Genomic amplification and high expression of EGFR are key targetable oncogenic events in malignant peripheral nerve sheath tumor

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Abstract

Background: The dismal outcome of malignant peripheral nerve sheath tumor (MPNST) highlights the necessity of finding new therapeutic methods to benefit patients with this aggressive sarcoma. Our purpose was to investigate epidermal growth factor receptor (EGFR) as a potential therapeutic target in MPNSTs.

Patients and methods: We performed a microarray based-comparative genomic hybridization (aCGH) profiling of two cohorts of primary MPNST tissue samples including 25 patients treated at the University of Texas MD Anderson Cancer Center (MD Anderson) and 26 patients from Tianjin Medical University Cancer Institute & Hospital (TMUCIH). Fluorescence in situ hybridization (FISH) method was used to validate the gene amplification detected by aCGH analysis. Another independent cohort of 56 formalin fixed paraffin embedded (FFPE) MPNST samples was obtained to explore EGFR protein expression by immunohistochemical analysis. Cell biology detection and validation were performed on human MPNST cell lines ST88-14 and STS26T.

Results: aCGH and pathway analysis of the 51 MPNSTs identified significant gene amplification events in EGFR pathway, including frequent amplifications of EGFR gene itself, which was subsequently validated by FISH assay. High expression of EGFR protein was associated with poor disease-free and overall survival of human MPNST patients. In human MPNST cell lines ST88-14 and STS26T, inhibition of EGFR by siRNA or Gefitinib led to decreased cell proliferation, migration, and invasion accompanied by attenuation of PI3K/AKT and MAPK pathways.

Conclusion: These results suggest that EGFR is a potential therapeutic target for MPNST.

Keywords: Malignant peripheral nerve sheath tumor, Epithelial growth factor receptor, Targeted therapy, Microarray-based comparative genomic hybridization, Gene amplification, Gefitinib
is a target for the existing anti-EGFR therapeutics in several types of cancers, such as Gefitinib and Erlotinib in lung cancer [8]. EGFR has been implicated in promoting peripheral nerve tumor formation and malignant transformation in neurofibromatosis type 1 (NF-1)-associated MPNST [2]. Furthermore, tumor-sphere formation requires signaling from EGFR tyrosine kinase, also exemplifying the importance of EGFR in neurogenic tumor transformation [9]. In a mouse model reminiscent of neurofibroma, EGFR blockade consistently prevented peripheral nerve disruption [10]. Along with these findings, the finding by Keizman and colleagues that EGFR expression has prognostic value in both NF-1-associated and sporadic MPNST suggests that EGFR-targeted therapy may be a potential treatment for MPNST [11].

With the working hypothesis that EGFR expression is a key targetable oncogenic event in MPNST, we performed aCGH profiling on 51 primary MPNST tissues. In addition, EGFR amplification status was specifically probed by fluorescence in situ hybridization (FISH) in 26 samples out of the 51 tissues. Another independent cohort of 56 formalin fixed paraffin embedded (FFPE) MPNST samples was obtained to explore EGFR protein expression by immunohistochemical analysis. We examined the effects of EGFR inhibition on cell proliferation and EGFR-associated downstream pathways in two human MPNST cell lines, ST88-14 and ST88-14. The findings from our integrated genomic and molecular studies suggest that EGFR is a potential therapeutic target for patients with MPNST.

Results
MPNST in diverse populations exhibited similar recurrent genetic aberrations that significantly altered multiple signaling pathways
We first compared the genomic aberrations of the two cohorts from Tianjin Medical University Cancer Institute & Hospital (TMUCIH) and the University of Texas MD Anderson Cancer Center (MD Anderson) (Figure 1A and B). The most significant difference is the higher overall aberration rate in the American patients, although the overall pattern of aberrations remains similar. The cause of the difference is unknown, possibly related to ethnicity and the minor differences in aCGH measurements between the institutions.

We also investigated the translational relevance of these genes by correlating the loci with several clinical parameters such as tumor location, clinical AJCC (American Joint Committee on Cancer) stage of tumor, tumor size, local recurrence, metastasis, and survival between the two cohorts. As reported in previous paper [3], we could not associate any individual aberration with patient survival, suggesting that multiple events might co-occur to affect survival. However, correlating the overall frequency of CNAs with survival did not implicate increased genomic instability in inducing statistically significant survival effects [3].

Given the minor difference in global aberration rates, we maintain that the cohorts were comparable and can be combined for the pathway analyses. Combining the cohorts from different institutions is critical because acquisition of MPNST samples is technically difficult.

