공격성 섬유종증에서 PTEN 종양 억제 인자의 발현

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공격성 섬유종증은 비교적 드문 연부 조직 종양이지만, 국소 증식 및 침윤성이 강한 종양으로 알려져 있다. 그렇지만 종양 증식에 관한 정확한 기전은 아직 보고되어 지지 않고 있다. 한편, 종양 억제 유전자 PTEN은 국소 침윤성과 전이를 보이는 여러 암 조직에서 발현의 감소 또는 돌연 변이가 보고되고 있다. 본 연구에서는 공격성 섬유종증에서 면역조직화학적 염색과 면역탁본법을 통해 종양 억제 유전자 PTEN의 발현의 양상을 알아보고자 하였다. 면역 탁본법과 면역조직화학적 염색상 PTEN의 발현은 정상 근막 조직에서는 균일하게 발현되었으나, 공격성 섬유종증 종양조직에서는 발현이 감소하였다. 공격성 섬유종증에서 PTEN발현의 감소는 급속한 증식과 재발 및 주위 조직의 침윤이 특징인 공격성 섬유종증의 병인에 관련이 있을 것으로 사료된다.

색인 단어: 공격성 섬유종증, PTEN, 침윤성

Introduction

Aggressive fibromatosis (AF) is a rare and slow-growing soft tissue tumor which arises from fascial sheaths and musculoaponeurotic structures[1]. While histologically benign in appearance, AF is locally invasive and tends to extend along fascial planes, infiltrate the surrounding muscles, and engulf blood vessels and nerves. Since AF can also involve the periosteum and may lead to bone erosion, thereby closely resembling desmoplastic fibroma of bone, as well as having a high incidence of local recurrence after attempted surgical excision , some authors have classified as a low-grade fibrosarcoma[6,13].

Although the etiology of AF remains unclear, like other fibromatosis, it is probably multifactorial, as genetic, endocrine, and physical factors seem to play an important role in its pathogenesis[17]. Until now, the understanding of the pathophysiology of AF is still unsatisfactory. Microscopic findings of AF depend on the age of the lesion and characterized by a strikingly cellular proliferation of plump, immature-appearing spindle-shaped fibroblasts that form one or

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more nodules. And, AF has locally invasive nature and destructive behavior is similar to that of low-grade fibrosarcomas. Therefore, as in the case of malignant tumors in other tissues, dysfunction of oncogenes and/or tumor suppressor genes might also be involved in the pathogenesis of AF.

PTEN/MMAC1/TEP1 is a tumor suppressor gene on chromosome 10q23 and is a lipid phosphatase that dephosphorylates the D3 position of phosphatidylinositol 3,4,5 triphosphate (PIP3), a downstream of phosphatidylinositol 3-kinase/Akt[10]. The gene product is a part of a complex signaling system that affects a variety important cell biologic functions[18] and has major roles not only suppressing cancer but also in embryonic development, cell migration and apoptosis[9]. It is well known that mutations in both alleles of the PTEN gene were shown in a remarkable variety of cancers, including brain, prostate, breast, endometrial cancers and melanoma[15]. Also, a lack of PTEN expression was found at the synovial lining and cultured fibroblast-like synoviocytes from the synovial tissues in rheumatoid arthritis, and suggested as a contributors to maintain aggressive phenotype at the sites of cartilage destruction[12]. Like these, mutations or reduction of PTEN seems to be associated with their invasive properties.

Although mutations or other changes of the PTEN gene have not been reported yet, we hypothesized that there are some changes of PTEN gene expression in AF. In the present study, we examined the pattern of the expression of PTEN in the tissues by immunohistochemical and immunoblotting analysis, and obtained the findings that down-regulation of PTEN in AF compared to the tissues of normal fascial and aponeurotic tissues.. Consequently, this paper provides the first evidence that the down-regulation of PTEN occur in AF.

