Tomasz Ferenc[1], Liliana Stalińska[1], Maria Turant[2], Jacek Sygut[3], Dariusz Tosik[4], Adam Dziki[5], Andrzej Kulig[2]

Analysis of TGF-β protein expression in aggressive fibromatosis (desmoid tumor)*

[1]Department of Biology and Genetics, Medical University, Łódź,
[2]Department of Clinical Pathomorphology, Institute Polish Mother's Health Centre, Łódź,
[3]Department of Neoplasm Pathology, Świętokrzyski Centre of Oncology, Kielce,
[4]Department of Histology and Tissues Ultrastructure, Medical University, Łódź,
[5]Department of General and Colorectal Surgery, Medical University, Łódź

Aggressive fibromatosis, usually termed desmoid tumor, develops from muscle connective tissue, fasciae and aponeuroses. Aggressive fibromatosis located in various parts of the body demonstrates differentiated biological behavior. Abnormalities in TGF-β expression are very common in many disease processes, including neoplasms. Immunohistochemical analysis employing a monoclonal antibody against TGF-β was performed on archival material, consisting of 38 cases of aggressive fibromatosis, among which 23 represented abdominal, 11 extra-abdominal and 4 intra-abdominal localizations. The sections for immunohistochemical study were stained using the streptavidin-biotin (ABC) method. The average percentage of cells positively stained for TGF-β protein was 40.2% in the group of extra-abdominal, 58.5% in the group of abdominal and 72.8% in the group of intra-abdominal localizations. There were significant differences observed between the analyzed groups of desmoid tumor ($p<0.05$). A positive cytoplasmic reaction for TGF-β was noted in 65.8% (25/38) of the aggressive fibromatoses. Overexpression of TGF-β protein was noted in 39.5% (15/38) of the aggressive fibromatoses. High expression noticed in desmoid fibroblasts might indicate that this protein plays a crucial role in the development of aggressive fibromatosis.

Introduction

Aggressive fibromatosis, also called a desmoid tumor, is a mesenchymal neoplasm and develops from muscle connective tissue, fasciae and aponeuroses. This neoplasm is composed of a clonal proliferation of spindle (fibrocyte-like) cells. Histologically it is composed of fibroblasts and myofibroblasts in a collagenous, often focally myxoid background [30]. During the proliferative phase of wound healing, mesenchymal (fibroblast-like) cells migrate into the healing wound, proliferate, and produce a disorganized matrix, providing the initial tensile strength [7]. Desmoid is a neoplasm rarely undergoing histological malignancy and lacks metastatic potential, but demonstrates the ability to local infiltration of tissues and is characterized by high risk of recurrence after surgical treatment [13, 19, 20, 24]. Aggressive fibromatosis may occur in extra-abdominal, abdominal and intra-abdominal locations [30]. Aggressive fibromatosis located in various parts of the body demonstrates differentiated biological behavior [20, 22].

The etiology of desmoid tumor is uncertain, however, trauma, endocrine and genetic factors have been considered causative factors (review Ferenc et al. [9]). This neoplasm occurs sporadically, it is also associated with familial adenomatous polyposis (FAP). Most of the sporadic cases of aggressive fibromatosis contain a somatic mutation in either the adenomatous polyposis coli (APC) or β-catenin genes [2, 7, 28, 29].

TGF-β is a multifunctional protein which affects many proteins, takes part in cell cycle regulation and, thanks to this it influences cell growth and differentiation. Protein products of TGF-β, same as *p16INK4A* and *Rb* genes, play crucial role in regulation of G1/S transition in the cell cycle [11]. TGF-β can stimulate the proliferation of mesenchymal

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cells, but it can also act as a growth-inhibitor factor for epithelial, lymphoid, hematopoietic, and endothelial cells. TGF-β plays an important role in the angiogenesis, the stimulation of extracellular matrix synthesis, including collagen type I, as well as in tissue repair and healing processes (review Stalińska and Ferenc [27]). Abnormalities in TGF-β expression are very common in many disease processes including neoplasms [7, 18, 21, 26, 27].

The aim of the study was to estimate immunohistochemically the expression of TGF-β protein in desmoid cells.

