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Epidermal Growth Factor Receptor Expression and Mutational Analysis in Synovial Sarcomas and Malignant Peripheral Nerve Sheath Tumors

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LEARNING OBJECTIVES

After completing this course, the reader will be able to:

1. Discuss the significance of deletions and mutations of the *EGFR* gene in cancer cell growth and survival.
2. Describe the significance of the role of EGFR in malignant peripheral nerve sheath tumors, especially those associated with neurofibromatosis 1.
3. Discuss the significance of why small molecule inhibitor therapy has been ineffectual in synovial sarcomas and malignant peripheral nerve sheath tumors.

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ABSTRACT

Background. **Synovial sarcomas (SnSrcs) and malignant peripheral nerve sheath tumors (MPNSTs) are rare mesenchymal tumors of adolescence and young adulthood. Previous work from our laboratory has demonstrated that SnSrcs express epidermal growth factor receptor (EGFR) and human EGFR (HER)-2/neu. The present study extends that work to examine the expression of EGFR in MPNSTs and the characterization of potential targets of the EGFR tyrosine kinase domain.**

Methods. **Tissue microarrays containing 48 cases of SnSrc and 32 cases of MPNST were stained for EGFR, EGFRvIII, and activated EGFR (pY1068-EGFR). Tumor DNA was extracted from fresh and formalin-fixed, paraffin-embedded tissue blocks and sequenced for exons 17–21 of *EGFR* and exon 2 of K-*ras* and b-*raf*.**

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Results. **Immunohistochemistry (IHC) demonstrated that EGFR is expressed in a majority of SnSrcs and MPNSTs (71% and 62.5%, respectively). EGFRvIII immunoreactivity was negative. IHC was weakly immunopositive for activated EGFR (18.7% and 3.1%, respectively). Sequence analysis of the *EGFR* genomic DNA did not demonstrate mutations in exons 17–21. No K-*ras* or b-*raf* mutations were observed in either tumor type.**

Conclusions. **Expression of EGFR in SnSrcs and MPNSTs with an intact EGFR/mitogen-activated protein kinase pathway has been hypothesized to contribute to the malignant potential of these tumors. Our study reveals the absence of known activating mutations in EGFR, which suggests that trials of small-molecule inhibitors would be of little clinical benefit. A clinical study of treatment with cetuximab is ongoing and may help elucidate whether blockade of EGFR with antibodies is likely to be more active.** ***The Oncologist*** 2008;13:459–466

INTRODUCTION

Synovial sarcomas (SnSrcs) and malignant peripheral nerve sheath tumors (MPNSTs) are rare mesenchymal tumors of adolescence and young adulthood [1]. Gene-expression profiling has suggested that these two tumors are similar and tend to cluster together in cluster analyses of microarray data [2, 3].

SnSrcs are malignant soft tissue tumors that account for approximately 7%–8% of all malignant mesenchymal tumors and tend to occur primarily in children and young adults [4]. Morphologically, they are biphasic, with spindle and epithelioid glandular cells, or monophasic, with a pure spindle-cell pattern. The majority of tumors possess a specific chromosomal translocation, where the proximal part of *SYT* (18q11) is translocated to the distal portion of one of several duplicated *SSX* genes (most notably *SSX1* and *SSX2*) on the short arm of chromosome X (Xp11). The *SYT/SSX1* translocation is present in the majority (>75%) of patients and is associated with a poor prognosis [5, 6]. The *SYT/SSX1* translocation is associated with both the biphasic and monophasic patterns, whereas the *SYT/SSX2* translocation is usually associated with only the sarcomatous (monomorphic) morphology.

Although surgery, radiotherapy, and adjuvant chemotherapy have improved the outcome of patients with local disease, management of distant metastasis remains problematic. Local disease recurrence, large tumors, lack of differentiation, older patient age, and pulmonary metastasis are all poor prognostic factors. Accordingly, there is a need for alternate therapies, including molecularly targeted agents such as those recently developed against the receptor tyrosine kinases (RTKs), such as imatinib for the treatment of gastrointestinal stromal tumor (GIST) and erlotinib for non-small cell lung cancer (NSCLC).

The MPNSTs account for approximately 5%–10% of all soft tissue sarcomas; about one fourth to one half occur with neurofibromatosis 1 (NF1) [7, 8]. Morphologically, most MPNSTs resemble fibrosarcomas in their overall organization, with certain modifications. Classically, the cells recapitulate the features of the normal Schwann cells. The cells have markedly irregular contours, and the nuclei are wavy, buckled, or comma shaped. The cytoplasm stains lightly and is usually indistinct. The cells can range from spindled in shape to fusiform or even rounded, such that the lesion can mimic a fibrosarcoma or even a round cell sarcoma [1].

