Targeted therapy in oncology: mechanisms and toxicity

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EGFR and ERBB2 expression in sarcomas: the search for new treatment options

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Abstract

Aims

Epidermal growth factor receptor (EGFR, Her1) and human epidermal growth factor receptor 2 (ERBB2, Her2) are members of the Her-family of transmembrane receptor tyrosine kinases. In various subtypes of sarcomas EGFR and ERBB2 overexpression has been reported. We studied different subtypes of sarcomas for EGFR and ERBB2 expression to evaluate possible candidates likely to benefit from EGFR and ERBB2 blocking therapies.

Methods

A tissue micro-array with 18 different types of soft tissue tumors was constructed, and immunohistochemical (IHC) analyzed for EGFR and ERBB2 expression.

Results

Positive membranous staining for EGFR was seen in various sarcoma subtypes, including liposarcomas (3/20), leiomyosarcomas (3/8), synovial sarcomas (4/5), malignant peripheral nerve sheath tumors (3/7), rhabdomyosarcomas (2/3), solitary fibrous tumors (1/2), and angiosarcomas (1/1). IHC staining for ERBB2 was negative in all subtypes.

Conclusions

Our results demonstrate that IHC staining for EGFR and ERBB2 shows cytoplasmatic staining in many subtypes of sarcomas, and membranous staining for EGFR in multiple subtypes of sarcomas. However, the immunohistochemical presence of growth factor receptors does not necessarily implicate that the subsequent pathway is activated, or is a potential subject to therapy. These results however open the possibility to study the effect of EGFR blocking therapies, and confirm previous results that ERBB2 is not a potential treatment target.

Introduction

Sarcomas are rare and complex malignant tumors of mesenchymal origin, with a broad histopathologic spectrum.[1] Sarcomas represent less than 1% of all malignancies. The overall prognosis of sarcomas depends on the possibility of complete surgical removal of the tumor, while in general the effects of radiation therapy and chemotherapy are limited. The search for new treatment modalities, especially in tumor types resistant to known cancer therapies, is focusing on identification and inhibition of molecular targets, such as growth factor receptors. Recent research has also focused on the Her-family of tyrosine kinases.

Epidermal growth factor receptor (EGFR, Her1) and human epidermal growth factor receptor 2 (ERBB2, Her2) are members of the Her-family of transmembrane receptor tyrosine kinases. Her-kinase activation deregulates growth, desensitizes cells to apoptotic stimuli and regulates angiogenesis. Overexpression of EGFR and ERBB2 is a factor of poor prognosis in a variety of malignancies, including breast cancer, ovarian cancer, and lung cancer.[2,3] Inhibiting EGFR with a tyrosine kinase inhibitor like erlotinib (Tarceva®) in non-small-cell lung cancer patients, with a monoclonal antibody like cetuximab (Erbitux®) in colorectal and head- and neck carcinoma, or blocking ERBB2 with a monoclonal antibody like trastuzumab (Herceptin®) in breast cancer patients are approved treatment options nowadays.

In breast cancer patients a higher level of membranous ERBB2 overexpression is a predictive factor for increased response to treatment with trastuzumab.[4] In normal clinical practice ERBB2 overexpression is classified as negative (0, 1+) or positive (2+, 3+) by immunohistochemical (IHC) staining. For EGFR the correlation between the level of EGFR expression with IHC staining and response to EGFR blocking therapies is not equally clear, sometimes demonstrating activity of EGFR inhibiting therapies in tumors that express low levels of EGFR.[5,6] Therefore EGFR levels are described as negative (0) and positive (1+, 2+, 3+).

In various subtypes of sarcomas EGFR and ERBB2 overexpression has been reported, however the number of studies are limited and sometimes contradictory.[7-18] For a better defining of the sarcoma subtypes that are overexpressing EGFR and/or ERBB2 and are therefore more likely to benefit from EGFR and/or ERBB2 blocking therapy, a tissue micro-array, with 19 different types of soft tissue tumors, was evaluated for EGFR and ERBB2 expression by IHC staining.