Integration of copy number profiles of the 51 individual samples yielded 4901 frequent deletions and 2599 amplifications in the primary MPNST tissue samples [3]. The most frequent deletion was 9p21.3 (harboring tumor suppressors cyclin-dependent kinase inhibitor 2A and 2B), with approximately 65% of patients affected. To investigate the potential effects of these alterations at the signaling pathway level, we computed pathway enrichment scores for each pathway by a method as reported previously [3,12]. This analysis identified 11 pathways that were statistically significantly altered in MPNST, including TFF, ERK, ARF, IGF1R, and EGFR signaling pathways. Taking into account previous reports that IGF1R pathway is a potential therapeutic target for MPNST patients, and the cross-talk between IGF1R and EGFR signaling pathways was detected in other types of cancers [3,13-17], the great success of EGFR targeted therapy in lung cancer prompted us to put emphasis on the EGFR pathway in this analysis, with the hypothesis that the EGFR signaling pathway is a potential therapeutic target and that blocking both IGF1R and EGFR simultaneously in MPNST might result in a synergistic antitumor effect.

Extensive EGFR pathway alterations and high expression of EGFR protein correlated with shorter patient survival EGFR, amplified in 37% (19/51) of our samples, has been suggested as a potential target in MPNST [18-20]. The comparison of the two cohorts indicated that the frequency of EGFR amplification did not differ significantly between TMUCIH samples (35%) and MD Anderson samples (40%). In addition to EGFR, we investigated the frequency (Figure 1C) and pattern (Figure 1D) of gene alterations in the EGFR signaling pathway genes. At least one EGFR pathway gene was altered in 84% of the samples. Some of the most significantly aberrated genes included growth factor receptor-bound protein 2 (GRB2) (amplified in 31%), Harvey rat sarcoma viral oncogene homolog (HRAS) (deleted in 35%), and mitogen-activated protein kinase 1 (MAPK1) (deleted in 41%) in ERK signaling branch, v-akt murine thymoma viral oncogene homolog 1 (AKT1) (deleted in 31%) in AKT signaling branch, and Janus kinase 2 (JAK2) (deleted in 47%) in JAK-STAT signaling branch (Figure 1C).

Interestingly, we found that there were a few co-aberrated genes in EGFR signaling pathway such as signal transducer and activator of transcription 1 (STAT1), CAMP responsive element binding protein 1 (CREB1), epidermal growth factor (EGF), nuclear factor of kappa light polypeptide gene...
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Figure 1 Copy number alterations in 26 MPNST samples from Tianjin Medical University Cancer Institute & Hospital (TMUCIH) and 25 MPNST samples from MD Anderson Cancer Center and genetic amplifications of the EGFR signaling pathway, including those of the EGFR gene itself. (A,B) Recurrent gene copy alteration patterns in 26 MPNST from TMUCIH patients (A) and 25 MPNST from MD Anderson Cancer Center patients (B). The x-axis numbered with 1–22 denotes chromosome numbers. The y-axis shows recurrence of gains (positive axis) and losses (negative axis) for each measured locus evenly distributed in chromosomal order. Recurrence rates that exceed the threshold (dashed line) are color-coded to emphasize the locations of significantly recurrent aberrations. Red denotes significantly recurrent amplifications and green denotes significantly recurrent deletions. Gray represents nonsignificant recurrence of aberrations. (C) Aberration rates of EGFR signaling pathway genes in all 51 MPNST samples. The number on the left side is the deletion frequency (Del) of the gene, and the number on the right side is the amplification rate (Amp). Color-coding indicates the type and frequency of aberration (red amplified, green deleted). (D) Hierarchical clustering of aberrations of EGFR signaling pathway genes in all 51 MPNST samples showed a panoramic view of genetic aberrations in EGFR signal pathway. Red means amplification and green means deletion.

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Inhibition of EGFR in STS26T and ST88-14 decreased tumor cell proliferation, invasion, and migration by blocking activation of AKT and PI3K pathway signaling

The next step in our investigation of EGFR as potential therapeutic target in MPNST was to evaluate the effect of EGFR inhibition in human MPNST cell lines STS26T and ST88-14. In the in vitro STS26T cell culture system, EGFR siRNA significantly decreased expression of EGFR and its phosphorylated form (Figure 3A). At the same time, this inhibition of EGFR expression significantly decreased the expression of the activated forms of AKT and PI3K signal pathway components pPI3K, pAKTS473, pERK, and pBad (Figure 3A). Functional experiments showed that inhibition of EGFR significantly reduced cell proliferation (Figure 3B), invasion (Figure 3C and D), and migration (Figure 3E and F) in contrast to the control siRNA. Similarly, in ST88-14 cells, the EGFR siRNA significantly decreased the expression of EGFR, phosphorylated EGFR, and the activated forms of AKT and PI3K signal pathway components, as well as tumor cell proliferation, invasion, and migration (Figure 4A–F).