Material and Methods

Material

Paraffin embedded archival tissues of 38 cases of aggressive fibromatosis (desmoid) were studied: 23 abdominal, 11 extra-abdominal and 4 intra-abdominal. All the sections were independently examined by two experienced pathologists (A.K. and J.S.), using a conference microscope and were histopathologically classified, as recommended by Weiss and Goldblum [30].

Immunohistochemical screening

Representative paraffin blocks containing tumor material from each case were sectioned at 4 μm, fixed on silanized slides and dried overnight at 56.7°C. Antigen retrieval was performed with citrate buffer (0.01 M, pH 6.0) in a standard microwave unit. The sections for immunohistochemistry were stained using the streptavidin-biotin (ABC) method according to Hsu et al. [12]. Deparaffinized sections were treated with 3% hydrogen peroxide (H_2O_2) for 5 min to block endogenous peroxidase activity. Nonspecific antibody binding was reduced by incubation of the sections for 10 min with normal horse serum. The slides were incubated with a 1:30 dilution of the primary mouse monoclonal anti-TGF-β antibody (clone: NCL-TGFβ, Novocastra, UK). In the negative control reaction the primary antibody was omitted. The reaction products were demonstrated using the Novocastra Universal Detection Kit (NCL-RTU-D) from Novocastra. 3,3'-diaminobenzidine (DAB) was used as chromogen, and the sections were counterstained with Mayers hematoxylin, dehydrated and mounted. The paraffin-embedded sections from placenta were used as positive control for TGF-β. For each case the immunohistochemical reaction for CD34 (clone: NCL-L-END, Novocastra, UK) was performed.

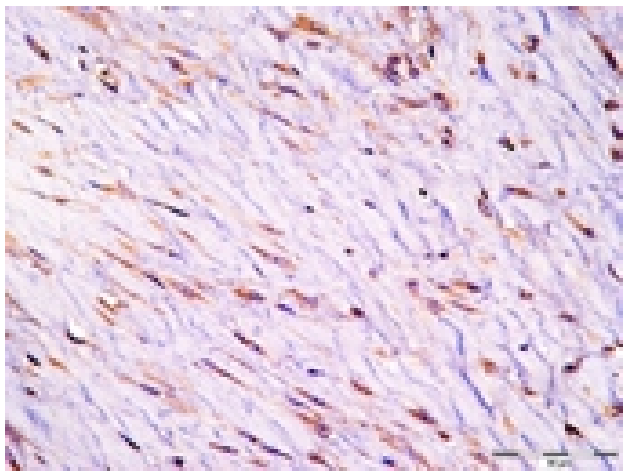

Fig. 1. Expression of TGF-β protein in aggressive fibromatosis. Lens magn. 200×.

The immunohistochemical staining of cells was estimated by means of the quantitative method using computer program IMAGEJ v. 1.34. The results were expressed as the percentage of positive cells per 250–900 aggressive fibromatosis cells, counted under magnification of objective lens (×40). The cases displaying cells with granular cytoplasmic staining with respect to TGF-β were considered as positive (Fig. 1). The relative number of immunoreactive cells was graded as follows: (–) – less than 10% of tumor cells stained positively; (+) – 10–50% of tumor cells stained positively; (++) >50% of tumor cells stained positively. Lesions scored as (++) were considered as showing high expression (overexpression) of the protein.

Statistical procedure

All the parameters represented as the mean percentages of positively stained cells were compared using Mann-Whitney test, where $p<0.05$ was considered significant.

Results

The average percentage of the cells positively stained for TGF-β protein was 40.2% (SD=11.0) in the group of extra-abdominal, 58.5% (SD=16.3) in the group of abdominal and 72.8% (SD=20.8) in the group of intra-abdominal localization (Table 1). The average values of the percentage of cells stained positively for TGF-β observed in the intra-abdominal

TABLE 1
The average per cent of TGF-β-positive cells in the study groups

Group (tumor localization)	N	M	SD
Abdominal	23	58.5	16.3
Extra-abdominal	11	40.2	11.0
Intra-abdominal	4	72.8	20.8

N – number of cases; M – arithmetic mean; SD – standard deviation

group were statistically higher than in the abdominal group ($p<0.05$) and in the extra-abdominal group ($p<0.05$). The average values of the percentage of cells stained positively for TGF-β observed in the abdominal group were statistically higher than in the extra-abdominal group ($p<0.05$).