Most MPNSTs are high-grade sarcomas, with a high likelihood of producing local recurrence and distant metastasis. In large series, the local recurrence rate varies in the range of 40%–65% and the metastatic rate is in the range of 40%–68% [8–10]. The 5-year survival rate based on a study of 134 patients with tumors from all sites was 52% [8]. Treatment with extensive surgery often coupled with adjuvant radiation or chemotherapy has improved survival. Prognostic factors in this disease include the size of the lesion, location, stage, grade, status of surgical margins, necrosis, and use of adjuvant radiation [9, 10]. In a multivariate analysis, the status of surgical margins and a history of irradiation emerge as independent prognostic variables.

MPNSTs arising in patients with NF1 syndrome are known to have aggressive clinical characteristics. The majority of patients have advanced disease at the time of diagnosis and the overall outcome of treatment is discouraging. A recently published large Italian and German series of children and adolescents with MPNST clearly demonstrated the poor survival rates of NF1 patients in comparison with cases without NF1, with 5-year progression-free survival (PFS) and overall survival rates of 19% and 32% in NF1 cases, respectively, versus 42% and 55% in non-NF1 cases, respectively [11]. Although some previous studies reported similar outcomes in NF1 and non-NF1 patients, most published adult MPNST series emphasize the worse prognosis of NF1 patients [7–9, 12, 13]. MPNSTs arising in NF1 cases appear more chemoresistant, with reported response rates in the Italian and German series of 17% in NF1 cases versus 55% in non-NF1 cases [11], suggesting biological differences between MPNST patients with and without NF1. A recent series by Ferrari et al. [14] confirmed

Table 1. Antibodies used in this study

Antibody	Manufacturer	Concentration	Epitope retrieval	Detection
EGFR	Zymed	1:50	Protease K, 10 minutes	LSAB+
pY-EGFR (Y1086)	Zymed	1:200	Trypsin, 10 minutes, 37°C	Polymer HRP
EGFRvIII	Zymed	1:100	HIER, pH 6	Polymer HRP

Abbreviations: EGFR, epidermal growth factor receptor; HIER, heat-induced epitope retrieval; HRP, horseradish peroxidase; LSAB, labeled streptavidin biotin.

this finding, with only 2 of 16 cases of MPNSTs arising in NF1 patients achieving partial responses to chemotherapy.

Previous work from our laboratory has demonstrated that SnSrcs express the RTKs epidermal growth factor receptor (EGFR) and human EGFR (HER)-2/neu [15]. The present study extends this work to examine the expression of EGFR in MPNSTs, the role of the activated EGFR/mitogen-activated protein kinase pathway in both tumor types, and the characterization of potential targets of the EGFR tyrosine kinase domain for which small-molecule inhibitors are available.

The human EGFR (ErbB) family of RTKs is an important group of mediators responsible for cell proliferation, survival, adhesion, migration, and differentiation [16]. The family comprises four distinct receptors: EGFR, HER-2, HER-3, and HER-4. EGFR, HER-3, and HER-4 are stimulated by a variety of ligands, whereas no known ligand has been identified for HER-2/neu. With the exception of HER-3, which lacks tyrosine kinase activity, all are transmembrane RTKs [17]. Once activated by their respective ligands, they rapidly dimerize either as homodimers or heterodimers and exert their biologic activity through several different signal transduction pathways. EGFR is expressed in a variety of neoplasms, including cervical, ovarian, bladder, and esophageal carcinomas. Deletions of the extracellular domain of EGFR also have an activating effect on the receptor, providing the cells that express these truncated receptors with a proliferative advantage [18].

Materials and Methods

Tumors and Patients

Fresh and formalin-fixed, paraffin-embedded (FFPE) tissue blocks of SnSrcs and MPNSTs were obtained from the files of the Department of Pathology, University of Michigan Medical Center, Ann Arbor, MI. Institutional review board approval was obtained and the diagnosis was confirmed by morphology. The diagnoses of SnSrc and MPNST were defined according to the recently published World Health Organization criteria [19]. After pathological review, tissue microarrays were constructed from the most representative area using the methodology of Nocito et al. [20]. These are the same patients described in our recently published cluster analysis paper [21] and HER-2/neu and EGFR expression manuscript [15].

Clinical data on all patients were obtained from the cancer registry and included age, gender, tumor type, date of diagnosis, dates and modalities of treatments received, time to recurrence, time to death or loss to follow-up, and vital status. Descriptive data were reviewed, and survival data were analyzed using the Kaplan–Meier method. All statistical analyses were performed using the STATA 9.2 statistical package (StataCorp, College Station, TX).

Immunohistochemical Staining

Immunohistochemical (IHC) staining was performed on the Dako Autostainer (Dako, Carpinteria, CA) using Dako labeled streptavidin biotin (LSAB™+) and diaminobenzadine (DAB) as the chromogen. Deparaffinized sections of formalin-fixed tissue at 5-μm thickness were labeled with several antibodies as detailed in Table 1. Appropriate negative (no primary antibody) and positive (breast carcinoma) controls were stained in parallel with each set of tumors studied. The immunoreactivity was scored by a three-tier modification of the normal grading scheme previously described by Wang et al. [22]. Our three-tier system assigns a score of zero to negative immunoreactivity, a low score (1+) to diffusely weak or focally strong immunoreactivity, and a high score (2+) to diffusely strong immunopositivity.