Material and methods

1. Patients and collection of specimen

Two patients with aggressive fibromatosis were included. This study protocol was performed with the informed consent from the patients and control subjects and the review and approval from our institutional Clinical Research Committee. They were diagnosed as on the basis of clinical, radiological findings and needle biopsy under the guidance of ultrasonography. Tissue samples were obtained under sterile conditions from above mentioned two patients with AF who underwent resection of tumor. Samples from tumor tissue (5 mm approximately) were shock frozen in liquid nitrogen and stored at -80℃. In parallel, fractions of the samples were formalin fixed and embedded in paraffin. For control specimens, normal musculoaponeurotic tissues were selected from healthy donor.

2. Immunohistochemistry for PTEN expression

Deparaffinized and rehydrated 3~5 μm sections were incubated in 10 mM citrate buffer pH 6 containing proteinase K (DAKO, Hamburg, Germany) for 10 min. Sections were incubated in 3% H_2O_2 for 10 min to quench endogenous peroxidase activity and blocked with 1.5% normal serum for 20 min. Rabbit anti-PTEN antibodies (Zymed, San Francisco, CA) were incubated in a dilution of 1:50 at room temperature for 1 hr. The secondary biotinylated anti-rabbit antibody, dilution 1:200 (Vector

Laboratories, Burlingame, CA) was applied for 30 min at room temperature. Immunohistological staining was performed with a commercially available kit (Vecta stain, Vector Laboratories) including a streptavidine-biotin-peroxidase amplification system and diaminobenzidine as a substrate. The sections were counterstained by hematoxylin. Normal musculoaponeurotic tissue was used as a positive control. The immunoreactivity was regarded as positive when brown staining was localized in the tumor cell nuclei or cytoplasm.

3. Immunoblotting for PTEN expression

Biopsy specimens were frozen in liquid nitrogen and then kept at -70℃ until homogenized. Specimens were homogenized on ice in a homogenization buffer [20 mM Tris (pH 7.4), 1% Triton X-100, 150 mM NaCl, 5 mM EDTA, 1 mmole/L PMSF, 2 μg/mL leupeptin, and 2 μg/mL aprotinin]. After incubation for 30 min on ice, cellular debris was removed by centrifugation at 100,000 g for 60 min, and supernatants were analyzed by SDS-PAGE (sodium dodecyl sulfate-polyacrylamide gel electrophoresis). Protein concentration was measured according to Bradford's method. SDS-PAGE was performed in 10% slab gel according to Laemmli[4]. Equal amounts of the protein extracts (15 μg per lane) were separated by SDS-PAGE (sodium dodecyl sulfate-polyacrylamide gel electrophoresis) of 10% polyacrylamide and transferred by semi-dry blotting onto polyvinylidene fluoride membranes (PVDF, Immobilon P, Millipore, Bedford, MA) for Western blotting. The membrane was blocked in 0.1% Triton X-100, 5% low fat milk powder in PBS for 1 hr. The primary polyclonal goat antibody against PTEN (Santa Cruz Biotechnology, Santa Cruz, CA) was incubated overnight at 4℃ in a dilution of 1:500 in 0.1% Triton, 1% low fat milk powder and 0.1% BSA (bovine serum albumin) in PBS. After washing, the membrane was incubated with horseradish peroxidase-conjugated secondary anti-goat-antibodies (Santa Cruz Biotechnology), diluted 1:2,000, for 1 hr at room temperature. The bound antibodies were visualized by an enhanced chemiluminescence detection system using Fuji medical X-ray films.

Results

1. Histologic and immunohistochemical findings

In Hematoxylin-Eosin staining for tissues of AF showed proliferation of elongated, slender, spindle-shaped cells of uniform appearance surrounded and separated from one another by abundant collagen with lack hyperchromatia and atypia (Fig. 1). To analyze the distribution of PTEN in a tissue of AF as well as in corresponding normal musculoaponeurotic tissue, paraffin-embedded tissue sections were immunohistochemically stained for PTEN expression by using PTEN-specific monoclonal antibody. In normal musculoaponeurotic tissue, PTEN was almost expressed in the fibroaponeurotic area, and scanty expressed in muscle cell. In contrast, PTEN expression was either absent or very faint in tumor tissue (Fig. 2).

