Expression Pattern of Smad Proteins in Diffuse Large B–cell Lymphomas

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Background : Smad proteins mediate the cellular signaling from members of transforming growth factor-β (TGF-β) family (TGF-βs). Smads 2 and 3 transmit signals from TGF-β and activin, and Smads 1, 5, and 8 transmit signals from the bone morphogenetic protein. Smad4 is known to be a common mediator of both pathways, yet little is known about the expression pattern of Smad proteins in normal lymphoid tissue and malignant lymphoma. Methods : Immunohistochemistry was performed for Smad3 and Smad4 on the paraffin-embedded tissue sections from 32 cases of diffuse large B-cell lymphomas. Results : In reactive lymphoid tissue, nearly all cells of the germinal centers were positive for Smad3 and more than 50% of paracortical cells were positive for Smad3. For Smad4 immunostaining, nearly all cells of the germinal centers showed diffuse cytoplasmic staining, and most of them revealed nuclear positivity as well. Most of the cells in the paracortex regions were positive for Smad4. For the malignant lymphomas, all the cases were positive for Smad3, but 26 cases were positive for Smad4 and 6 cases (19%) were negative for Smad4. Conclusions : These results suggest that TGF-β-specific Smads may be actively involved for signal transduction in lymphoid organs, and the TGF-β signaling pathway through Smads is operative in malignant lymphoma. The loss of Smad4 expression might be associated with development of some diffuse large B-cell lymphomas.

Key Words : Smad-B lymphocyte-Lymphoma-Pathogenesis

The transforming growth factor-β (TGF-β) family is a multifunctional group of cytokines that includes three mammalian TGF-βs, activins/inhibins and a number of bone morphogenetic proteins (BMPs), and these proteins can regulate a number of processes such as cell growth, terminal differentiation, apoptosis, immune responses and the expression of the extracellular matrix through the transcriptional regulation of a diverse members of gene targets.1,3

The TGF-β superfamily members signal through a family of transmembrane receptors with intrinsic cytoplasmic serine/threonine kinase activity. Ligand binding to type II receptor (TGF-β RI) results in the recruitment and transphosphorylation of type I receptors (TGF-βRI) that then signal downstream responses and induce carboxy-terminal serine phosphorylation of a set of cytoplasmic signal-transducing proteins collectively referred to as the Smad proteins.1,4 Receptor-activated Smads interact transiently with specific, ligand activated TGF-βRI, and they are then phosphorylated. Pathway-specific Smads consist of Smads 1, 5 and 8 that mediate BMP signaling, and Smads 2 and 3 mediate TGF-β and activin signaling. A phosphorylated pathway-specific Smad heterodimerizes with Smad4, and this complex then translocates to the nucleus to transactivate specific target genes. Smad4 is functionally unique among the Smads, and it is not regulated by phosphorylation; rather, it acts as a common mediator of all of the pathway-specific Smads and then it acts as a common mediator of TGF-β, activin and the BMP signaling responses. Inhibitory Smads disrupt signal transduction by preventing phosphorylation of pathway-specific Smads. Smad6 appears to inhibit BMP signaling, while Smad7 is more involved in inhibiting TGF-β dependent signaling. In contrast to the pathway-specific Smads, the inhibitory Smads are primarily localized at the nucleus in the absence of ligand, but they accumulate in the cytoplasm upon receptor activation.3,4

TGF-β is a potent growth inhibitor of most cells,5 and cellular insensitivity to growth inhibition by TGF-β is a hallmark in the genesis and progression of human cancer; this can be directly linked to inactivating mutations in or the loss of expression of various signaling molecules whose activities are regulated by TGF-β. TGF-β and its intracellular signaling proteins are regarded as widely established tumor suppressors.6 Mutation in or loss of the gene for TGF-β receptor expression has been found in cutaneous and noncutaneous T-cell lymphoma, B-cell lymphoma and Hodgkin’s lymphoma.5,7,8 Thus, the loss of receptors for TGF-β in lymphocytes has removed the immunosup-
pressive properties of TGF-β and so enhances cell proliferation, and this may be an important step in the development of malignant lymphoma.