A positive cytoplasmic reaction for TGF-β protein was noted in 65.8% (25/38) of the aggressive fibromatoses. Overexpression of TGF-β protein (more than 50% positively stained cells) was noted in 39.5% (15/38) of the aggressive fibromatoses (Table 2).

TABLE 2
The number of TGF-β-positive and negative cases in the study groups

Antibody/ antigen	N	++ n (%)	+ n (%)	– n (%)
TGF-β	38	15 (39.5)	10 (26.3)	13 (34.2)

(++) >50%; (+) 10–50%; (–) <10% of positive cells; N – number of cases studied; n (%) – number (per cent) of positive or negative cases

Discussion

Aggressive fibromatosis is a mesenchymal neoplasm, which despite benign nature and lack of metastatic potential demonstrates the tendency to local infiltration of tissues. This tumor develops from muscle connective tissue, fasciae and aponeuroses. On microscopy, fibroblasts are observed dispersed in extracellular matrix, the main component of which is collagen type I [30].

It has been postulated that growth factors e.g. transforming growth factor β (TGF-β), play an important role in the development of numerous neoplasms including aggressive fibromatosis [18, 25]. TGF-β is produced by nearly all types of cells in an organism, among others by blood platelets, macrophages and the resultant effect of its activity depends on the kind of tissue and the context this factor is active in. During the transmission of a signal induced by TGF-β through TβRI and TβRII receptors and Smad proteins, the type of the induced cellular response depends on which cofactor proteins Smad proteins would bind in a nucleus and on the expression of which genes they stimulate or inhibit [17, 23]. The effect of TGF-β on the development of a neoplasm may be either positive or negative and depends on the kind of cells creating the environment of the given type of a tumor or the context of the mutation which contributed to its formation. The TGF-β gene may act as the tumor suppressor because its protein product inhibits the division of numerous cells e.g. epithelial, endothelial, hematopoietic or, as protooncogene, because is necessary for the development of metastases [1]. In both these cases TGF-β-stimulated signal is transmitted through Smad proteins [21]. In general, it is assumed that in the early phase of the neoplasm development TGF-β acts as a suppressor, whereas in the stage of progression its activity is promoting [3]. Also alterations in TGF-β signaling can be the base for neoplasm development. These alterations most frequently concern the mutation of *TGF-β1* gene, the genes coding TβRI, TβRII (TGFBR1, TGFBR2) receptors or Smad proteins [3, 25, 27]. Furthermore, transduction of signal through TGF-β in fibroblasts is indicated to affect epithelial cells. Such interactions between stromal fibroblasts and epithelial cells in the environment of tumor tissue have a significant effect on its initiation and further progression [3].

TGF-β has also the ability to induce fibroblasts transformation into myofibroblasts, which demonstrate features characteristic both for fibroblasts and for smooth muscles, initiating among others the synthesis of α-actin, caldesmon or myosin heavy chains. These myofibroblasts take part in the formation of a foundation on the wound site in the course of healing process and collagen synthesized by them ensures strength and elasticity of this construction [8]. On the other hand, the formation of myofibroblasts often contributes to tumor progression although the process of fibroblasts transformation is not limited only to fibroblasts originating from the tumor but may also concern the cells in healthy tissues [3].

As it has already been mentioned, TGF-β despite inhibitory effect on epithelial and endothelial cell division is a stimulator of mesenchymal cells division [25, 27]. Moreover, TGF-β may facilitate the effect of other growth factors such as: EGF, PDGF or FGF [18] and also stimulates directly and indirectly the synthesis of extra-cellular matrix components including proteoglycans, glycosaminoglycans, collagen and fibronectin [5, 10, 15, 18]. TGF-β was found to be a stimulator of glycosaminoglycans synthesis in the desmoid fibroblast cells [16]. Furthermore, TGF-β inhibits the synthesis of proteases degrading extra-cellular matrix components and stimulates the production of their inhibitors [4, 6, 8].

