequence in fibronectin type III repeats may be responsible for Tn-mediated cell attachment (9). Many cells attached to Tn remain rounded and do not flatten and spread as it is seen on fibronectin coated substrate (45). Several cellular receptors that spread on Tn and mediate Tn action have been suggested and include integrins a2(3l, a8(il, a9(il, aVp3, and aVp"6 (46), contactin (47) and annexin II (48). Another binding site in Tn molecule was found to be the fibrinogen-like C-terminal knob. It has been claimed that only the binding of the fibrinogen-like C-terminal knob of Tn to a2 (i 1 integrin may play a biologically significant role based on the fact that there are species variation of RGD sequence and cryptic location of some RGD sites (49). Recent studies show that the interaction of integrins a9(31 or aVp3 with the third fibronectin type III repeat of Tn can directly stimulate cell proliferation (50)

Many earlier studies demonstrated that Tn was present in mesenchyme, but not in epithelium, at sites of mesenchymal-epithelial interactions. It has once been suggested that epithelial cells do not synthesize Tn, whereas fibroblasts do. This assumption needs to be revised since an *in situ* hybridization study has showed that epithelial cells are also able to produce Tn (51). In cell culture, both amnion epithelial cells and bronchial epithelial cells synthesize Tn and assemble it to extracellular matrix (52, 53). Given the appropriate environment and stimuli, almost all cells can produce Tn although such an ability has not been described, at least not yet, for blood cells and muscle cells.

The highly regulated pattern of Tn expression suggests that its synthesis may be modulated, at least in part by mechanical forces and a series of soluble factors such as hormones and cytokines. The structure of the 5'-region of the human tenascin gene contains several potential binding sites for transcription factors, which indicates that complex mechanisms control the transcriptional regulation of Tn gene. Transforming growth factor-p was the first one shown to upregulate Tn expression (54). To date, a long list of growth factors and cytokines have been found to upregulate Tn expression (Table 1).

Elevated Tn expression has been shown to be induced by mechanical force exerted on fibroblasts cultured in restrained collagen gel. while Tn synthesis was decreased in a floating, contracting gel (64). The results suggest that mechanical force may have an important role in the regulation of Tn expression during developmental, regenerative and morphogenetic processes. Thus, mechanical force exerted on tissues is translated to an altered protein pattern of the extracellular matrix. This can also partly explain the mechanism of the generation of bone (spicules) along the line of force acting on the bone, which would help to remodel the bone according to mechanical demands, according to the law of Wolff.

Table 1. Regulation oftenascin expression

Effects	Regulatory factors	Refs
Upregulation	Transforming growth factor-α	54
	Basic fibroblast growth factor	55
	Epidermal growth factor	56
	Nerve growth factor	57
	Platelet-derived growth factor	58
	Angiotensin II	58
	Interleukin-1	59
	Interleukin-4	59
	Tumor necrosis factor-α	59
	Activin	60
Downregulation	Glucocorticoids	61-63

Tn can be readily catabolized by extracellular matrix proteinases. Only matrix metalloproteinase (MMP)-7, also known as matrilysin, can degrade small Tn isoform while MMP-7, MMP-2 and MMP-3 are all involved in the degradation of the larger Tn isoform. Cathepsin G and leukocyte elastase can also digest Tn (65, 66). Glucocorticoids are shown to downregulate Tn expression in bone marrow stroma (61), tumor stroma (62) and healing wound (63).

TENASCIN EXPRESSION IN DEVELOPING CARTILAGE AND BONE

During cartilage embryogenesis, Tn is produced by chondrogenic cells in the condensing mesenchyme of cartilage anlagen while it is absent from the surrounding mesenchyme. Tn expression decreases with chondrocyte differentiation and is undetectable in mature and hypertrophic cartilage (24). In day 7 chick embryo, Tn was found in newly formed vertebral cartilage with a homogenous distribution. Tn could not be detected in mature cartilage but persisted in surrounding perichondrium (67). It is commonly hold that in early stages of chondrocyte development. Tn is only expressed by poorly differentiated chondrocytes at epiphyseal ends while fully-differentiated chondrocytes do not produce Tn (68).

During articular cartilage development, Tn was first detected in cell condensation region of day 4 chick embryo limb bud. With development, Tn was only present in the articular cap of the models. Tn persisted in articular cartilage throughout postnatal life but decreased with age. There was a tissue-specific distribution of Tn isoforms around articular cap. The shortest Tn isoform (Tn 190) was almost exclusively detected in the cartilage, whereas the peripheral articular cartilage, the region near fibrocartilage, fibrocartilage and the outer layer of perichondrium contained both Tn 190 and Tn 200. The largest Tn isoform (Tn 230) was only detected in the articular cartilage associated tissues, ligaments and meniscus. Tn was absent from growth plate cartilage undergoing endochondral ossification and was barely detectable in the secondary ossification center within the articular cap (69).