To investigate the therapeutic role of EGFR in MPNST, STS26T and ST88-14 cells were treated with EGFR tyrosine kinase inhibitor Gefitinib. Gefitinib (ZD1839) is often referred to as a “specific” or “selective” inhibitor of EGFR and the maximum plasma concentrations resulting from clinically relevant doses are 0.5–1 μM or more, well within the IC50 values of several tyrosine kinases [24]. However, the selectivity of Gefitinib for inhibition of EGF-driven cell growth was demonstrated by the large difference in IC50 in the presence or absence of EGF, such as cytotoxicity was not observed at Gefitinib concentrations up to 25 μM [24]. To get the effective concentration in MPNST cell line, IC50 data were interpolated by nonlinear regression.
(four-parameter logistic equation) using Microcal Origin software (version 3.78; Microcal Software Inc., Northampton, MA). At a median inhibitory concentration (IC\textsubscript{50}) of 10 μM, Gefitinib significantly inhibited STS26T cell proliferation in the presence of EGF (10 ng/ml) (Figure 5A-C). Furthermore, Gefitinib inhibited the activation of EGFR by decreasing expression of pEGFR\textsubscript{Y1068} (Figure 5C), along with decreases of pPI3K, pAKT\textsubscript{S473}, pERK, and pBad (Figure 5D). In ST-8814 cells, Gefitinib treatment had similar effects [3].

**Inhibition of EGFR did not induce activation of IGF1R signaling pathway**

Inspired by the reported cross-talk between the IGF1R and EGFR signaling pathways [13-17], we blocked both EGFR and IGF1R in MPNST cells to evaluate the possibility of synergistic or antagonistic effects. Because no IGF1R protein expression was detected in STS26T, while ST88-14 cells expressed both IGF1R and EGFR, we chose ST88-14 cells to explore the effect of inhibiting EGFR and IGF1R individually and in combination. In ST88-14 cells, inhibition of EGFR with siRNA or Gefitinib did not induce activation of the IGF1R signal pathway. Furthermore, inhibition of EGFR and IGF1R with siRNA or Gefitinib/MK-0646 did not induce any synergistic effects [3].

**Discussion**

MPNST occurs either sporadically or in association with NF-1, and in 2002 the World Health Organization coined the term “malignant peripheral nerve sheath tumor” to
replace previous heterogeneous and often confusing terminologies [25]. It is a highly malignant sarcoma for which more effective therapeutic strategies are urgently needed [26]. In this study, we carried out genomic and molecular studies of MPNST, both human tumors and cell lines, to identify potential therapeutic targets. Our findings

Figure 3 Down-regulation of EGFR by EGFR siRNA in STS26T MPNST cells significantly decreased tumor cell proliferation, invasion, and migration by blocking the PI3K/AKT and MAPK pathways. (A) EGFR siRNA decreased EGFR expression and activation. Activated forms of PI3K/AKT and MAPK pathway factors decreased with EGFR inhibition. (B) EGFR siRNA significantly decreased tumor cell proliferation compared with the nonspecific control siRNA. (C, D) EGFR siRNA significantly decreased tumor cell invasion compared with the nonspecific control siRNA: cell invasion (C) and cell counts (D). (E, F) EGFR siRNA significantly decreased tumor cell migration compared with the nonspecific control siRNA: cell migration (E) and cell counts (F). * indicate P-values <0.05.
Figure 4 Down-regulation of EGFR by EGFR siRNA in ST88-14 MPNST cells significantly decreased tumor cell proliferation, invasion, and migration by blocking PI3K/AKT and MAPK pathways. (A) EGFR siRNA decreased EGFR expression and activation. Activated forms of PI3K/AKT and MAPK pathway factors decreased with EGFR inhibition. (B) EGFR siRNA significantly decreased tumor cell proliferation compared with the nonspecific control siRNA. (C, D) EGFR siRNA significantly decreased tumor cell invasion compared with nonspecific control siRNA: cell invasion (C) and cell counts (D). (E, F) EGFR siRNA significantly decreased tumor cell migration compared with nonspecific control siRNA: cell migration (E) and cell counts (F). * indicate P-values <0.05.
not only provide evidence of genetic aberrations of the EGFR signaling pathway in these tumors, but also indicate that genomic amplification and high expression of EGFR are key targetable oncogenic events in MPNST patients.