Methods

A tissue micro-array (TMA) with 18 different types of soft tissue tumors, was constructed at the Department of Pathology of the Leiden University Medical Center, and used for the

immunohistochemical (IHC) analyses. Triplicate tissue cores with a diameter of 0.6 mm, as selected by two pathologists (SR and PCWH), were taken from each specimen using a tissue arrayer (Beecher Instruments, Silver Springs, MD, USA) and arrayed on a recipient paraffin block, using standard procedures.[19] Table 1 shows the tumor types that are present on the TMA. All leiomyosarcomas were of deep soft tissue origin. Immunohistochemical staining was performed on 5 μm sections of the tissue array, using a paraffin sectioning aid system (Instrumedics Inc, Hackensack, NJ, USA).

Staining for EGFR was performed using the EGFR detection system (Zymed, San Francisco, California, USA), according to the manufacturer's instructions. The samples were dewaxed, rehydrated, and washed with phosphate-buffered saline (PBS). Antigen retrieval with pepsine was employed before incubation with the primary antibody, anti-EGFR (mouse monoclonal antibody, clone 31G7, 1:100). After washing with PBS, the samples were incubated with the biotinylated secondary antibody, followed by incubation with labeled streptavidin-biotin complex. After the final washing with PBS, staining was performed by means of 3,3'-diamino-benzidine (DAB), followed by counterstaining with Mayer–haematoxylin for 30 sec.

Staining for ERBB2 was performed using the ERBB2 detection system (Lab Vision Corporation Fremont, California, USA), according to the manufacturer's instructions. Microwave citric acid antigen retrieval was employed before incubation with the primary antibody, anti- ERBB2 (mouse monoclonal antibody, clone 3B5 1:1000).

As a negative control sections were stained without adding the primary antibody. Positive controls (placenta for EGFR and 3+ overexpressing breast carcinoma for ERBB2) were present on the TMA. Both EGFR and ERBB2 staining was scored as 0 (negative), 1+ (weak), 2+ (moderate) or 3+ (strong), according to the scoring system provided by the manufacturer. For EGFR levels were described as negative (0) and positive (1+, 2+, and 3+). For ERBB2 expression was classified as negative (0, 1+) or positive (2+, 3+).

Slides were examined and scored blind by two of the investigators (NS, SR) independently. Conflicting assessments were reviewed until final agreement was achieved. Where duplicate cores gave discordant results, the higher score was used.

Results

A tissue micro-array with 18 different soft tissue tumors, including 12 types of sarcomas, and 6 types of benign soft tissue tumors (table 1), was immunohistochemically (IHC) stained for epidermal growth factor receptor (EGFR) and human epidermal growth factor receptor 2 (ERBB2, Her2). Not all cases were evaluable, because tissue cores may be lost from the slides. The IHC results are also shown in table 1.

Table 1. Contents of the tissue micro-array (evaluable tissue samples) and results of the immunohistochemically (membranous) staining for epidermal growth factor receptor (EGFR) and ERBB2.

Tissue type	membranous IHC staining		
	EGFR		ERBB2
	positive/ total number cases	positive cells (%)	positive/ total number cases
Liposarcoma	3/20		0/19
Myxoid liposarcoma	2/8	50-75	0/8
Pleomorphic liposarcoma	1/2	25-50	0/2
Dedifferentiated liposarcoma	0/2		0/2
Atypical lipomatous tumor	0/8		0/7
Leiomyosarcoma	3/8	50-75	0/8
MPNST	3/7	50-75	0/6
Synovial sarcoma	4/5	75-100	0/5
Rhabdomyosarcoma	2/3		0/3
Pleomorphic	1/1	75-100	0/1
Embryonal	1/2	0-25	0/2
Gastrointestinal stromal tumor	0/3		0/3
Myxofibrosarcoma	0/3		0/3
Solitary fibrous tumor	1/2	25-50	0/2
Myxoinflammatory fibroblastic sarcoma	0/2		0/2
Dermatofibrosarcoma protuberans	0/2		0/2
Angiosarcoma of soft tissue	1/1	25-50	0/1
Undifferentiated high grade pleomorphica sarcoma	1/4	0-25	0/4
Lipoma	0/5		0/4
Desmoid type fibromatosis	0/4		0/4
Myxoma	0/4		0/4
Schwannoma	0/2		0/2
Synovial chondromatosis	0/1		0/1
Diffuse type giant cell tumor of soft tissue	0/1		0/1

MPNST: malignant peripheral nerve sheath tumor

In all positive cases ERBB2 staining was diffusely present throughout the cell, consistent with cytoplasmatic rather than membranous expression. The positive controls showed clear membranous staining. Positive membranous staining for EGFR was seen in multiple malignant soft tissue tumor types and in one of the benign soft tissue tumors.