2. Western Blotting for PTEN

To quantify the expression of PTEN in AF, protein extracts from tumor tissue and corresponding normal musculoaponeurotic tissue were analyzed by Western blot. In

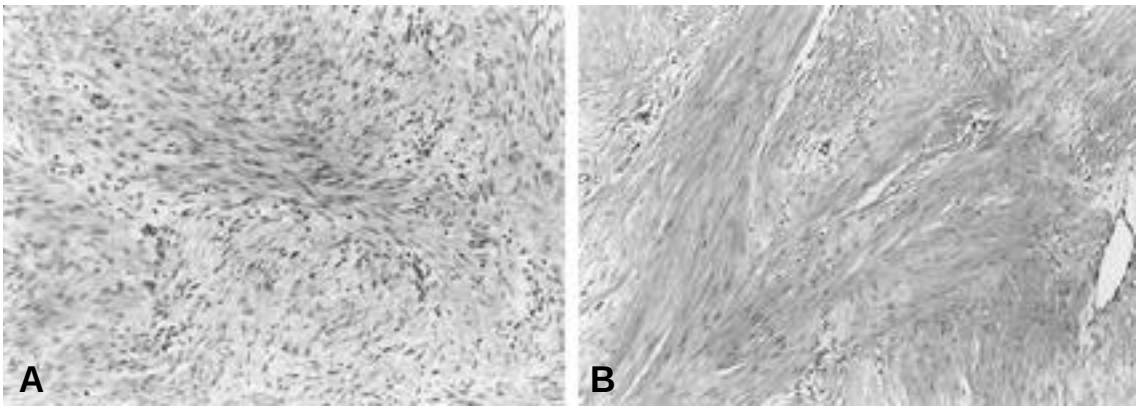

Fig. 1. Hematoxylin-Eosin staining of tissues of aggressive fibromatosis. The proliferation consists of elongated, slender, spindle-shaped cells of uniform appearance surrounded and separated from one another by abundant collagen with lack hyperchromatia and atypia in patient 1 (**A**) and patient 2 (**B**).

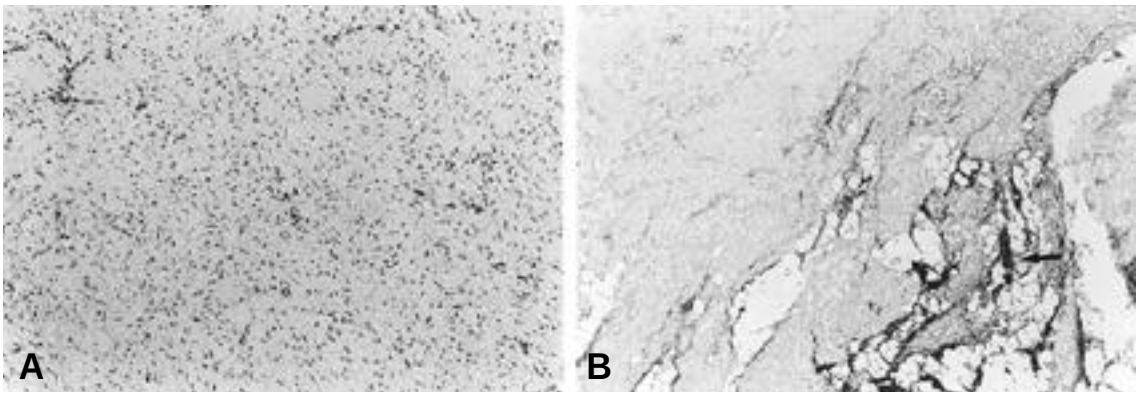

Fig. 2. PTEN expression by immunohistochemical staining using specific monoclonal antibody against PTEN. Pannel **A** (patient 1) and **B** (patient 2) show PTEN-immunohistochemical stain in the normal musculoaponeurotic tissues (black arrow) and aggressive fibromatosis tissue respectively.

