Absence of Expression of c-*sis* and Transforming Growth Factor-β mRNA in Malignant Fibrous Histiocytoma

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Total RNA was extracted from five malignant fibrous histiocytomas and two benign fibrohistiocytic lesions and assayed for mRNA expressions for transforming growth factor beta (TGF-β) and c-*sis* by Northern blot analysis. Production of both of these has been associated with cells of monocyte-macrophage lineage, and these factors have been shown to be important in physiologic mesenchymal cell proliferation. No mRNA expression of either TGF-β or c-*sis* was identified in any of the fibrohistiocytic tumor samples. The lack of expression of TGF-β and c-*sis* may be consistent with a nonhistiocytic origin of malignant fibrous histiocytoma, or may reflect transformation-associated loss of the normal molecular mechanisms of mesenchymal proliferation. The absence of c-*sis* mRNA expression can be reconciled with the prior immunohistochemical demonstration of platelet-derived growth factor in tumor cells of malignant fibrous histiocytoma. *Int J Surg Pathol(2):117–122, 1993*

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Transforming growth factor beta (TGF-β) is a highly conserved family of peptides[1] that is constitutively expressed in both resting and activated monocytes and is secreted by the latter.[2,3] Although the effects of TGF-β are quite heterogeneous,[4] TGF-β is chemotactic for both fibroblasts and macrophages,[5] and TGF-β production by monocytes is thought to play a role in wound healing by stimulating angiogenesis and connective tissue elaboration.[2,3,6,7] One of the effects of TGF-β is autocrine stimulation of production of c-*sis* by macrophages as well as by fibroblasts.[3] c-*sis* encodes a precursor of the B chain of platelet-derived growth factor (PDGF),[8] which is a powerful mitogen of mesenchymal cells.[9] In fact, the mitogenicity of TGF-β is believed to be mediated by the induction of c-*sis* and PDGF.[4] Activated human peripheral blood monocytes,[10] monocytes matured *in vitro*,[11] and activated alveolar and peritoneal macrophages[12] have all been shown to express c-*sis* mRNA. Thus, TGF-β and c-*sis* mRNA are expressed by non-neoplastic cells of the monocyte-phagocyte system and appear to play an important role in mesenchymal proliferation.[6] Since the cellular components and histologic appearance of malignant fibrous histiocytoma (MFH) overlap with those of the inflammatory response, we have investigated whether the molecular mechanisms of proliferation in MFH are analogous to wound repair and thus involve expression of TGF-β and c-*sis*. We have analyzed samples of fresh-frozen tissue from five cases of MFH and two cases of benign fibrohistiocytic tumors for mRNA expression of TGF-β and c-*sis*.

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Table 1. Clinical Information

Case No.	Diagnosis	Sex	Age (years)	Site	Diameter
1	MFH	F	71	Thigh	8 cm
2	MFH	M	29	Thigh	13.5 cm
3	MFH	M	67	Tibia	Not available
4	MFH	M	88	Buttock	Not available
5	MFH	M	65	Chest	4 cm
6	Fibrous histiocytoma (benign)	F	57	Cheek	5.5 cm
7	Atypical fibrohistiocytic lesion (benign)	F	39	Shoulder	3.2 cm

MFH, malignant histiocytoma.

Materials and Methods

Materials

Frozen tissue was available from 11 fibrohistiocytic lesions; intact mRNA was obtained from 7 of these. Five of these seven were MFHs, one was a benign fibrous histiocytoma, and one was diagnosed as an "atypical fibrohistiocytic lesion." The five MFHs, designated samples 1–5 (Table 1, Fig. 1), were from four men and one woman whose ages ranged from 29 to 88 years (average, 64 years) and tumor size (where known) ranged from 4 to 13.5 cm. All of the MFHs were high grade. Four cases were of storiform-pleomorphic type, and one was a giant cell variant (case 2). The benign fibrous histiocytoma (case 6) was from a 57-year-old woman and measured 5.5 cm, and the atypical fibrohistiocytic lesion (case 7) was from the shoulder of a 39-year-old woman and measured 3.2 cm. The clinical data are summarized in Table 1.