Among a number of studies that implicated EGFR as an important molecule in MPNSTs, the most important contribution of our investigation is the exhaustive demonstration of the genetic evidence that the EGFR signaling pathway can serve as a potential therapeutic target in MPNST. EGFR expression in neurogenic tumors has been reported by several investigators, and the data showed that it is a very important receptor in neurofibromatosis 1, neurofibroma, and MPNST [11,19,27,28]. The improving understanding of the role of EGFR in the pathogenesis of MPNST, the limitations of available treatments for MPNST, and the successful use of EGFR-targeted therapy in non-small cell lung cancer make a strong case for EGFR as a potential therapeutic target in MPNST [11,19,27,28]. Huang and colleagues reported that the MPNST cell lines from the NF-1-p53 mouse model can be blocked by an antagonist of EGFR or inhibition of its downstream target PI3K [29]. Holtkamp and colleagues observed dose-dependent inhibition of MPNST cell proliferation mediated by erlotinib, an EGFR-targeted tyrosine kinase inhibitor [19]. By now, nine of the approximately 55 finished or ongoing clinical trials in MPNST are phase I-III clinical trials involving a tyrosine kinase inhibitors such as imatinib, erlotinib, PLX3397, dasatinib, sunitinib, and sorafenib (http://clinicaltrials.gov/ct2/results?term=MPNST&Search=Search) [30-33]. In the present study, integrated genetic and molecular profiles confirmed genetic alterations of EGFR signaling pathway, including amplification of EGFR gene itself and the high protein expression of EGFR, are key targetable oncogenic events in MPNSTs. Our solid genetic data including aCGH, pathway analysis, and FISH validation provided genetic evidence of this target therapy.

The reported rates of EGFR protein expression in MPNST vary from 43% to 86% [2,11,27,28]. This variation in expression pattern might have been due to several factors; the most important one might be the gene dosage of EGFR. In the study by Holtkamp et al., FISH analysis revealed increased EGFR dosage in 28% of MPNST, and level of EGFR protein expression was significantly associated with increased EGFR gene dosage [19]. In the present study, the level of EGFR protein expression was also correlated to EGFR gene amplification as evaluated by FISH and immunohistochemical assays, indicating that EGFR dosage plays an important role in aberrant EGFR protein expression. However, Tabone-Eglinger et al. detected EGFR expression in 86% of MPNST and no amplification of the EGFR locus, and the EGFR expression was
more frequent in NF-1 specimens and was closely associated with high-grade and p53-positive areas [28,34]. Therefore, other factors might be involved in EGFR expression, such as NF-1, p53 mutation, and MDM2 expression [27,28,34]. EGFR gene mutation also may be one of the factors, in MPNST a portion of EGFR expression appears as EGFR VIII and is linked to exon 17–21 deletion [27]. Somatic mutations of the EGFR gene were more sensitive to Gefitinib, being completely inhibited at 0.2 μmol/L, whereas wild-type EGFR required 2 μmol/L gefitinib for complete inhibition [35]. In this sense, EGFR expression and/or mutational status, which had been frequently observed, might be proposed as signatures to identify MPNST patient subtypes that might be more sensitive to EGFR targeted therapy.

Inhibition of EGFR in colon carcinoma cells promotes activation of the IGF1R signaling pathway, and inhibition of EGFR-directed MAPK shifts regulation of Akt from EGFR toward IGF1R [15]. Furthermore, acquired resistance to EGFR tyrosine kinase inhibitors in cancer cells is mediated by loss of IGF-binding proteins, as was shown in A431 squamous cancer cells [36]. In rhabdomyosarcoma cell line Rh36, which is resistant to BMS-536924 (a small molecule inhibitor of IGF1R), combined analysis of targeting EGFR and IGF1R pathways revealed enhanced inhibitory activities [29]. However, in neither the present study nor our previous study was any additive antitumor effect observed with combined inhibition of IGF1R and EGFR, suggesting a lack of cross-talk between IGF1R and EGFR pathways in MPNST [3]. Thus, any insight and conclusions drawn from these cell line results would need more circumspect investigations considering several issues such as tumor types, culture conditions, and the host environment. Therefore, our investigation of EGFR/IGF1R-targeted therapy highlighted the urgent need to clarify the possible crosstalk mechanisms in MPNST.

In summary, integrated genetic and molecular profiles confirm genetic alteration of the EGFR signaling pathway, including amplification of the EGFR gene itself and the high expression of EGFR protein, as potential key targetable oncogenic events in MPNST. Inhibition of EGFR in vitro induced inhibition of MPNST tumor cell proliferation, invasion, and migration via inhibition of the PI3K/AKT and MAPK pathways. Though need more investigation and clinical trials to confirm, these findings suggested that inhibition of EGFR might be a valid therapeutic choice, supplementing routine treatments such as surgery and radiotherapy for MPNST patients.