DNA Extraction and Mutational Analysis

DNA was extracted from three 5-μm thick sections of each SnSrc and MPNST FFPE specimen using a Nucleon HT DNA extraction kit (Amersham Biosciences, Piscataway, NJ) according to the manufacturer's instructions. Genomic exons 17–21 of the *EGFR* gene, exon 2 of the cK-*ras* gene, and exon 2 of the b-*raf* gene were separately amplified according to the methods of Yantiss et al. [23] and detailed in Table 2. Amplicons were purified using a Wizard SV PCR clean-up kit (Promega, Madison, WI) and sequenced directly within the University of Michigan Medical Center DNA sequencing core using an ABI 377 DNA sequencer

Table 2. Primer sequences and reaction conditions

Gene	Exon	Forward primer	Reverse primer	Annealing temperature	Amplicon size (bp)
EGFR	17	TCCTTGTTCCTCCACCTCAT	TATGTATCTAACATACACAAC TGCTAAT	55	270
EGFR	18	GCTGAGGTGACCCTTGTCTC	ACAGCTTGCAAGGACTCTGG	55	246
EGFR	19	CCCAGTGTCCCTCACCTTC	CCACACAGCAAAGCAGAAAC	55	239
EGFR	20	TTCTGGCCACCATGCGA	CCGTATCTCCCTTCCCTGATTA	55	258
EGFR	21	TGATCTGTCCCTCACAGCAG	TCAGGAAAATGCTGGCTGAC	55	231
cK-*ras*	2	GGCCTGCTGAAAATGACTGA	GTCCTGCACCAGTAATATGC	60	162
b-*raf*	2	TGCACAGGGCATGGATTACTTA	TTCTGGTGCCATCCACAA	52	190

Abbreviation: EGFR, epidermal growth factor receptor.

(ABI, Foster City, CA). Chromatograms were downloaded directly to CodonCode Aligner software (v1.4.4, CodonCode Corp., Dedham, MA), and the sequence was compared with the reference sequence downloaded from the National Center for Biotechnology Information. The reference sequence numbers are NM_005228 for *EGFR*, NM_033360 for cK-*ras*, and NM_004333 for b-*raf*.

Results

Clinicopathologic Data

Forty-eight specimens of SnSrc from 35 patients were studied. The average age of the patients was 33 years (range, 7–70), with a male-to-female ratio of 1:1.2. There were 29 patients with the *SSX1* translocation and six with the *SSX2* translocation. No patients with the *SSX4* translocation were observed.

Thirty-two cases of MPNST from 28 patients were used for this study. Ten patients had documented NF1 by standard clinical criteria, although mutational analysis for NF1 was not performed. The average age was 38.6 years (range, 5–76) with a male-to-female ratio of 1:1.15.

Expression of EGFR, EGFRvIII, and Phosphorylated EGFR in SnSrc and MPNST by IHC

IHC analysis of 48 SnSrc specimens representing primary and metastatic lesions using the anti-EGFR polyclonal antibody demonstrated the characteristic membranous staining associated with membrane-bound tyrosine kinases in 34 specimens (71%). The majority had weak staining (1+); only two samples had strong staining (2+), similar to positive controls (Fig. 1A). IHC analysis of 32 MPNST specimens demonstrated EGFR expression in 20 specimens (62.5%), with six specimens staining strongly positive (19%) (Fig. 2A), while the rest were weakly positive. All positive and negative IHC controls were appropriate.