Fibroblasts isolated from fibromatous lesions are characterized by intensified secretion of glycosaminoglycans, collagen and TGF-β as compared to normal fibroblasts [14]. In the carried out by us studies, positive cytoplasmic reaction for TGF-β protein was observed in 65.8% (25/38) of desmoid cases. Overexpression of TGF-β (more than 50% positively stained cells) was noted in 39.5% (15/38) of the aggressive fibromatoses. Then, mean values of the percentage of cells positive for TGF-β protein were highest in intra-abdominal form and were 72.8% (SD=20.8). Locci et

al. [16] observed 6-fold increase of TGF-β1 in desmoid fibroblast cultures as compared to normal fibroblasts. The increase of TGF-β level in aggressive fibromatosis cells in comparison to skin fibroblasts was also noted in immunohistochemical examinations of Mills et al. [18]. Elevated level of TGF-β is also characteristic for many other types of neoplasms in which the process of tissue fibrosis occurs [4].

Despite the fact that desmoid etiology is not fully recognized, an injury after surgical procedure associated with the disruption of tissues (about 25% of cases) is thought to be one of the causes of this neoplasm development [24]. Some desmoid tumors occur in the scar after appendectomy, laparotomy or other surgical procedures in the area of abdominal cavity (*cicatrical fibromatosis*) [30]. Especially in FAP patients, there is a strong correlation between prophylactic proctocolectomy and the subsequent development of desmoid tumors [24]. According to Weiss and Goldblum [30], the injury of tissue in patients with genetic predisposition to fibrous tissue overgrowth is an important cause of the development of intra-abdominal fibromatosis (fibromatosis of pelvis, mesentery in Gardner's syndrome).

In the case of surgical disruption of the tissue line, it is natural that the organism initiates the repair process, which is associated with the secretion of numerous cytokines. TGF-β indispensable for the initiation and finalizing of this process plays a key role in the repair of the damaged tissues. However, further TGF-β production by autoinduction after the end of the repair process of the damaged tissues may result in the initiation of a cascade of molecular events leading to the development of diseases including neoplastic diseases in which a process of normal or pathological fibrosis is the basic occurrence.

The noted in our studies highly positive immunohistochemical reaction for TGF-β in desmoid cells may point to the share of TGF-β in the development of this neoplasm.