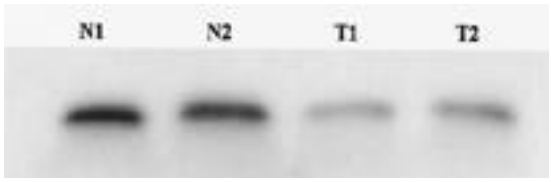

Fig. 3. Status of PTEN expression in aggressive fibromatosis. Western blot analysis of equal amounts of protein from musculoaponeurotic tissue lysates of normal and aggressive fibromatosis was probed with an antibody to PTEN.(N1, N2; normal musculoaponerotic tissue, T1,T2; tumor tissue)

AF, PTEN expression was markedly reduced compared to normal tissue as evidenced by Western blot analysis (Fig. 3). These findings strongly suggest that high proliferating capacity of AF is associated with reduction of PTEN expression.

Discussion

In this study, we found that PTEN expression in AF was markedly reduced compared with normal musculoaponeurotic tissues. These results suggest that lack of the expression of PTEN might be involved in the growth of the AF, and associated with phenotype of AF that have been shown to invade surrounding muscles and adjacent

bone. Moreover, the present findings may have some similar findings with rheumatoid arthritis because of fibroblasts are involved in the pathogenesis of both diseases. So, it also has an impact on further investigations of other tumor suppressors such as p53 and oncogenes including, myc, ras, fos and erg-1, which is demonstrated rheumatoid arthritis[11].

Transforming growth factor-β1 (TGF-β1) is expressed abundantly in the fibroblast isolated from human desmoid tumor and involved in the pathogenesis of this disease [24] as in the Dupuytren's contracture[3]. It has been reported that TGF-β1 downregulate gene transcription of PTEN[7]. Therefore, it could be hypothesized that TGF-β1 might be involved in the downregulation of PTEN.

Over the last two decades, endocrine aspects of AF have attracted attention. Most of AF occurs in women in their childbearing years, often during or shortly after pregnancy, and animal models have demonstrated that development of fibrous tumors after prolonged estrogen administration and subsequent tumor regression after prolonged progesterone treatment[5]. Overall, there is mounting evidence that AF cells proliferate under estrogen influence. The presence of estrogen receptors in the tumor tissue was detected in 33~50% and microsomal antiestrogen binding sites were detected in 79% of AF[8,14]. However, the molecular mechanism by which estrogen stimulate the growth of fibroblasts to form tumors is still unknown. Some authors speculate that estrogens may activate the transcription of genes involved in tumor growth[2,16]. Although the mechanism of antiestrogen in the inhibition of fibroblasts in desmoid tumor was proven to be not associated with estrogen receptor, further studies are need about the correlation between PTEN and estrogen receptor and antiestrogen binding sites.

Surgery with wide local excision is the mainstay of therapy and local recurrence is one of the major problems. The major predictor of local recurrence is the extent of complete operative excision of tumor, but histologic findings are not associated to it. So, more comprehensive analysis in a large study group about the association between the pattern of PTEN and clinical course, response to several therapeutic agents is warranted. And, this paper consequently provides the first evidence that the downregulation of PTEN occur in AF. It also should be documented that the relation between the lack of PTEN expression and the invasive phenotype of fibroblasts of AF is really significant or only phenomenological.

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Abstract

Expression of PTEN, Tumor Suppressor Protein, in Aggressive Fibromastosis

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Aggressive fibromatosis is a rare soft tissue tumor with locally invasive and infiltrative characteristics. The mechanism of this invasive nature was not reported until now. Mutations or reduction of PTEN, tumor suppressor gene, in cancer tissues, have been found to be associated with invasiveness and metastatic properties of cancer cells. To know the pattern of expression of PTEN in aggressive fibromatosis, we analysed the expression of PTEN with immunohistochemical stain and immunoblotting. PTEN was homogeneously expressed in the normal musculoaponeurotic tissues, but absent or very faint in tissues of patients with aggressive fibromatosis as evidenced by western blot analysis and immunohistochemical examinations. Although the meaning of decreased PTEN expression in aggressive fibromatosis is not certain, it might be involved in the growth of the aggressive fibromatosis, and associated with phenotype of aggressive fibromatosis.

Key Words: Aggressive fibromaosis, PTEN, Invasiveness

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