Materials and methods

Patients and primary tumors

Fifty-one archived MPNST samples and matching patient records were acquired from The University of Texas MD Anderson Cancer Center (MD Anderson; 25 FFPE tumor samples) and Tianjin Medical University Cancer Institute & Hospital (TMUCIH; 26 fresh-frozen tumor samples with matched FFPE tissues) [3]. All samples were evaluated by two pathologists (one from each institution) to confirm the diagnosis and ensure that each specimen contained at least 90% of tumor. TMUCIH cohort was also used for FISH validation. An independent cohort of 56 FFPE tumor samples was acquired from TMUCIH for immunohistochemical validations only.

Patient information collected included age, sex, tumor location, largest diameter of the tumor, clinical AJCC (American Joint Committee on Cancer) stage of the tumor, time to recurrence, metastatic status, treatments, and outcome [3]. The presence of the NF-1 syndrome was determined by the NIH criteria [37]. Tissue and information collection for this retrospective study were approved by the Institutional Review Boards (IRBs) at Tianjin Medical University Cancer Institute & Hospital (TMUCIH) and The University of Texas, MD Anderson Cancer Center and with patients’ consent.

Array CGH hybridization and bioinformatic analysis

The genome-wide copy number levels were mapped by aCGH for the 51 primary tumor samples using commercially available normal genomic DNAs as reference (Clontech Laboratories, Inc., Mountain View, CA) [3]. The tumor genomic DNAs were isolated according to standard procedures and the labeled genomic DNAs were hybridized by using the Agilent 4 × 44 k Human Genome CGH Microarray kit (Agilent Technologies, Santa Clara, CA). The aCGH data analysis was conducted as described previously [3,12].

FISH analysis

The Vysis LSI EGFR SpectrumOrange/CEP 7 SpectrumGreen Probe kit was used for the FISH detection of EGFR (Abbott Laboratories, Abbott Park, IL). The CEP 7 probe showing green signal indicates the chromosome 7 centromere, and the EGFR probe shows orange signal representing the EGFR gene copy number.

Twenty-six FFPE tissues of 51 samples from TMUCIH were subjected to FISH (matching fresh-frozen MPNST tissues were used in the aCGH analysis) as described in our previously published paper [12]. Staining of experimental slides was accompanied by concurrent staining of positive and negative control slides to monitor assay performance and to assess the accuracy of signal enumeration.

Alterations of EGFR gene copy number were evaluated according to the established methods by two pathologists in a blinded fashion [12,38,39]. In the informative cases (>90% of nuclei showed hybridization signals), the presence of more than two orange and green signals in each tumor cell with a ratio of orange signals to green signals greater than 1 was considered focal EGFR amplification.
The presence of more than two orange and green signals in each tumor cell with a ratio equal to 1 was considered large-fragment amplification. The presence of only two orange and green signals in each tumor cell or a ratio less than 1 was considered no EGFR amplification.

**Immunohistochemical analysis**

EGFR protein expression was detected in another independent TMUCIH cohort of 56 FFPE tissues by immunohistochemical methods using the EGFR antibody (Santa Cruz Biotechnology, Santa Cruz, CA) in 1:100 dilutions as described previously [3,16,17]. Nonimmune rabbit serum at the same concentration was used as negative control. The expression levels of EGFR were estimated according to criteria previously reported [17,40]. Scoring was performed according to the percentage of positive cells: <5% was classified as negative, 6-30% was classified as a weak positive, 31-60% as a moderate positive, and >60% as a strong positive. In the survival analysis, the negative and weak positives were considered low EGFR expression, the moderate and strong positives as high EGFR expression.

**Cell culture and reagents**

MPNST cell lines ST88-14 and STS26T were authenticated by short tandem repeat DNA fingerprinting. The ST-8814 line is NF-1/s and STS26T is NF-1/s. The cell lines were maintained in Eagle’s minimum essential medium and incubated at 37°C in a humidified atmosphere containing 7.5% CO₂. Gefitinib was stored at −20°C as a 20 mM concentration solution in dimethyl sulfoxide (DMSO). MK-0646, a monoclonal antibody against insulin growth factor-1 receptor (IGF1R), was dissolved in sterile water at a concentration of 20 mg/mL and stored at −20°C.

**Small-interfering RNA transfections**

For the siRNA studies, an EGFR siRNA (sc-29301, Santa Cruz Biotechnology) previously proven specific and effective was used to block EGFR expression in MPNST cells according to the manufacturer’s instructions. Because of the cross-talk of the IGF1R and EGFR pathways [13-15], a smart pool of three double-stranded siRNAs against IGF1R (NM-000875) was used as previously reported [3,12,40]. In all siRNA transfection experiments, nonspecific siRNA (D-001206-01-05) purchased from Dharmacon (Lafayette, CO) was used as a control.