Figure 1 shows examples of positive cytoplasmatic staining in ERBB2 (rhabdomyosarcoma) and positive membranous staining in EGFR (solitary fibrous tumor, synovial sarcoma, angiosarcoma).

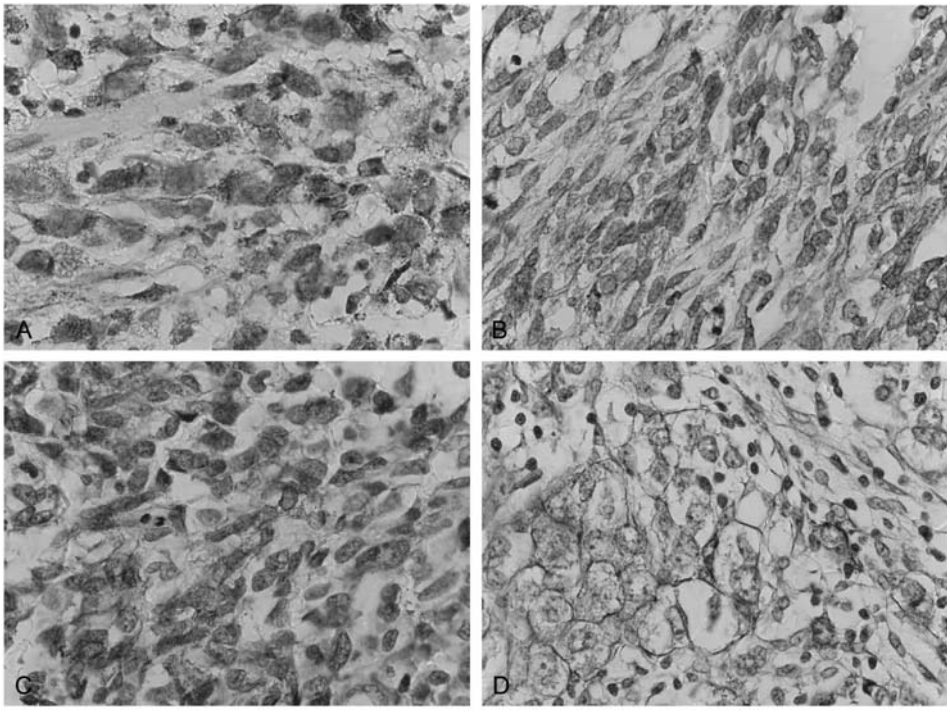

Figure 1. Epidermal growth factor receptor (EGFR) and ERBB2 immunostaining results. All original magnification 630X.
A Diffuse cytoplasmic staining for ERBB2 in rhabdomyosarcoma.
B Membranous staining for EGFR in solitary fibrous tumor.
C Membranous staining for EGFR in synovial sarcoma.
D Membranous staining for EGFR in angiosarcoma.

Discussion

Most sarcomas are resistant to radiotherapy and many commonly used chemotherapy agents, and therefore investigators are searching for other treatment options in sarcoma patients. In the oncology field over the last years there has been great interest in the growth factor receptor blocking therapies. Both the epidermal growth factor receptor (EGFR) inhibitors and ERBB2 inhibitors have been proven beneficial in respectively non-small-cell lung, head- and neck, pancreatic and colorectal cancer (all EGFR) and breast cancer (ERBB2). In sarcoma patients EGFR or ERBB2 inhibitors have never been evaluated systematically.

Our results show positive membranous staining for EGFR in a variety of sarcoma subtypes, including liposarcomas (myxoid and pleomorphic), leiomyosarcomas (intra-

muscular), synovial sarcomas, malignant peripheral nerve sheath tumors (MPNST), rhabdomyosarcomas (pleomorphic and embryonal), solitary fibrous tumors, and angiosarcomas (deep). Membranous IHC staining for ERBB2 was negative in all sarcoma subtypes. These results show that treatment of these EGFR positive subtypes of sarcomas with EGFR blocking therapies can theoretically be effective.