During the healing of cartilage injury, Tn was undetectable in the initially formed blastema, i.e. in the primitive granulation tissue formed after injury, consisting mainly of hematoma, newly generated blood vessels and ingrowing mesenchymal

tissue. With differentiation of chondroblasts and osteoblasts, Tn was detected in the matrix surrounding these cells. After the regenerating cartilage matured, Tn staining disappeared (70).

In vitro study showed that addition of Tn to substrate, in which the chondrocytes were cultured, stimulated chondrogenesis (24). When anti-tenascin antibody was added to culture media, chondrogenesis was inhibited (71). The promotion of chondrogenesis is thought to be through the effect of Tn on cell shape, i.e. by the anti-adhesive effect of Tn, which counteracts fibronectin-mediated adhesion, since round morphology is necessary for the maintenance of chondrocyte phenotype (72).

During endochondral bone formation, Tn is detected around the osteogenic cells invading the cartilage model. During membranous bone formation, Tn is first present in condensing mesenchyme and later present around the spicules of forming bone. Tn is absent from mineralized bone matrix (24). In membranous bone of growing rat skull, Tn was present in immature bone matrix and was most prominent in areas where the periosteum and perichondrium were thickened such as sites of some muscle attachment (73).

In human fetal long bone, Tn was present in a clearly defined and characteristic beaded pattern, i.e. granular distribution along type III collagen. At the sites of active bone formation, Tn expression on collagen type III fibers was particularly strong. In the middle region adjacent to the basophilic cortical bone, staining of Tn on collagen type III was also conspicuous. In the mature bone of adult mandible, there was no staining of Tn on collagen type III fiber. However, in adult mandible bone with dysplastic lesion, Tn was present with a diffuse and irregular pattern in the soft fibrous tissue adjacent to the site of early ossification. Different Tn expression may represent different stages of osteogenic differentiation. The regular, characteristically beaded pattern of Tn distribution on type III collagen implicates that bone tissue is developing toward maturity (74).

Alkaline phosphatase (AP) is thought to be involved in matrix mineralization by catalyzing the hydrolysis of phosphate ester bonds at alkaline pH in hard connective tissues. In growing membranous bone, Tn and AP were detected in periosteum and perichondrium. Activity of AP was confined to the inner layer of periosteum while Tn expression extended to more superficial layer. The wider distribution of Tn suggests that it may function at an earlier stage of hard tissue formation than AP (75).

Although Tn is undeteclable in mature mineralized bone matrix, osteoblasts express Tn both *in vivo* and *in vitro.* Cultured osteoblasts from chicken, human and rat synthesize exclusively the largest Tn isoform, whereas fibroblast cultures from periostea of chicken calvariae synthesized approximately equal amounts of all three isoforms. The results suggest that progressive osteoblast differentiation from multipotential precursor cell correlates with the loss of Tn diversity. The additional fibronectin type III repeats in the large Tn isoform may play a role in the maintenance of round shape of osteoblasts by its antiadhesive effect (76, 77).

TENASCIN EXPRESSION IN ARTHRITIC SYNOVIUM AND CARTILAGE

The histopathologic features of arthritis include synovial lining cell hyperplasia, mononuclear cell infiltration, vascular changes and fibrosis (78, 79). These changes result in degradation of extracellular matrix in the synovial tissue itself and in the articular cartilage covered by an abnormal extension of the synovial tissue known as *pannus* (from Latin, a piece of cloth). Local production of a variety of matrix molecules is of vital importance for the return of tissue to normal after injury. In normal synovium, Tn is often found around blood vessels and just beneath the synovial lining cell layer, but is rarely detected in the normal synovial lining layer itself (Table 2).

Extensive staining of Tn was seen in synovium specimens from patients with osteoarthritis. Tn was diffusely distributed in the subintimal connective tissue with the expression being particularly strong in areas of sclerosis and fibrosis. Tn was also prominently expressed in perivascular regions, and in the synovial lining layer (82,83) (Table 2, Fig.4a).

In rheumatoid arthritis, strong and diffuse Tn expression was detected in the synovial lining layer, in particular among the fibroblast-like type B synovial cells, in perivascular areas and in areas of fibrosis. In inflammatory cell infiltration with densely packed lymphocytes, Tn expression was described to be either weak (83) or increased (84) (Table 2, Fig.4b).