**Western blot analysis and cell proliferation, invasion, and migration assays**

Western blot analysis of treated MPNST cells was performed as previously described [3]. Antibodies to EGFR, AKT, PI3K, IRS-1 ERK, and their phosphorylated forms were obtained from Abcam (Cambridge, MA), Sigma Chemical (St. Louis, MO), Santa Cruz Biotechnology, and Cell Signaling Technology (Beverly, MA). Cell proliferation was analyzed by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay, and cell invasion and migration were analyzed by Transwell migration assays (EMD Biosciences, San Diego, CA) according to procedures reported previously [3].

**Statistical analyses**

The statistical analyses were performed as described previously [3,12]. The SPSS software (version 16.0; SPSS, Chicago, Ill) and Matlab (R2012b 64-bit, MathWorks Inc, Natick, MA) were used in the analyses. The clinical and pathologic features of the 25 MD Anderson and 26 TMUCIH MPNST cases were compared via the chi-square test, an analysis of variance, the Student’s t-test, or the Fisher’s exact test, as appropriate. The relationships between survival rates and EGFR gene amplification or EGFR protein expression were evaluated by comparing the differences of Kaplan-Meier survival estimators by Mantel-Cox test. Associations between copy number alterations and clinical variables were computed by using the Fisher’s exact test. Enrichment analysis was performed on the genes that were either amplified or deleted in at least 20% of the samples by a standard hypergeometric test. Enrichment P-values were computed for all signaling pathways included in Biocarta (http://www.biocarta.com/). A value less than 0.05 was considered as the threshold of statistical significance in all tests.

**Competing interests**

The authors have declared no conflicts of interests.

**Authors’ contributions**

JY, XD and AY carried out the genetic studies, molecular experiments, participated in the aCGH analysis and drafted the manuscript. AY, ZZ and JY designed the experiments and edited the manuscript. All authors read and approved the final manuscript.

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References

1. Lin CT, Huang TW, Nieh S, Lee SC: Treatment of a malignant peripheral nerve sheath tumor. Oncology 2009, 32:503–505.

2. Zou C, Smith KD, Liu J, Zheng H, Chen K, Nykter M, Trent J, Ratner N, Lev DC, Zhang W: Genomic and molecular characterization of malignant peripheral nerve sheath tumor identifies the IGFIR pathway as a primary target for treatment. Clin Cancer Res 2011, 17:7563–7573.

3. Yu J, Dethmsahl H, Payton JE, Dunham C, Scheithauer BW, Thitan P, Prason RA, Guha A, Bridge JA, Ferner RE, et al: Array-based comparative genomic hybridization identifies CDK4 and FOXM1 alterations as independent predictors of survival in malignant peripheral nerve sheath tumor. Clin Cancer Res 2011, 17:2942–1943.

4. Kresse SH, Skam M, Oanh TD, Nomlos HM, Bjerkehsagen B, Myklebost O, Meza-Zepeda OA: DNA copy number changes in high-grade malignant peripheral nerve sheath tumors by array CGH. Mol Cancer 2008, 7:469.

5. Meningrappada K, Susick G, Kluwe L, Chushanov N, Ferner RE, Frayling IM, Dumaspi J, Guha A, Mautner V, Upadhyaya M: High-resolution DNA copy number profiling of malignant peripheral nerve sheath tumors used targeting microarray-based comparative genomic hybridization. Clin Cancer Res 2008, 14:1015–1024.

6. Endo M, Kobayashi C, Seto H, Takahashi Y, Kobashi K, Yarnamoto H, Tamiya S, Matuda S, Iwamoto Y, Tsurenushi M, Oda Y: Prognostic significance of p14ARF, p15INK4B, and p16INK4A inactivation in malignant peripheral nerve sheath tumors. Clin Cancer Res 2011, 17:3771–3782.

7. Lee Y, Shin HG, Park MS, Kim JH, Ha SJ, Kim SH, Cho BC: High EGFR gene copy number and skin rash as predictive markers for EGFR tyrosine kinase inhibitors in patients with advanced squamous cell lung carcinoma. Clin Cancer Res 2012, 18:1760–1766.

8. Williams JPW, Johanssen G, Gori TA, Miller SC, Geiger H, Malin L, Li Y, Mokuryaue YS, Canela JA, Ratner N: NF1 mutation expands an EGFR-dependent peripheral nerve progenitor that cooperates with neurofibromin tumorigenic potential. Cell Stem Cell 2008, 3:585–596.