Published reports on EGFR and especially ERBB2 expression in sarcomas have been contradicting.[7-18] Positive IHC staining for EGFR in soft tissue sarcomas ranges from 50 – 100%, with the highest number of positive cases in synovial sarcoma, myxofibrosarcoma, MPNST, and so-called malignant fibrous histiocytoma.[7-9,12,13,20,21] Yang et al reported EGFR positive staining in 3 out of 4 angiosarcomas, which is consisted with our findings. Positive IHC staining for ERBB2 ranges from 0-60%.[7-12,14-16] The results of IHC staining depend highly on the types of antibodies used, the time-span and method of fixation, and the absence of a uniform scoring system leads to a high interobserver variability.[22] This, together with the low number of sarcoma samples investigated, may be the reason for the previously described contradicting results.

In our study EGFR was considered positive when membranous staining was present. In most cases membranous staining was focal rather than diffuse in all malignant cells. When IHC staining for a growth factor receptor is focal a beneficial effect of receptor inhibiting therapies is less likely, while staining is not present in all malignant cells of that patient. However in our study, EGFR and ERBB2 staining was also diffusely present throughout the cell, consistent with cytoplasmatic rather than membrane expression. Previous reports in osteosarcoma cell-lines describe that even in tumors that have no membrane pattern on IHC staining, EGFR and ERBB2 receptors are located on the cell membrane.[9] The difference is that receptor levels expressed are much lower than in the epithelial malignancies in which the IHC staining for EGFR and ERBB2 was first used, which can prevent a clear immunodetection of the protein on the cell surface. Other possible explanations for the cytoplasmatic immunostaining are that the antibody binds to precursor forms of the EGFR/ ERBB2 protein in the cytoplasm or that the activated antibody-EGFR/ ERBB2 complex is internalized.[23]

Previous reports show that EGFR expression is more frequently found in synovial sarcomas than in other soft tissue tumors.[7,17,18] This resulted in the initiation of a phase II trial treating patients with synovial sarcoma with gefitinib therapy (an EGFR small molecule tyrosine kinase inhibitor) by the European Organization for Research and Treatment of Cancer (EORTC). However in recent reports in IHC positive synovial sarcomas, and other soft tissue sarcomas, there was no EGFR gene amplification seen by FISH, and positive effects of gefitinib therefore seemed less likely.[20,24] The results of this Phase II trial were reported recently and no substantial benefit of gefitinib monotherapy compared to conventional chemotherapy was seen.[25]

Questions are still rising whether IHC staining for EGFR is the correct method to evaluate EGFR status.[5] It is possible that other markers, like the level of activated phosphorylated EGFR or the presence of activating EGFR mutations, are more important. In non-small cell lung cancer (NSCLC) total EGFR expression does not relate to other clinical prognostic indicators, and does not relate to clinical response to EGFR inhibitors like erlotinib or gefitinib.[26] Phosphorylated EGFR (p-EGFR), EGFR gene copy number, and EGFR activating mutations have been shown to be better markers then EGFR overexpression by IHC for prediction of poor prognosis in NSCLC.[27,28] Future research should focus on the determination of markers that can predict a favorable outcome, like the association between activating mutations in the ATP-binding site of EGFR and response on gefitinib (Iressa) in non-small-cell lung cancer patients, or the association between KRAS mutations and resistance to cetuximab (Erbitux®) in colorectal carcinoma patients.[29,30]

In breast cancer there is a correlation between ERBB2 gene amplification and ERBB2 protein overexpression.[2,31] Studies in other tumor types, including osteosarcoma, Ewing sarcoma and synovial sarcoma report a ERBB2 protein overexpression with positive, mainly cytoplasmatic, IHC staining without amplification of ERBB2 gene by fluorescence in situ hybridization (FISH) evaluation, indicating that ERBB2 overexpression can be independent from gene amplification.[16,17] There was no effect of trastuzumab (ERBB2 inhibition) in osteosarcoma and Ewing sarcoma cell-lines.[15,17] Further studies are required to increase insight in the role of IHC and FISH evaluation of ERBB2 as predictive markers for response to ERBB2 inhibiting therapies in sarcomas.

Our results demonstrate that IHC staining for EGFR and ERBB2 shows cytoplasmatic staining in many subtypes of sarcomas, and membranous staining for EGFR in multiple subtypes of sarcomas. Therefore there is a possibility that sarcoma patients may benefit from EGFR inhibiting therapies. Benefit from ERBB2 blocking therapies is highly unlikely.

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