The Smads for TGF-β also appear to function as tumor suppressors. For instance, mutation that inactivates Smad2 has been identified in human colorectal and lung cancers, while those mutations leading to the inactivation or loss of expression of Smad4 have been found in human pancreatic, breast, colorectal, lung, ovarian, and head and neck cancers. Moreover, the gene for Smad3 functions as a tumor suppressors in mice, so that the targeted disruption of Smad3 leads to the formation of colorectal adenocarcinomas that are capable of penetrating the intestinal wall and metastasizing to distant locations; this suggests that the gene for Smad3, like those for Smad2 and Smad4, is a tumor suppressor. However, little is known about the expression of endogenous Smad proteins during lymphomagenesis. In this study, we compare the localization of Smad3 and Smad4 in diffuse large B-cell lymphoma tissues, which is the most common type of malignant lymphoma, in order to understand the roles of Smads in lymphomagenesis.

MATERIALS AND METHODS

Paraffin-embedded tissue blocks of surgically resected lymphoma tissues were retrieved from the surgical files. The histologic diagnosis of diffuse large B-cell lymphoma was based on the new WHO classification. The B-cell nature of these tumors was confirmed by the immunohistochemical detection of the B-cell (CD20) and T-cell (CD3) markers by using the paraffin sections. Thirty-two cases of diffuse large B-cell lymphomas were included in this study. The patients’ ages ranged from 24 to 80 years (mean: 53.75), and the male to female ratio was 21:11. Seventeen cases arose from lymph nodes and 15 cases arose from extranodal sites (8 tonsils, 3 paranasal sinus, 1 nasopharynx, 1 brain, 1 ovary and 1 gingiva).

Immunohistochemistry

Immunohistochemical analysis was performed on the formalin-fixed, paraffin-embedded materials using a primary antibody for Smad3 (diluted 1:50) (Zymed Laboratories, San Francisco, CA, USA (51-1,500)) and Smad4 (diluted 1:50) (Santa Cruz Biotechnology, Santa Cruz, CA, USA (B-8)). Briefly, the sections were deparaffinized in xylene, rehydrated, washed in distilled water, immersed in 10 mM citrate buffer of pH 6, and then microwaved for 10 min. The sections were treated with a 3% H2O2 solution to reduce endogenous peroxidase activity, and then washed in phosphate buffer saline; they were subsequently subjected to the incubation with the primary antibodies. Detection of the immunoreactive staining was obtained by the streptavidin biotin method using an LSAB kit (DAKO, Carpinteria, CA, USA). The sections were subjected to a color reaction with diaminobenzidine and counterstained with Mayer’s hematoxylin. Tumors were considered to be positive only when a distinct nuclear staining could be demonstrated that was comparable to normal lymphoid cells.

RESULTS

Normal squamous epithelium was positive for Smad3 and Smad4 included in this study. The patients’ ages ranged from 24 to 80 years (mean: 53.75), and the male to female ratio was 21:11. Seventeen cases arose from lymph nodes and 15 cases arose from extranodal sites (8 tonsils, 3 paranasal sinus, 1 nasopharynx, 1 brain, 1 ovary and 1 gingiva).

Fig. 1. (A, B) In reactive lymphoid tissue, nearly all cells of the germinal center (A) are positive and more than 50% of paracortical cells (B) are positive for Smad3.
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and therefore, it was used as the positive control. In the reactive lymphoid tissue, nearly all cells of the germinal centers were positive for Smad3 (Fig. 1A) and more than 50% of paracortical cells were positive for Smad3 (Fig. 1B). For the Smad4 immunostaining, nearly all the cells of the germinal centers showed diffuse cytoplasmic staining, with most of them revealing nuclear positivity as well (Fig. 2A), and most of the cells in paracortex were positive (Fig. 2B). For the malignant lymphomas, all the cases were positive for Smad3 (Fig. 3), but 26 cases (81%) were positive for Smad4 (Fig. 4) and 6 cases (19%) were negative for Smad4. Most Smad3 and Smad4 positive tumors showed nuclear positivity in nearly all the tumor cells. Among the six Smad4-negative lymphomas, three of these cases arose from lymph nodes and three cases arose from the tonsils.