9. Wu J, Ciminini JT, Monk KR, Williams JP, Fitzgerald JF, Tellesco S, Norm K: Perinatal epidermal growth factor receptor blockade prevents peripheral nerve disruption in a mouse model reminiscent of benign world health organization grade I neurofibroma. Am J Pathol 2011, 178:169–1696.

10. Reiman D, Isakov J, Moller I, Maimon Y, Ish-Shalom-Singer O, Merikins O: Expression and significance of EGFR in malignant peripheral nerve sheath tumor. J Neurooncol 2009, 94:385–391.

11. Yang J, Yang D, Sun Y, Sun B, Gu G, Tang J, Araclio DM, Chen K, Zhang W: Genetic amplification of the vascular endothelial growth factor (VEGF) pathway genes, including VEGFA, in human osteosarcoma. J Neurooncol 2009, 93:355–366.

12. Ueda S, Hattori A, Yodashi H, Ogata K, Kawarabayashi N, Takigawa T, Einama T, Monta D, Fukuda K, Sato T, et al: Potential crosstalk between insulin-like growth factor-I receptor and epidermal growth factor receptor in progression and metastasis of pancreatic cancer. Mod Pathol 2006, 19:29–39.

13. Bredeman MC, Takitchi M, Solhail M, Macaulay VM: The EGFR receptor interacts with type I IGF receptor and regulates its stability. Biochem Biophys Res Commun 2007, 355:707–714.

14. Liu Y, Hsu SF, Panaviacius M, Li W, Hauser J, Humphrey LE, Brattain MG: Aberration of receptor function in colon carcinoma cells determined by crosstalk between insulin-like growth factor-I receptor and epidermal growth factor receptor. Cancer Res 2008, 68:8004–8013.

15. Rudovini V, Bellezza G, Pistola L, Bianconi F, Di Carlo L, Sidini A, Serenaro A, Del Sordo R, Tofanetti FR, Mameli MG, et al: High coexpression of both insulin-like growth factor receptor-1 (IGFIR)-1 and epidermal growth factor receptor (EGFR) is associated with shorter disease-free survival in resected non-small-cell lung cancer patients. Ann Oncol 2009, 20:842–849.

16. Kauffuss S, Burfleid P, Gaedcke J, Schaf JG: Dual silencing of insulin-like growth factor-I receptor and epidermal growth factor receptor in colorectal cancer cells is associated with decreased proliferation and enhanced apoptosis. Mol Cancer Ther 2009, 8:821–833.

17. Kohli L, Kaza N, Lavalley NJ, Turner KL, Byer S, Carroll SL, Roth KA: The pan erbB inhibitor PD168393 enhances lysosomal dysfunction-induced apoptotic death in malignant peripheral nerve sheath tumor cells. Neuro-oncology 2012, 14:266–277.

18. Holkamp N, Malzer E, Zieroth J, Ondukova AF, Mucha J, Mawrin C, Mautner F, Schildhaus HU, von Deimling A: EGFR and erb2 in malignant peripheral nerve sheath tumors and implications for targeted therapy. Neuro-oncology 2008, 10:946–957.

19. Dilworth JT, Wujick MJ, Mathieu P, Taizska MA, Reiners J, Mottingly RR, Hancock CN: Suppression of proliferation of two independent, malignant peripheral nerve sheath tumor cell lines by the pan-inhibitor Cr1033. Cancer Biol Ther 2008, 7:1938–1946.

20. Pilay V, Allaf L, Wilding AL, Donoghue JJ, Court NW, Small SA, Jastrz BM, Johns TG: The plasticity of oncogene addiction: implication for targeted therapies directed to receptor tyrosine kinases. Neoplasia 2008, 11:348–453. 442 p following 458.

21. Kawaguchi K, Murakami H, Taniguchi H, Fujimura M, Kawata T, Fukui T, Kondo Y, Osada H, Usami N, Yokoi K, et al: Combined inhibition of MET and EGFR suppresses proliferation of malignant melanotheloma cells. Carcinogenesis 2009, 30:1097–1105.

22. Peghini PL, Iwamoto M, Pirke SA, Chen YJ, Ceelb SJ, Serrano A, Jensen RT: Overexpression of epidermal growth factor and hepatocyte growth factor receptors in resection of gastrinomas correlates with aggressive growth and poor curability. Clin Cancer Res 2002, 8:8273–8285.

23. Cohen MH, Williams LM, Hamblin R, Chen G, McGuinn WD Jr, Morse D, Abraham S, Rahmalia C, Udango G, Loffredo R, et al: United States Food and Drug Administration Drug Approval summary: Gefitinib (ZD1839; Iressa) tablets. Clin Cancer Res 2004, 10:1212–1218.