RNA Isolation and Northern Blot Hybridization Analysis

Tissue was obtained at surgery and snap-frozen in liquid nitrogen and stored at $-70°C$ until used. From each frozen sample approximately 0.5 cm^3 of tissue was available for analysis. Total RNA was isolated from each sample using acid guanidinium thiocyanate-phenol-chloroform extraction.[13] The amount of RNA extracted ranged from 17 to 210 μg (average yield, 77 μg). Twenty micrograms of each RNA sample were used for Northern blotting (except for case 7 from which only 16 μg were available). Also included was a positive control consisting of 20 μg of total cellular RNA from cultured human pulmonary endothelial cells that express c-*sis*[14] and TGF-β (Kern J., verbal communication, May 1989). In addition, 1 μg of each cDNA from which the TGF-β and c-*sis* probes had been prepared were loaded onto the gel as "hybridization controls." A negative control consisted of 20 μg of cellular RNA from unstimulated cultured Jurkat T lymphocytes. The samples were electrophoresed through a glyoxyl-agarose gel[15] and photographed to determine position of the 28S and 18S bands, as well as the RNA size marker, and to assess the integrity of the extracted cellular RNA. The RNA was transferred to Gene Screen Plus nylon membranes (New England Nuclear, Boston, MA) following published protocols[16] and hybridized overnight to 5×10^6 counts per minute of ^{32}P-labeled cDNA probes at 42°C in a prewarmed, fresh hybridization buffer. ^{32}P-labeled probes were prepared by random priming[17] of the following cloned DNA fragments: human c-*sis*[18] in pBR322 (obtained from the Cell Center at the University of Pennsylvania), human TGF-β1[19] (plasmid sp64, gift of R. Derynck), and human β_2-microglobulin[20] to check for equal loading of mRNA. After hybridization, filters were washed and exposed to Kodak XAR-2 film (Rochester, NY) at $-70°C$ with two intensifying screens for 3–15 days. Between hybridizations to different probes, filters were stripped with boiling water and reexposed to film for several days to ascertain complete removal of the original probe. Hybridization for the human β_2-microglobulin probe was performed last.

Results

Intact RNA was obtained from 7 of 11 patients from whom tissue was available, as evidenced by direct visualization of 28S and 18S bands under ultraviolet fluorescence, as well as by the presence of a band for β_2-microglobulin at the expected size of 1.0 kilobase (kb)[20] (Fig. 1, bottom). Hybridization for β_2-microglobulin demonstrated comparable amounts of mRNA in each sample lane except for case 7 ("atypical fibrohistiocytic lesion"), from which only 16 μg were available. As illustrated in Figure 1 (top), a 4.2 kb c-*sis* mRNA transcript[21] was detected in the positive control cells as expected, but not in the RNA extracted from the MFH samples or the benign fibrohistiocytic tumors. Most of the pa-