DISCUSSION

TGF-β and BMP are widely expressed during embryonic development, and especially in tissues where the inductive factors produced at mesenchymal-epithelial interfaces influence cell differentiation and organogenesis. In the normal lymph node, TGF-β...
is normally produced by follicular dendritic cells within the microenvironment of the secondary lymphatic follicles. In the reactive lymphoid tissues, less than 5% of small lymphocytes were found to be positive for TGF-β. Most small lymphocytes, granulocytes and germinal centers were non-reactive. In one previous experiment using a mouse model, all the tissues that were examined expressed at least one of the BMP-specific Smads (1 or 5) and one of the TGF-β/activin-specific (2 or 3) Smads, but the amount of nuclear staining of these Smad proteins varied depending upon the tissue and cell type. In lymphoid tissue, the stromal cells and T cells of the developing thymus show widespread expression of the common mediator Smad4, and moderate expression of TGF-β-specific Smads 2 and 3 with Smad3 being found mostly in the nucleus. In the spleen, Smads 2 and 4 are the most highly expressed Smads. However, little has been discovered regarding the expression pattern of Smad proteins in human lymphoid tissue. In this study, nearly all cells of the germinal centers were positive and more than 50% of paracortical cells were also positive for Smad3. Most of the cells in the germinal centers and the paracortex regions showed nuclear positivity for Smad4. TGF-β plays an important role in regulating the balance between proliferation and differentiation for hematopoietic cells and it is also an important regulator of immune cell development and function. The generalized expression patterns of Smad3 and Smad4 found in lymphoid tissues suggest that TGF-β-specific Smads may be actively involved in signal transduction of the TGF-β signaling pathway, and that they function to maintain the homeostasis of lymphoid cells in the human peripheral lymphoid organs.

In non-Hodgkin’s lymphomas, only a few small lymphocytes were positive for TGF-β. No TGF-β was detected in the tumor cells of the follicular lymphomas or the peripheral T-cell lymphomas. In contrast, 30% of Reed-Sternberg cells were positive for TGF-β, and a large number of medium sized T-lymphocytes were also positive for TGF-β in the Hodgkin’s lymphoma samples. All the cases of low- and high-grade gastric B-cell lymphomas were negative or weakly positive for TGF-β. So far, there have been no data regarding the expression pattern of Smads in malignant lymphoma tissues. In this study, all the cases of diffuse large B-cell lymphomas were positive for Smad3, but 26 of the cases were positive for Smad4 and 6 cases (19%) were negative for Smad4. Most Smad3 and Smad4 positive tumors showed nuclear positivity in nearly all the tumor cells. These results suggest that the TGF-β signaling pathway through the Smads proteins is mostly operative in this type of lymphoma because the nuclear localization of Smad proteins pinpoints the areas where signaling is particularly active.

Although our results suggest that receptor activated Smads signaling is active in the malignant lymphoma cells, high expression of inhibitory Smads may ameliorate or attenuate the strong signaling by receptor-activated Smads, and the expression of other factors, such as BAMBI/Eyb/nma, Ski, SnoN and SARA may modulate this signaling. Moreover, signaling by members of the TGF-β superfamily has been shown not to be exclusively Smad dependent. Further studies investigating these factors in normal lymphoid tissues and lymphoma tissues are needed to ascertain the meaning of Smad3 and Smad4 expressions in development of lymphoid organs and for lymphomagenesis.

Disturbance of TGF-β signaling pathway in lymphocytes enhances cell proliferation and may cause the development of malignant lymphoma. In this study, a small portion of the lymphomas revealed the loss of Smad4 expression. Smads can function as tumor suppressors to ameliorate the TGF-β signal in lymphoid tissues, although mutations of the Smad genes are seemingly rare in hematopoietic tumors. Further study with a larger series is certainly warranted to correlate the loss of Smad4 expression and other clinicopathologic parameters, and to determine its clinical significance in diffuse large B-cell lymphomas.

REFERENCES