24. Gupta R, Mahalinga M, Manier A: Malignant peripheral nerve sheath tumors. Neurosurg Clin N Am 2008, 19:533–543.

25. Tang J, Du J: Genomic and molecular aberrations in malignant peripheral nerve sheath tumor and their roles in personalized target therapy. Surg Oncol 2013, 22:653–657.

26. Tabone-Eglinger S, Bahleda R, Cote JF, Terrier P, Vidaud D, Cayre A, Beauchet A, Theou-Anton N, Terrier-Lacombe M, Lemoine A, et al: Frequent EGFR Positivity and Overexpression in High-Grade Areas of Human MPNSTs. Sarcoma 2008, 2008:49156.

27. Huang F, Greer A, Hurwitl W, Han X, Hafizi R, Wittenberg GM, Reeves K, Chen J, Robinson D, Li A, et al: The mechanisms of differential sensitivity to an insulin-like growth factor-I receptor inhibitor (BMS-536924) and rationale for combining with EGFR/HER2 inhibitors. Cancer Res 2008, 69:161–170.

28. Sbiglia V, Spols J, Hong DC, Ketson LM, Hamilton J, McCueon FE, Kurczok R: Treatment of patients with advanced neurofibromatosis type 2 with novel molecularly targeted therapies: from bench to bedside. J Clin Oncol 2013, 31:64–648.

29. Maki RG, D’Adamo DR, Keohan ML, Saulle M, Schuetze SM, Undevia SD, Livingston NB, Cooney MW, Himsley ML, Mita MW, et al: Phase II study of sorafenib in patients with metastatic or recurrent sarcomas. J Clin Oncol 2009, 27:1313–1319.

30. George S, Merriam P, Maki RG, Van der Abbeele AD, Yap JT, Akhurst T, Hamond DC, Buchar G, O’Mara MM, D’Adamo DR, et al: Multicenter phase II trial of sunitinib in the treatment of nongastrointestinal stromal tumor sarcomas. J Clin Oncol 2009, 27:1314–1316.

31. Chugh R, Wathen JK, Maki RG, Benjamin RS, Patel SR, Meyers PA, Prebhat OA, Reineke DK, Thomas DG, Keohan ML, et al: Phase II multicenter trial of imatinib in 10 histologic subtypes of sarcoma using a bayesian hierarchical statistical model. J Clin Oncol 2009, 27:1318–13135.

32. Carroll SL, Ratner N: How does the Schwann cell lineage form tumors in NF1? Genes 2008, 56:1590–1605.

33. Lynch TJ, Bell DW, Sordella R, Gurubhagavatula S, Liokets MA, Brannigan BW, Harris PL, Haselrat LM, Hamilton J, McCueon FE, et al: Activating mutations in the epidermal growth factor receptor underlying responsiveness of non-small-cell lung cancer to gefitinib. N Engl J Med 2004, 350:2129–2139.

34. Guin X, Faber AC, Wang SC, Olives MG, Song Y, Qu S, Rinearh C, Seidel B, Yee D, Arteaga CL, Engelman J: Acquired resistance to EGFR tyrosine kinase inhibitors in cancer cells is mediated by loss of IGF-binding proteins. J Clin Invest 2008, 118:2609–2619.
37. Anghileri M, Miceli R, Fiore M, Mariani L, Ferrari A, Mussi C, Lozza L, Collini P, Olmi P, Casali PG, et al: Malignant peripheral nerve sheath tumors: prognostic factors and survival in a series of patients treated at a single institution. Cancer 2006, 107:1065–1074.

38. Freeman SS, Allen SW, Ganti R, Wu J, Ma J, Su X, Neale G, Dome JS, Daw NC, Khoury JD: Copy number gains in EGFR and copy number losses in PTEN are common events in osteosarcoma tumors. Cancer 2008, 113:1453–1461.

39. Kersting C, Tidow N, Schmidt H, Liedtke C, Neumann J, Boecker W, van Diest PJ, Brandt B, Buerger H: Gene dosage PCR and fluorescence in situ hybridization reveal low frequency of egfr amplifications despite protein overexpression in invasive breast carcinoma. Lab Invest 2004, 84:582–587.

40. Pantaleo MA, Astolfi A, Di Battista M, Heinrich MC, Paterini P, Scotrandi K, Santini D, Catena F, Manara MC, Nannini M, et al: Insulin-like growth factor 1 receptor expression in wild-type GISTs: a potential novel therapeutic target. Int J Cancer 2009, 125:2991–2994.

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