In situ hybridization showed that inRNA^{Tn} was detected at a low level in normal synovium in synovial lining and in perivascular regions. A striking increase of Tn expression was seen in synovium from patients with arthritis. In arthritic synovium, there was an increase in the number of cells expressing mRNA^{Tn} and an increase in the level of mRNA^{To} expressed in individual cells. Interleukin-1 induced cultured synovial fibroblasts to produce Tn (84).

The localization of Tn in normal and arthritic synovium suggests that it may have a regulatory effect on the interaction between synovial lining and sublining tissue and between blood vessels and perivascular connective tissue matrix. Due to its effects on immune function and cell adhesion, Tn may have a role in the pathogenesis of arthritis. Locally produced cytokines may modulate the expression and thus effects of Tn.

Table 2. Tenascin expression in normal and pathologically altered synovial membrane, hyaline cartilage, ligament, and tendon

Tissue	Normal	Pathological	Refs
Synovium	Small amount underneath the synovial lining cell layer	In osteoarthritis (OA) and rheumatoid arthritis (RA), increased expression spreadingto synovial lining layer and deep subsynovial tissue zone	80-84
Articular cartilage	Small amount in the surface zone	In OA and RA, extensive distribution with a diffuse pattern in the surface zone and pericellular pattern in the deep layers	81,83,85
Ligament	Small amount at the sites of attachment to bone (enthesis)	Intense expression in ligaments with injury or degeneration	86, 87
Tendon	Weak expression in organized, fibrous region of tendon matrix	Strong expression associated with some round cells in disorganized fibrocartilaginous tissue	88

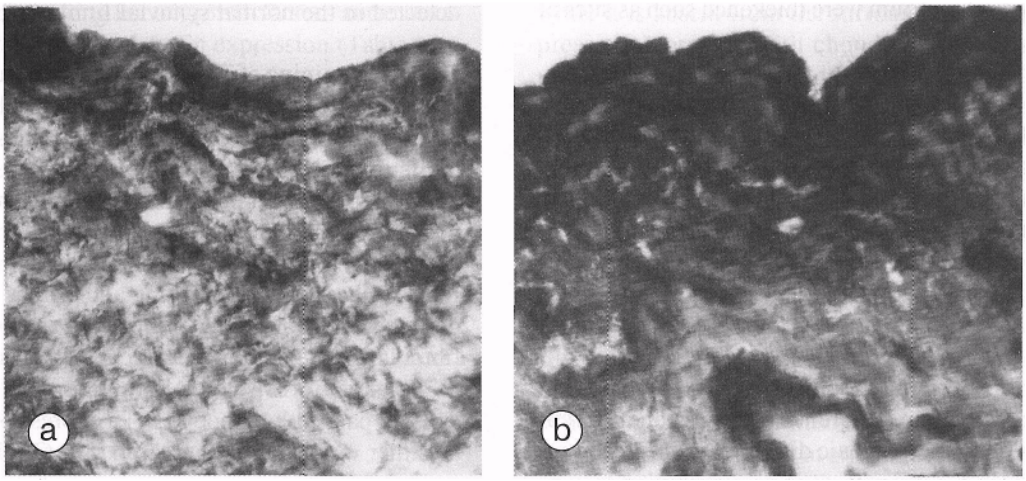


Figure 4. Tenascin staining of synovial membrane from osteoarthritis (a) and rheumatoid arthritis (b). Strong lenascin staining can be seen in the synovial lining cell layer and in the sublining connective tissue. Note also the prominent staining of the perivascular connective tissue matrix. '

In normal human cartilage, Tn is present in small amount and usually confined to superficial zone. Sometimes Tn expression extends focally to the matrix surrounding chondrocytes of upper mid zone. Normal cartilage explant treated with interleukin-1 expressed Tn in almost all of the pericellular areas of all cartilage layers, but not in the matrix at sites distant from chondrocytes (81,85).

In the articular cartilage from patients with osteoarthritis, Tn was found mainly in the superficial layer, but also in the transitional and deep zones of the undecalcified hyaline articular cartilage. In the extracellular matrix near surface, Tn expression was diffuse and irregular, whereas in the deep layer of cartilage, it was expressed in a pericellular pattern. In the areas showing severe pathological changes such as vertical fissure, superficial cartilage loss and cell clustering, Tn expression was particularly strong and extended to both the interterritorial and pericellular regions of the deep zone, sometimes even to subchondral bone undergoing osteoid remodeling. In the articular cartilage from patients with rheumatoid arthritis,

Tn was seen in all layers of the cartilage and in the juxtasubchondral (i.e. in the bone just below the subchondral bone plate) zones of bone in a pericellular pattern. Tn expression was strong also in *pannus,* adjacent residual hyaline articular cartilage and reparative fibrocartilage (81, 83, 85).