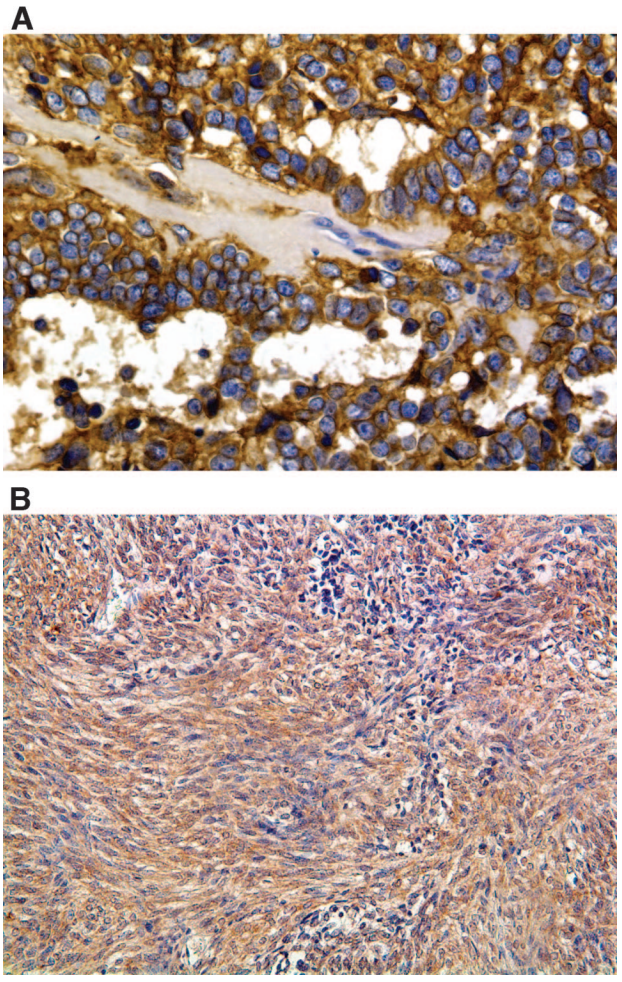

Figure 1. Immunohistochemical stain for epidermal growth factor receptor (EGFR) and its activated form (pY1068-EGFR) in two cases of synovial sarcoma. **(A):** Mixed epithelial–spindle cell pattern of *SYT/SSX1* translocation synovial sarcoma demonstrating diffuse strong immunoreactivity for EGFR (anti-EGFR, avidin-biotin peroxidase complex/hematoxylin; original magnification ×400). **(B):** Diffuse immunoreactivity for pY1068-EGFR in a spindle-cell synovial sarcoma with *SYT/SSX1* translocation (anti-pY1068-EGFR, avidin-biotin peroxidase complex/hematoxylin; original magnification ×200).

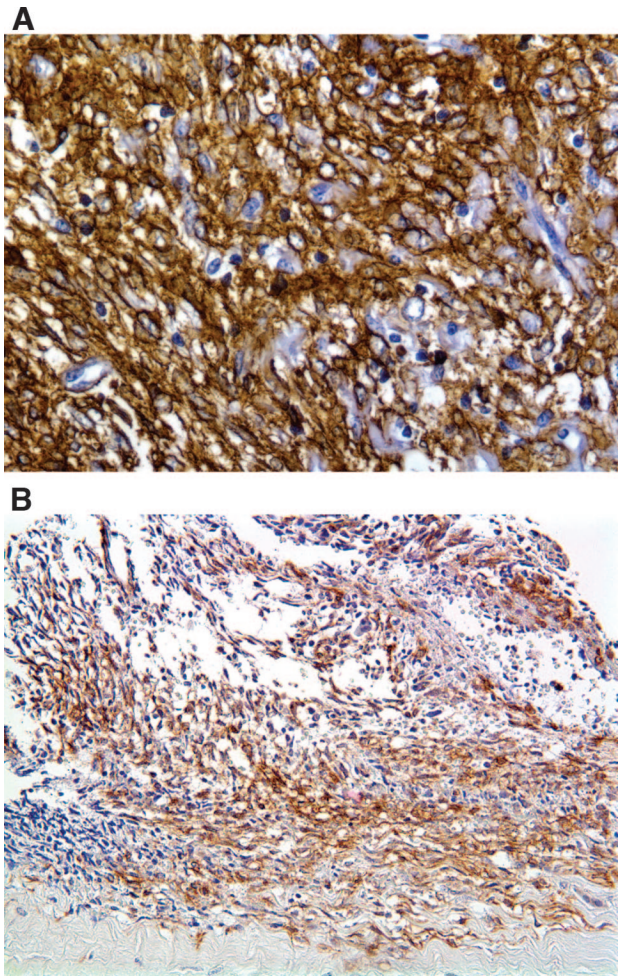

Figure 2. Immunohistochemical stain for epidermal growth factor receptor (EGFR) and its activated form (pY1068-EGFR) in two cases of malignant peripheral nerve sheath tumor (MPNST). **(A):** MPNST demonstrating diffuse strong immunoreactivity for EGFR (anti-EGFR, avidin-biotin peroxidase complex/hematoxylin; original magnification ×400). **(B):** Diffuse positive immunoreactivity for pY1068-EGFR in an MPNST (anti-pY1068-EGFR, avidin-biotin peroxidase complex/hematoxylin; original magnification ×200).

IHC was next performed with a polyclonal antibody specific to the phosphorylated form of EGFR (pY1068-EGFR). Nine of 48 (18.7%) SnSrc specimens demonstrated positive membranous staining (Fig. 1B), while only one of 32 MPNSTs (3.1%) was positive (Fig. 2B). IHC analysis for EGFRvIII was performed on MPNSTs and was uniformly negative, suggesting the absence of this modification of the receptor in MPNSTs.

Mutational analysis of the *EGFR* genomic DNA demonstrated no evidence of point mutations in the exons evaluated. However, a presumptive 20-base pair insertion was seen in exon 18 in 17 of 48 samples of SnSrc (14.5%) and 8 of 32 MPNSTs (25%). No K-*ras* mutations in codons 12 or 13 or in exon 2 of the b-*raf* gene were identified in either tumor type.