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References

1. *Akhurst RJ, Derynck R:* TGF-beta signaling in cancer – a double-edged sword. Trends Cell Biol 2001, 11, S44–51.
2. *Bhattacharya B, Dilworth HP, Iacobuzio-Donahue C et al:* Nuclear beta-catenin expression distinguishes deep fibromatosis from other benign and malignant fibroblastic and myofibroblastic lesions. Am J Surg Pathol 2005, 29, 653–659.
3. *Bierie B, Moses HL:* TGF-beta and cancer. Cytokine Growth Factor Rev 2005, [Epub ahead of print].
4. *Border WA, Ruoslahti E:* Transforming growth factor-beta in disease: the dark side of tissue repair. J Clin Invest 1992, 90, 1–7.
5. *Border WA, Noble NA:* Transforming growth factor β in tissue fibrosis. N Engl J Med 1994, 331, 1286–1292.
6. *Border WA, Noble NA:* Fibrosis linked to TGF-beta in yet another disease. J Clin Invest 1995, 96, 655–656.
7. *Cheon SS, Cheah AY, Turley S et al:* beta-Catenin stabilization dysregulates mesenchymal cell proliferation, motility, and invasiveness and causes aggressive fibromatosis and hyperplastic cutaneous wounds. Proc Natl Acad Sci USA 2002, 99, 6973–6978.
8. *Dominguez-Malagon H:* Intracellular collagen and fibronexus in fibromatosis and other fibroblastic tumors. Ultrastruct Pathol 2004, 28, 67–73.
9. *Ferenc T, Sygut J, Kopczyński J et al:* Aggressive fibromatosis (desmoid tumors): definition, occurrence, pathology, diagnostic problems, clinical behavior, genetic background. Pol J Pathol 2006, 57, 5–15.
10. *Gerdin B, Hallgren R:* Dynamic role of hyaluronan (HYA) in connective tissue activation and inflammation. J Intern Med 1997, 242, 49–55.
11. *Hirama T, Koeffler HP:* Role of the cyclin-dependent kinase inhibitors in the development of cancer. Blood 1995, 86, 841–854.
12. *Hsu SM, Raine L, Fanger H:* Use of avidin-biotin-peroxidase complex (ABC) in immunoperoxidase techniques: a comparison between ABC and unlabeled antibody (PAP) procedures. J Histochem Cytochem 1981, 29, 577–580.
13. *Lewis JJ, Boland PJ, Leung DHY et al:* The enigma of desmoid tumors. Ann Surg 1999, 299, 866–873.
14. *Lilli C, Marinucci L, Bellocchio S et al:* Effects of transforming growth factor-beta1 and tumor necrosis factor-alpha on cultured fibroblasts from skin fibroma as modulated by toremifene. Int J Cancer 2002, 98, 824–832.
15. *Locci P, Baroni T, Lilli C et al:* TGF beta and TGF alpha, antagonistic effect in vitro on extracellular matrix accumulation by chick skin fibroblasts at two distinct embryonic stages. Int J Dev Biol 1999, 43, 157–165.
16. *Locci P, Bellocchio S, Lilli C et al:* Synthesis and secretion of transforming growth factor-beta1 by human desmoid fibroblast cell line and its modulation by toremifene. J Interferon Cytokine Res 2001, 21, 961–970.
17. *Massague J, Blain SW, Lo RS:* TGF beta signaling in growth control, cancer, and heritable disorders. Cell 2000, 103, 295–309.
18. *Mills BG, Frausto A, Brien E:* Cytokines associated with the pathophysiology of aggressive fibromatosis. J Orthop Res 2000, 18, 655–662.
19. *Nuyttens JJ, Rust PF, Thomas CR, Turrisi AT:* Surgery versus radiation therapy for patients with aggressive fibromatosis or desmoid tumors: A comparative review of 22 articles. Cancer 2000, 88, 1517–1523.
20. *Peterschulte G, Lickfeld T, Moslein G:* Das desmoid problem. Chirurg 2000, 71, 894–903.
21. *Roberts AB, Wakefirld LM:* The two faces of transforming growth factor β in carcinogenesis. Proc Natl Acad Sci USA 2003, 100, 8621–8623.

22. *Schlemmer M:* Desmoid tumors and deep fibromatoses. Hematol Oncol N Am 2005, 19, 565–571.
23. *Shi Y, Massague J:* Mechanism of TGF-β signaling from cell membrane to the nucleus. Cell 2003, 113, 685–700.
24. *Shields CJ, Winter DC, Kirwan WO, Redmond HP:* Desmoid tumors. Eur J Surg Pathol 2001, 27, 701–706.
25. *Siegel PM, Massague J:* Cytostatic and apoptotic actions of TGF-β in homeostasis and cancer. Nat Rev Cancer 2003, 3, 807–821.
26. *Singer AJ, Clark RA:* Cutaneous wound healing. N Engl J Med 1999, 341, 738–746.
27. *Stalińska L, Ferenc T:* The role of TGF-β and cell cycle regulation. Postępy Hig Med Dośw 2005, 59, 441–449. [article in Polish]
28. *Sturt NJ, Gallagher MC, Bassett P et al:* Evidence for genetic predisposition to desmoid tumors in familial adenomatous polyposis independent of the germline APC mutation. Gut 2004, 53, 1832–1836.
29. *Tejpar S, Nolllet F, Li C et al:* Predominance of beta-catenin mutations and beta-catenin dysregulation in sporadic aggressive fibromatosis (desmoid tumor). Oncogene 1999, 18, 6615–6620.
30. *Weiss SH, Goldblum JR:* Enzinger and Weiss's Soft Tissue Tumors (fourth edition). St. Luis: Mosby 2001, 309–346.

Address for correspondence and reprint requests to:
Prof. Andrzej Kulig, M.D., Ph.D.
Institute Polish Mother's Health Centre
Rzgowska 281/289
93-338 Łódź