The mechanism of Tn accumulation in arthritic cartilage remains to be elucidated. Strong expression in surface zones suggests passive absorption from synovial fluid. Nevertheless, its pericellular location in deeper layer of articular cartilage indicates that production by chondrocytes may contribute to increased local levels. Increased local Tn production in arthritic cartilage is likely to be a part of reparative and inflammatory response in injured joints. Elevated Tn levels may antagonize the adverse effects of increased fibronectin to chondrocyte metabolism, i.e. fibronectin-mediated inhibition of synthesis of the cartilage matrix, and fibronectin mediated inhibition of chondrocyte migration to the site of lesions.

TENASCIN EXPRESSION IN TUMORS OF THE MUSCULOSKELETAL SYSTEM

• Tn and proliferating cell nuclear antigen (PCNA) were investigated in benign and malignant cartilage tumors. Tn was barely detectable in the fully differentiated cartilage of enchondroma and low grade chondrosarcoma. In the high grade chondrosarcoma, Tn was strongly expressed at the periphery of tumor lobules and matrix. Intense staining of PCNA was found in the spindle-shaped cells of high-grade chondrosarcoma. The distribution of PCNA-positive cells always corresponded to the regions with Tn reactivity. Surprisingly, Tn and PCNA were present in all chondroblastoma specimens, indicating that there is a relatively high proliferative activity in chondroblastoma (89). These data suggest that Tn plays a role in promoting tumor cell proliferation and its synthesis seems to be the result of tumor development. However, further studies are needed before determination of the significance and prognostic value of Tn in benign and malignant cartilage tumors can be done.

Synovial sarcoma is a soft tissue tumor with characteristic biphasic structure consisting of epithelial and spindle mesenchymal cells. Strong Tn expression was found in the mesenchyme immediately around epithelial cells. Tn was barely detectable in the monophasic tumors and in the mesenchymal tissue distant from epithelial elements in the biphasic tumors. This peculiar pattern of Tn distribution suggests that it is involved in neoplastic epithelial-mesenchymal interactions (90) (Table 1).

TENASCIN EXPRESSION IN OTHER CONDITIONS

• In human yellow ligament, Tn was present in the matrix around fibroblasts of entheses, particularly in transitional region between the cartilage and ligament. The appearance of Tn in human yellow ligament varied with age and was frequent in spinal disease with ossification (86). Tn was sparsely distributed in normal anterior cruciate ligament (ACL), but strongly expressed in ruptured ligament with a time-restricted pattern. The data suggest that Tn is an important factor during healing of ACL (87). In human skeletal muscle, Tn was found in a short segment of the muscle spindle fibers, in the equatorial region where the sensory nerve endings are found, and in the outer layers of the spindle capsule. Tn was also found in the neuromuscular junctions of the extrafusal fibers. The close spatial

Table 3. Tenascin expression in tumors of the musculoskeletal system

Type of tumor	Tenascin expression	Refs
Enchondroma	Rarely detected	89
Chondroblastoma	Commonly detected	89
Chondrosarcoma	Grade I: weak staining in tumor lobules, interlobular septa and fibrous capsule Grade II: increased staining at the margins of the lobules Grade III: intensive staining throughout the matrix	89
Synovial sarcoma	Strong staining in mesenchymal tissue around epithelial elements and weak staining in mesenchymal tissue distant from the epithelial elements and in monophasic tumors	90
Osteomacutis Rhabdomyosarcoma Osteogenic sarcoma	Strongly expressed	91

relationship between Tn and nerve endings implicates that Tn is of functional importance in adult nerve-muscle contacts in human skeletal muscle (92). Tn production may become stimulated by the regenerative process of skeletal muscle and remains upregulated until the muscle undergoes successful regeneration (93). In normal human tendons, Tn was associated with organized, fibrous regions of the tendon matrix, while in the degenerative tendons, Tn was strongly associated with some round cells in disorganized fibrocartilagenous regions (88).

CONCLUSION

• Tenasein expression is associated with developmental, regenerative, degenerative, inflammatory and neoplastic events of musculoskeletal system, and almost always present at the sites with active tissue turnover (see Table 2,3).

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