Survival by EGFR, Phosphorylated EGFR, and EGFRvIII Status

Survival data were analyzed using the Kaplan–Meier method. Patients with SnSrcs that expressed EGFR, phosphorylated EGFR, or EGFRvIII did not have any statistically significant difference in time to first recurrence or death when compared with patients that lacked expression.

Similarly, there was no difference in the time to recurrence or survival time in patients with MPNSTs that had positive expression of EGFR as compared with those without EGFR expression. The presence or absence of phosphorylated EGFR expression did not correlate with disease outcome in MPNST.

Discussion

In an earlier report, we examined the expression of the *EGFR* and *HER-2/neu* genes in 38 samples of SnSrc representing 30 patients using IHC and molecular methods. We demonstrated that *EGFR* and *HER-2/neu* were expressed in the majority of cases, albeit at relatively low levels [15].

In this report, we extend our previous findings on the expression of EGFR in SnSrcs to examine the expression of the activated form of EGFR (phosphorylated EGFR). We also report the largest series of MPNST tissue examined for the expression of EGFR, its phosphorylated form p-EGFR, as well as the prevalence of the mutated form EGFRvIII.

Deletions of the extracellular domain of EGFR have an activating effect on the receptor, providing the cells that express the truncated receptors with a proliferative advantage [24].

The most common truncated receptor is the variant III EGFR deletion mutant (EGFRvIII), which contains an in-frame deletion of exons 2–7 from the extracellular domain and is commonly observed in glioblastomas. EGFRvIII has recently been found to be present in a small percentage (5%) of human NSCLCs [25]. Murine data confirm that overexpression of EGFRvIII is oncogenic in lung tissue. In addition, the abrogation of EGFRvIII expression by withholding doxycycline causes regression of the lung tumors, showing that these tumors are dependent on the activated EGFR pathway [26]. It has also been shown that HKI-272, an irreversible inhibitor that covalently binds to the EGFR kinase domain cleft, is effective in the treatment of EGFRvIII-dependent mouse lung tumors. Gefitinib and erlotinib also inhibit the growth of cells harboring EGFRvIII mutations, although at much higher concentrations than HKI-272. It is reasoned that this partial activity may provide an explanation for the reported response seen with erlotinib in a small percentage of squamous cell lung carcinomas [25].

Our findings have a direct clinical implication because tyrosine kinase inhibitors are already being examined in early clinical trial settings for activity in SnSrcs and MPNSTs. While this approach appears to be warranted in SnSrcs given the high expression of EGFR, MPNSTs may be less amenable to show therapeutic benefit because they have a lower prevalence of EGFR expression and lower activation.

Preclinical evidence has suggested a central role for EGFR in the tumorigenesis of MPNSTs, particularly associated with NF1. As early as 1992, Basu et al. [27] provided evidence that Ras proteins in malignant tumor cell lines from patients with NF1 are in a constitutively activated state. These cells were shown to contain $p21^{ras}$ and p120GAP that are both functionally wild type, but barely any functional NF1 protein. The authors concluded that NF1 protein is normally essential for correct negative regulation of Ras proteins in the cell, and thus acts as a tumor suppressor whose product acts upstream of Ras [27].

DeClue et al. [28] provided additional evidence for the role of the EGFR pathway when they used immunoblotting, Northern analysis, and IHC to demonstrate that each of three MPNST cell lines from NF1 patients expressed the EGFR, as did seven of the seven other primary MPNSTs, a non-NF1 MPNST cell line, and the S100+ cells from each of nine benign neurofibromas. Furthermore, transformed derivatives of Schwann cells from *NF1–/–* mouse embryos also expressed EGFR. All the cells or cell lines expressing EGFR responded to EGF by activation of downstream signaling pathways. The authors concluded that EGFR expression may play an important role in NF1 tumorigenesis and Schwann cell transformation. This hypothesis was further tested by demonstrating that growth of NF1 MPNST lines and the transformed *NF1−/−* mouse embryo Schwann cells was greatly stimulated by EGF in vitro and could be blocked by agents that antagonize EGFR function [28].

EGFR was also found to be expressed in 23 of 24 cell lines derived from malignant soft tissue sarcomas from *Nf1:p53* compound heterozygous mice [29]. *EGFR* gene amplification is also observed in MPNSTs [30]. Ling et al. [31] showed that expression of EGFR in transgenic mouse Schwann cells elicits features of neurofibromas such as Schwann cell hyperplasia, excess collagen, mast cell accumulation, and progressive dissociation of non–myelin-forming Schwann cells from axons. Mating *EGFR* transgenic mice to *Nf1* hemizygotes did not enhance this phenotype. Genetic reduction of *EGFR* in *Nf1(+/−):p53(+/−)* mice that develop sarcomas significantly improved survival. Thus, gain- and loss-of-function experiments support the relevance of EGFR to peripheral nerve tumor formation [31].