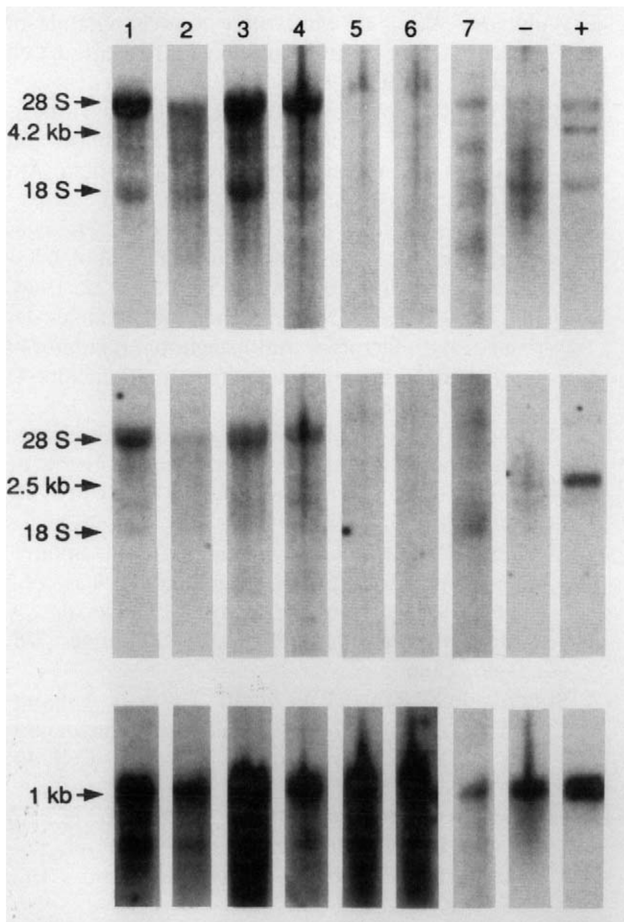

Fig. 1. Northern blot for transforming growth factor-β and c-*sis* expression in fibrohistiocytic neoplasms. Hybridization is seen to c-*sis* at 4.2 kb (top) and transforming growth factor-β at 2.5 kb (middle) in the positive control only (+); the tumor samples do not demonstrate specific binding to the probe. Hybridization for β_2-microglobulin (bottom) was performed to ensure comparable loading of the tumor samples (note the lesser amount in sample 7). Exposure times were 15 days for transforming growth factor-β, five days for c-*sis*, and 7 days for β_2-microglobulin. Lanes 1–5 consist of 20 μg of total cellular RNA from malignant fibrous histiocytoma tumor tissue (cases 1–5; Table 1). Lane 6 contains 20 μg of RNA from a fibrous histiocytoma, and lane 7 contains 16 μg of RNA from an atypical fibrohistiocytic lesion. Twenty micrograms of total cellular RNA from cultured human pulmonary endothelial cells and cultured Jurkat T cells served as positive (+) and negative (−) controls, respectively.

tient samples hybridized to RNA species at approximately 5 and 2 kb, which was interpreted as nonspecific hybridization of the probe to 28S and 18S rRNA due to the relatively nonstringent hybridization conditions and the large amount of total RNA used for each sample. A faint band running between 3.0 and 4.0 kb in the atypical fibrohistiocytic lesion (case 7) was similarly interpreted as nonspecific binding. None of the patient samples contained detectable TGF-β mRNA at 2.5 kb[19] (Fig. 1, middle). A band hybridizing to the probe at approximately 5 kb in these lanes was interpreted as cross-hybridization to rRNA as above. Although we considered the possibility that this band might represent hybridization to a 5.1 kb TGF-β2 transcript,[22] the absence of the other TGF-β2 bands at 4.1 and 6.5 kb, and the presence of the same size band cross-hybridizing with the c-*sis* probe indicated that this 5 kb band was nonspecific.

Discussion

Although TGF-β[5,23] and c-*sis*[24,25] mRNA expression have been previously reported in human mesenchymal tumors, to our knowledge no one has studied their expression in resected MFHs. Since TGF-β and c-*sis* are clearly established as mediators of physiologic cellular proliferation,[3,6] their absence in our MFH samples implies that the normal molecular mechanisms that mediate mesenchymal cell growth are bypassed in MFH.

These findings may have implications regarding the cell of origin of this neoplasm. Malignant fibrous histiocytoma was originally thought to be a true histiocytic neoplasm derived from cells of monocytic cell lineage.[26] Since TGF-β and c-*sis* expression are closely associated with such cells,[6,12] the absence of their expression in MFH may reflect a nonhistiocytic lineage for this neoplasm. This interpretation is consistent with other studies using a variety of methods, such as electron microscopy,[27] cell culture,[28] and immunohistochemistry,[29–33] that do not support histiocytic derivation of MFH.[27,34–38] However, the absence of TGF-β and c-*sis* expression in MFH may simply reflect transformation-associated alterations in gene expression rather than characteristics of the premalignant cell of origin. The true significance of our findings with regard to the histogenesis of MFH would be further supported by a comparative analysis of mRNA expression in proliferating fibroblasts or fibrosarcoma.

One of the authors of this study earlier reported the immunohistochemical identification of PDGF in MFH.[39] Since c-*sis* encodes a precursor of the B chain of PDGF, the data presented here might seem to contradict these earlier observations. One possibility is that the presence of PDGF by immunohistochemistry is an artifact due to the endocytotic[33,40,41] properties of the MFH cell. However, the presence of PDGF in the absence of c-*sis* is not necessarily a contradiction. The antibodies used in the study cited above were polyclonal to PDGF and are, therefore, likely against

both A and B chains. c-*sis* encodes only the B chain of PDGF,[8] and, in fact, the relationship between c-*sis* mRNA expression and PDGF secretion is not as straightforward as was originally thought.[42] For example, it is increasingly recognized that A-chain homodimers of PDGF-A have biologic activity.[43] In fact, there is evidence in a number of tumor cell lines,[44] as well as in human fibroblasts,[45] that expression of the PDGF A chain is independent of that of the PDGF B chain. We believe that the immunohistochemical identification of PDGF expression by polyclonal antibodies may reflect the presence of A-chain unaccompanied by B-chain (c-*sis*) mRNA expression. However, Franklin et al.[46] and Palman et al.[47,48] recently reported the immunohistochemical identification of the β-subunit of PDGF receptor, which specifically binds the B chain of PDGF, in several cases of MFH. According to the autocrine hypothesis of soft tissue tumor growth,[39] the presence of PDGF B-chain receptors in MFH might imply that the tumor cells also produce the PDGF B chain. Further analysis with newer monoclonal antibodies specific for the PDGF B chain, as well as analysis for PDGF A chain and PDGF receptor α-subunit expression, will be necessary to fully elucidate the role of PDGF in MFH.

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