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Lack of ErbB-2 Oncogene Product Overexpression in Soft Tissue Sarcomas

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The c-*erb*B-2 gene and its products (also designated *HER*-2 and c-*neu*) encode for a 185-kd transmembrane glycoprotein with intracellular tyrosine kinase activity. c-*erb*B-2 belongs to the epidermal growth factor receptor family, of which there are four known members, and has molecular homology to the epidermal growth factor receptor. It seems that this family is critical in control of growth, differentiation, and mobility of many normal and transformed epithelial cell types. We have looked for overexpression of c-*erb*B-2 gene product in paraffin-embedded material from 230 cases of soft tissue sarcoma, in order to establish a possible new prognostic marker and a potentially new treatment option. In all the cases, irrespective of the sarcoma histological type, the immunostaining for erbB-2 was negative. Applications of erbB-2 for prognostication as well as the option of receptor targeting by trastuzumab monoclonal antibodies were aborted.

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The most important factors affecting control of disease and survival of patients with soft tissue sarcomas (STS) are tumor size and tumor grade. Additional accepted prognostic factors are age, sex, tumor site, and depth, status of surgical margins, and presence or absence of distant metastases (1–4). Immunohistochemical markers such as DNA ploidy, bcl-2 oncoprotein, mutant p-53, proliferating cell nuclear antigen (PCNA), Ki-67, and P-Glycoprotein (Pgp) have been widely applied for more precise prognostication of STS.

Over the past few years, advances in molecular biology have led to investigation of a variety of tumor-associated markers. The erbB family attracted clinicians' attention, especially after the introduction of this molecule as a predictive factor for breast cancer chemotherapy, and even more after successful targeting of the receptor by trastuzumab (5). The c-*erb*B-2 gene and its products (also designated *HER*-2 and c-*neu*) encode for a 185-kd transmembrane glycoprotein with intracellular tyrosine kinase activity. c-*erb*B-2 belongs to the epidermal growth factor receptor family, of which there are four known members, and has molecular homology to the epidermal growth factor receptor (6). Although no ligand has been identified for c-*erb*B-2, several peptide growth factors bind to the other members of the family (7–9). The role of this family is critical in the control of growth, differentiation, and mobility of many normal and transformed epithelial cell types (10). We have looked for overexpression of c-*erb*B-2 gene product in our patients with soft tissue sarcomas, in order to establish a possible new prognostic marker, and a potentially new treatment option for this disease.

MATERIAL AND METHODS

The histological material of 230 cases of STS was available for immune staining for c-erbB-2 receptors. The types of STS are listed in Table 1. Patients' age ranged from 1 month to 87 years. Immunohistochemical staining was performed on paraffin-embedded material with monoclonal mouse anti-HER2 (c-erbB-2)—Zimed Laboratories Inc. Three μm sections mounted on Super Frost/Plus glass (Menzel, Glaser, Braunschweig, Germany) were processed by a labeled-(strept) avidin-biotin (LAB-SA) method using a HISTOSTAINTM plus kit (Zymed, San Francisco, USA). Heat-induced antigen retrieval was performed by 10-min protease treatment using an H2800 model proces-

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sor (Energy Beam Sciences, Inc., Agawan, MA, USA) in 10 mM citrate buffer, pH 6.0, for 12 min at 97°C. The sections were treated with 3% H_2O_2 for 5 min, followed by a 10-min incubation with the universal blocker, CAS-BLOCKTM (Zymed, San Francisco, USA). The sections were incubated for 1 h with 1:150 diluted erb-4 (C-18) antibody. A biotinylated second antibody was applied for 10 min, followed by incubation with horseradish peroxidase-conjugated streptavidin (HRP-SA) for 10 min. The slides were washed thoroughly with Optimax wash buffer (Biogenix, San Ramon, CA, USA) following each incubation. The immunoreaction was visualized by an HRP-based chromogen/substrate system, including DAB (brown) chromogen (Liquid DAB substrate kit—Zymed, San Francisco, USA). The sections were then counterstained with Mayer's hematoxylin (which stains the nuclei a blue color), dehydrated in ascending ethanol concentration, cleared in xylene and mounted for microscopic examination. Substitution of preimmune serum for the primary antibody served as a negative control.

RESULTS

In all 230 tissue samples, irrespective of the sarcoma histological type, the immunostaining for erbB-2 was negative. Consequently, no attempt was made to correlate this marker with the clinical course of the sarcoma.

DISCUSSION

Our results were disappointingly negative. None of the 230 sarcoma specimen showed any trace of erbB-2 staining. Therefore erbB-2 has no clinical role in soft tissue sarcomas, nor can it be targeted by monoclonal antibodies.

Table 1

Soft tissue sarcoma (STS) types

Type of STS	No. of cases
Malignant fibrous histiocytoma (MFH)	70
Liposarcoma (all types)	63
Synovial sarcoma	25
Leiomyosarcoma	14
Rhabdomyosarcoma	11
Dermatofibrosarcoma protuberans (DFSP)	8
Extraskeletal small blue round cell (Ewing family)	7
Malignant peripheral nerve sheath tumor (MPNST)	7
Angiosarcoma	5
Epithelioid sarcoma	5
Fibrosarcoma	4
Clear cell sarcoma	3
Extraskeletal osteosarcoma	3
Alveolar soft part sarcoma (ASPS)	2
Kaposi's sarcoma	1
Pleomorphic sarcoma	1
Congenital fibrosarcoma	1

Our results are in accordance with other reports. In these reports, there was almost no evidence of c-erbB-2 protein overproduction in a spectrum of STS (11, 12).

The expression of erbB-2 in bone sarcoma is still controversial. In one study, HER2 was not expressed in any specimen, while EGFR was overexpressed in 16%. Overexpression of EGFR did not correlate with prognosis or with any significant prognostic variables (13). In a second group study, erbB-2 seemed to be an important tissue marker and a prognostic indicator. Higher frequencies of expression were observed in samples from patients with metastatic disease at presentation and at the time of relapse. Expression of HER2/erbB-2 correlated with a significantly poorer histologic response (14, 15).

There may be, however, some role for erbB-2 in the pathogenesis of soft tissue sarcomas. It is known that the erbB-2 oncogene is predominantly leukemogenic (16), while the v-src oncogene induces sarcoma (17, 18). A chimeric oncogene, erbB/Src, contains the src catalytic domain inserted into the erbB backbone. The src kinase domain of the chimera converted the erbB into a sarcoma oncogene. The chimeric oncogene produced sarcomas, but not leukemias, in young birds (19). Thus, it is plausible that erbB-2 may interact with the src oncogene to yield a sarcoma, or may undergo a mutation in its catalytic domain that induces the development of sarcoma. As a mutated gene that causes sarcoma, it is possible that its product, the receptor itself, may not be detected by the immunostain used.

In conclusion, our results indicate that HER-2/neu protein overexpression seems to be a rare event in soft tissue sarcomas. The potential uses of erbB-2 for prognostication as well as the option of receptor targeting by trastuzumab monoclonal antibodies are therefore aborted.

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