A xenograft model for MPNST was developed by Mahller et al. [32], and the effect of EGFR inhibition with erlotinib was tested. When grown in the presence of erlotinib, the MPNST cell lines STS26T and S462 showed lower expression of proangiogenic genes and demonstrated antiproliferative effects. s.c. tumors treated with erlotinib daily, for five consecutive days, illustrated a lower vascular density ($p < .01$), and analysis of vessel size distribution showed a trend toward fewer small vessels within erlotinib-treated tumors. These results suggest that erlotinib has antiproliferative and antiangiogenic effects against MPNSTs [32].

The role of EGFR in MPNST development was compelling in preclinical settings and led to the evaluation of tyrosine kinase inhibitors in patients with MPNSTs. Erlotinib was given at 150 mg/day in a phase II study of the Southwest Oncology Group in metastatic or unresectable MPNSTs [33]. Twenty-four patients were enrolled over 22 months, from 13 institutions. The median age was 45.3 years; 50% had neurofibromatosis. At enrollment, 15 had performance status scores of 0–1, 18 had metastatic disease, and 19 had unresectable disease. Twenty patients were evaluable for response: one had stable disease after first evaluation and 19 had no response. The median PFS time observed was 2 months. Fourteen patients have died; the median overall survival time was 4 months. Because no objective responses were observed in the first stage of the study (designed as a Simon two-stage trial), it was closed to further accrual.

While our findings in MPNST are consistent with the reported literature, we still found only 62% of tumors to express EGFR. But, more importantly, the downstream effect of EGFR activation, namely, phosphorylation, was present in only one tumor (3.1%), suggesting that, despite the expression of EGFR, this pathway does not appear to be constitutively active and hence is not necessarily driving the malignant machinery of the cell. This is more consistent with the lack of a clinical benefit of EGFR tyrosine kinase inhibition in MPNST. Furthermore, we have demonstrated the absence of mutated forms of *EGFR*, which suggests that a monoclonal antibody approach to EGFR inhibition may be more effective in this tumor type. This approach warrants preclinical and/or clinical evaluation of anti-EGFR monoclonal antibodies in the treatment of MPNST. In fact, an ongoing trial of cetuximab at the University of Michigan is open to patients with SnSrcs and MPNSTs as well as other soft tissue and bony sarcomas.

The first report of EGFR expression in SnSrc was in 1985, when an immunocytochemical study of EGFR in 35 human soft tissue sarcomas showed particularly strong staining in one epithelioid sarcoma and in the spindle-cell component of an SnSrc [34]. Subsequently, Barbashina et al. [35] reported that 13 of 19 SnSrcs (68%) were immunoreactive with EGFR. Also, 10 of 19 tumors (52%; seven

monophasic and three biphasic) showed positive cytoplasmic and membranous staining with HER-2/neu (HerceptTest™, Dako). However, cellular expression of HER-2/neu was independent of EGFR positivity and showed no association with the proliferative activity of the tumors [35].

The expression of EGFR was examined in a cohort of 13 SnSrc patients. All specimens showed strong diffuse or focal EGFR expression. No amplifications of the *EGFR* gene were found. In contrast, several point mutations were identified in exons 18–21 of two SnSrcs. Whereas one of these tumors carried only a synonymous mutation, two missense mutations in exons 19 and 21 of the *EGFR* gene (P733S and A840 T, respectively) could be demonstrated in the second sample. The authors concluded that strong EGFR expression in SnSrcs is unrelated to gene amplification, and that the existence of mutations in the tyrosine kinase domain of the *EGFR* gene in a small subset of SnSrcs suggests that only few patients may benefit from tyrosine kinase inhibitor therapy [36].

Terry et al. [37] assessed the effect of gefitinib on two SnSrc cell lines (monolayer Fuji and SYO-1). The gefitinib 50% inhibitory concentration was 265.1 μmol/l for Fuji and 266.4 μmol/l for SYO-1. These concentrations are significantly higher than those described to inhibit the proliferation of gefitinib-sensitive cell lines. Activating mutations in EGFR affecting the kinase domain have recently been identified to bestow sensitivity to gefitinib in NSCLC. Similar sensitizing mutations in exons 18, 19, and 21 of *EGFR* were sought in each SnSrc cell line and 16 archival SnSrc tumor specimens that strongly expressed the EGFR protein. However, no such mutations were found, consistent with our findings [37].

Small-molecule tyrosine kinase inhibitors that target EGFR have been used to treat patients with SnSrc. In a phase II European Organization for Research and Treatment of Cancer trial of second-line treatment of patients with metastatic or locally advanced SnSrc, gefitinib was given at 500 mg/day until progression or intolerance. The primary endpoint was the PFS rate at 3 months. Forty-eight patients were included, 27 (56%) men and 21 (44%) women, with a median age of 42 years (range, 19–66). Metastatic sites were lung in 92% and soft tissue or lymph nodes in 42% of the patients. Thirty-seven patients were evaluable for the primary endpoint, with a median treatment duration of 11 weeks (range, 2–25). There were no objective responses reported. Seven (18%) patients achieved stable disease as their best response. At 3 months, five of the 39 (13%) evaluable patients achieved PFS; the 6- and 12-month PFS rates were 10% and 3%, respectively [38].

The role of the EGFR pathway has been of great scientific interest and, more importantly, offers great potential for improving clinical outcomes in patients with various malignancies. The first member of the Erb family whose inhibition has shown impressive clinical activity was HER-2/neu with the advent of trastuzumab in the treatment of breast cancer. The monoclonal antibody approach to EGFR has also found application in head and neck cancer, NSCLC, and colon cancer. Small-molecule tyrosine kinase inhibitors have been quite effective in a subset of patients with EGFR mutations in NSCLC. These findings have the potential of being transposable to the treatment of soft tissue sarcomas. Soft tissue tumors are rare, thus limiting the feasibility of large, randomized, phase III trials that allow detection of small but clinically significant therapeutic improvements. Hence, there is value in building on preclinical findings and the experience obtained from other tumors to guide therapy in soft tissue tumors. Such an approach has met monumental success in the therapy of GISTs with imatinib. Our results support the role of EGFR in the carcinogenesis of SnSrcs, but the lack of mutations may explain the absence of a clinical benefit of tyrosine kinase inhibitors. Similarly, the role of EGFR appears to be of less value in the carcinogenesis of MPNST, because we found a low prevalence of its activated form. The absence of EGFRvIII mutations in both tumor types suggests that EGFR blockade using a monoclonal antibody may offer the optimal approach for targeting this pathway in SnSrcs and MPNSTs.

The absence of any correlation between the expression of EGFR pathway proteins and clinical outcome, as well as the lack of observed clinical benefit with approaches designed to abrogate this pathway, indicate that translating laboratory findings into clinically oriented interventions needs to happen only after careful examination of all available data and thorough evidence for the role of this pathway in each specific tumor type has been obtained.

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REFERENCES

1 Weiss SW, Goldblum JR. Enzinger and Weiss's Soft Tissue Tumors, Fourth Edition. St. Louis: Mosby, Inc., 2001:1–1622.

2 Francis P, Namlos HM, Muller C et al. Diagnostic and prognostic gene expression signatures in 177 soft tissue sarcomas: Hypoxia-induced transcription profile signifies metastatic potential. BMC Genomics 2007;8:73.

3 Baird K, Davis S, Antonescu CR et al. Gene expression profiling of human sarcomas: Insights into sarcoma biology. Cancer Res 2005;65: 9226–9235.

4 Brennan MF, Casper ES, Harrison LB. Soft tissue sarcoma. In: DeVita VT Jr, Hellman S, Rosenberg SA et al., eds. Cancer: Principles and Practice of Oncology, Fifth Edition. Philadelphia: Lippincott-Raven, 1997:1738–1852.

5 Kawai A, Woodruff J, Healey JH et al. SYT-SSX gene fusion as a determinant of morphology and prognosis in synovial sarcoma. N Engl J Med 1998;338:153–160.

6 Inagaki H, Nagasaka T, Otsuka T et al. Association of SYT-SSX fusion types with proliferative activity and prognosis in synovial sarcoma. Mod Pathol 2000;13:482–488.

7 Ducatman BS, Scheithauer BW, Piepgras DG et al. Malignant peripheral nerve sheath tumors. A clinicopathologic study of 120 cases. Cancer 1986; 57:2006–2021.

8 Wong WW, Hirose T, Scheithauer BW et al. Malignant peripheral nerve sheath tumor: Analysis of treatment outcome. Int J Radiat Oncol Biol Phys 1998;42:351–360.

9 Hruban RH, Shiu MH, Senie RT et al. Malignant peripheral nerve sheath tumors of the buttock and lower extremity. A study of 43 cases. Cancer 1990;66:1253–1265.

10 Kourea HP, Bilsky MH, Leung DH et al. Subdiaphragmatic and intrathoracic paraspinal malignant peripheral nerve sheath tumors: A clinicopathologic study of 25 patients and 26 tumors. Cancer 1998;82:2191–2203.

11 Carli M, Ferrari A, Mattke A et al. Pediatric malignant peripheral nerve sheath tumor: The Italian and German soft tissue sarcoma cooperative group. J Clin Oncol 2005;23:8422–8430.

12 Ghosh BC, Ghosh L, Huvos AG et al. Malignant schwannoma. A clinicopathologic study. Cancer 1973;31:184–190.

13 Wanebo JE, Malik JM, VandenBerg SR et al. Malignant peripheral nerve sheath tumors. A clinicopathologic study of 28 cases. Cancer 1993;71: 1247–1253.

14 Ferrari A, Bisogno G, Macaluso A et al. Soft-tissue sarcomas in children and adolescents with neurofibromatosis type 1. Cancer 2007;109:1406–1412.

15 Thomas DG, Giordano TJ, Sanders D et al. Expression of receptor tyrosine kinases epidermal growth factor receptor and HER-2/neu in synovial sarcoma. Cancer 2005;103:830–838.

16 Yarden Y. The EGFR family and its ligands in human cancer: Signalling mechanisms and therapeutic opportunities. Eur J Cancer 2001;37(suppl 4): S3–S8.

17 Ullrich A, Schlessinger J. Signal transduction by receptors with tyrosine kinase activity. Cell 1990;61:203–212.

18 Rosell R, Taron M, Reguart N et al. Epidermal growth factor receptor activation: How exon 19 and 21 mutations changed our understanding of the pathway. Clin Cancer Res 2006;12:7222–7231.

19 Fletcher CDM, Unni KK, Mertens F. Pathology and Genetics of Tumours of Soft Tissue and Bone. Lyon, France: IARC Press, 2002:1–427.

20 Nocito A, Kononen J, Kallioniemi OP et al. Tissue microarrays (TMAs) for high-throughput molecular pathology research. Int J Cancer 2001;94:1–5.

21 Olsen SH, Thomas DG, Lucas DR. Cluster analysis of immunohistochemical profiles in synovial sarcoma, malignant peripheral nerve sheath tumor, and Ewing sarcoma. Mod Pathol 2006;19:659–668.

22 Wang S, Saboorian MH, Frenkel E et al. Laboratory assessment of the status of Her-2/neu protein and oncogene in breast cancer specimens: Comparison of immunohistochemistry assay with fluorescence in situ hybridisation assays. J Clin Pathol 2000;53:374–381.

23 Yantiss RK, Rosenberg AE, Sarran L et al. Multiple gastrointestinal stromal tumors in type I neurofibromatosis: A pathologic and molecular study. Mod Pathol 2005;18:475–484.

24 Nicholas MK, Lukas RV, Jafri NF et al. Epidermal growth factor receptor-mediated signal transduction in the development and therapy of gliomas. Clin Cancer Res 2006;12:7261–7270.

25 Ji H, Zhao X, Yuza Y et al. Epidermal growth factor receptor variant III mutations in lung tumorigenesis and sensitivity to tyrosine kinase inhibitors. Proc Natl Acad Sci U S A 2006;103:7817–7822.

26 Tsao MS, Sakurada A, Cutz JC et al. Erlotinib in lung cancer—molecular and clinical predictors of outcome. N Engl J Med 2005;353:133–144.

27 Basu TN, Gutmann DH, Fletcher JA et al. Aberrant regulation of Ras proteins in malignant tumour cells from type 1 neurofibromatosis patients. Nature 1992;356:713–715.

28 DeClue JE, Heffelfinger S, Benvenuto G et al. Epidermal growth factor receptor expression in neurofibromatosis type 1-related tumors and NF1 animal models. J Clin Invest 2000;105:1233–1241.

29 Li H, Velasco-Miguel S, Vass WC et al. Epidermal growth factor receptor signaling pathways are associated with tumorigenesis in the Nf1:p53 mouse tumor model. Cancer Res 2002;62:4507–4513.

30 Perry A, Kunz SN, Fuller CE et al. Differential NF1, p16, and EGFR patterns by interphase cytogenetics (FISH) in malignant peripheral nerve sheath tumor (MPNST) and morphologically similar spindle cell neoplasms. J Neuropathol Exp Neurol 2002;61:702–709.

31 Ling BC, Wu J, Miller SJ et al. Role for the epidermal growth factor receptor in neurofibromatosis-related peripheral nerve tumorigenesis. Cancer Cell 2005;7:65–75.

32 Mahller YY, Vaikunth SS, Currier MA et al. Oncolytic HSV and erlotinib inhibit tumor growth and angiogenesis in a novel malignant peripheral nerve sheath tumor xenograft model. Mol Ther 2007;15:279–286.

33 Albritton KH, Rankin C, Coffin CM, et al. Phase II study of erlotinib in metastatic or unresectable malignant peripheral nerve sheath tumors (MPNST). J Clin Oncol 2006;24(18 suppl):524s.

34 Gusterson B, Cowley G, McIlhinney J et al. Evidence for increased epidermal growth factor receptors in human sarcomas. Int J Cancer 1985;36:689–693.

35 Barbashina V, Benevenia J, Aviv H et al. Oncoproteins and proliferation markers in synovial sarcomas: A clinicopathologic study of 19 cases. J Cancer Res Clin Oncol 2002;128:610–616.

36 Bode B, Frigerio S, Behnke S et al. Mutations in the tyrosine kinase domain of the EGFR gene are rare in synovial sarcoma. Mod Pathol 2006;19:541–547.

37 Terry J, Lubieniecka JM, Kwan W et al. Hsp90 inhibitor 17-allylamino-17-demethoxygeldanamycin prevents synovial sarcoma proliferation via apoptosis in in vitro models. Clin Cancer Res 2005;11:5631–5638.

38 Blay J, Le Cesne A, Whelan J et al. Gefitinib in second line treatment of metastatic or locally advanced synovial sarcoma expressing HER1: A phase II trial of EORTC Soft Tissue and Bone Sarcoma Group. J Clin Oncol 2006;24(18 suppl):524s.