

Original Article

Expression of pERK and pAKT in pediatric high grade astrocytomas: Correlation with YKL40 and prognostic significance

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The Ras signaling pathway, consisting of mitogen-activated protein kinase (MAPK) and PI3K/AKT signaling, is a prominent oncogenic pathways in adult diffuse gliomas, but few studies have evaluated such pathways in pediatric malignant gliomas. We investigated by immunohistochemistry MAPK and AKT signaling in a series of 28 pediatric high-grade gliomas (WHO grade III and IV). We sought a possible association of phospho-ERK (p-ERK) and phospho-AKT (p-AKT) with expression of other proteins involved in the Ras pathway, that is, YKL40, epidermal growth factor receptor (EGFR), EGFR vIII and c-Met. Moreover we correlated the expression of p-ERK and p-AKT with prognosis. No cases showed expression for c-Met and EGFR, and only one case was positive for EGFR vIII. YKL-40 protein was expressed in 43% of cases. We detected expression of p-ERK and p-AKT in 61% and 57%, respectively, of pediatric high grade gliomas. Statistical analysis comparing the two groups in term of high and low p-ERK and p-AKT expression showed a trend toward worse overall survival in patients with high expression of p-AKT. The activation of ERK and AKT suggest a possible role of this protein in inducing activation of the Ras signaling pathway in pediatric high-grade gliomas. Moreover high levels of p-AKT are associated with worse overall survival.

Key words: AKT, children, high grade gliomas, MAPK, prognosis, YKL40.

INTRODUCTION

The Ras signaling pathway, consisting of mitogen-activated protein kinase (MAPK) and PI3K/AKT signaling, represents a prominent oncogenic pathways in adult diffuse gliomas.¹ Activity of Ras is aberrantly increased in most adult glioblastomas (aGBM) and AKT activation is observed in 70% of aGBM,² being correlated to proliferation, invasiveness,³ radiation resistance⁴ and survival.⁵ Aberrant activation of the Ras signaling pathway results in most cases from mutations or deletions involving PTEN, with loss of its suppressive function and consequent AKT phosphorylation.⁶ Few studies have reported a constitutive AKT activation in pediatric high grade gliomas (pHGGs),⁷ highlighting the importance of the RAS signal also in pediatric gliomagenesis. However, pediatric malignant gliomas infrequently exhibit PTEN mutations, suggesting that other mechanisms are involved in the constitutive activation of AKT.⁸ Ras gene mutations, able to activate the signal pathway, are rarely found in gliomas.⁹ It is therefore likely that mechanisms other than RAS mutations activate this pathway. RAS pathway can be activated by growth factors (platelet-derived [PDGF], hepatocyte [HGF], epidermal [EGF]), by some components of the signal cascade which can be mutated or aberrantly expressed, that is, Ras and B-Raf, or by mutations or overexpression occurring at genes encoding upstream receptors (EGFR, c-Met).^{10,11} Adult high-grade gliomas frequently exhibit overexpression of the EGFR

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which is detected in about 30% of adult glioblastomas and is associated with shorter survival and poor treatment response.²⁴ However, our data failed to demonstrate any correlation, because of the absence of expression of this marker in all cases tested. In a previous study we observed that YKL-40 protein is overexpressed in pHGG.¹⁵ On the basis of this observation, we evaluated the possible association of this protein with Ras signaling activation. YKL40 protein is able to activate the Ras pathway, initiating MAPK and PI3K signalling cascades in human connective-tissue cells and leading to the phosphorylation of ERK1/ERK2 and AKT.¹⁴ YKL-40 protein is involved in proliferation of fibroblasts and modulation of collagen formation,¹⁴ facilitating tumor invasion and metastasis. Such mesenchymal functions have been supported in a microarray analysis in high-grade gliomas, where YKL-40 gene was found upregulated together with other genes activated in mesenchymal tissues and are associated with poor prognosis,²⁵ as suggested in previous reports where the overexpression of YKL-40 in adult malignant gliomas is associated with an adverse prognosis.^{26,27} Moreover, a previous study in a series of adult high-grade gliomas describes a positive correlation between YKL-40 protein expression and p-AKT and p-ERK, suggesting that YKL-40 plays a pivotal role in glioma cell proliferation through activation of the MAPK and AKT pathways.²⁷ However, we did not find correlation between YKL40 protein and p-AKT and p-ERK in the series of pediatric malignant astrocytomas. In the literature AKT activation has been described in about 60% of pHGGs, and this was related to adverse outcome.^{7,20} In our study, even without reaching statistical significance, there was a trend toward an association between pAKT and shorter overall survival and progression-free survival, suggesting that, as in adult, activation of AKT protein is associated with worse prognosis. In support of this, patients with activated AKT showed a shorter median survival than patients with non-activated AKT. The next step was to search for tyrosine kinase receptors, potentially involved in activation of this pathway: our results were not able to identify activation of EGFR, EGFR vIII and c-Met. A possible explanation is the presence of other tyrosine kinase receptors involved in pediatric gliomagenesis, such as PDGFR. In this regard, Paugh *et al.*²⁸ showed how pediatric high-grade astrocytomas have a different genetic profile with adult cases, as suggested by the predominant target of amplification of PDGFR in pediatric cases rather than adults.

In conclusion, our results suggest that in pediatric high-grade gliomas:

1 high levels of p-AKT are associated with a decrease in overall survival and progression-free survival, even if the relatively small number of cases suggests the need to confirm these observation in further studies;

- 2** tyrosine kinase receptors, that is, EGFR, EGFR vIII and c-Met are not expressed in pHGGs and are not correlated with ERK or AKT activation; and
- 3** YKL40 overexpression in pHGGs is not correlated with ERK and AKT phosphorylation as in adults.

These observations highlight that the biological features of high-grade gliomas in children are different from those of adults.

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REFERENCES

- Norris JL, Baldwin AS Jr. Oncogenic Ras enhances NF-kappaB transcriptional activity through Raf-dependent and Raf-independent mitogen-activated protein kinase signaling pathways. *J Biol Chem* 1999; **14**: 13841–13846.
- Rajasekhar VK, Viale A, Socci ND, Wiedmann M, Hu X, Holland EC. Oncogenic Ras and Akt signaling contribute to glioblastoma formation by differential recruitment of existing mRNAs to polysomes. *Mol Cell* 2003; **12**: 889–901.
- Lakka SS, Jasti SL, Gondi C *et al.* Downregulation of MMP-9 in ERK-mutated stable transfectants inhibits glioma invasion in vitro. *Oncogene* 2002; **21**: 5601–5608.
- Gupta AK, Bakanauskas VJ, Cerniglia GJ *et al.* The Ras radiation resistance pathway. *Cancer Res* 2001; **15**: 4278–4282.
- Aeder SE, Martin PM, Soh JW, Hussaini IM. PKC-eta mediates glioblastoma cell proliferation through the Akt and mTOR signaling pathways. *Oncogene* 2004; **2**: 9062–9069.
- Endersby R, Baker SJ. PTEN signaling in brain: neuropathology and tumorigenesis. *Oncogene* 2008; **18**: 5416–5430.
- Faury D, Nantel A, Dunn SE *et al.* Molecular profiling identifies prognostic subgroups of pediatric glioblastoma and shows increased YB-1 expression in tumors. *J Clin Oncol* 2007; **25**: 1196–1208.
- Pollack IF, Hamilton RL, James CD *et al.* Children's Oncology Group. Rarity of PTEN deletions and EGFR amplification in malignant gliomas of childhood: results from the Children's Cancer Group 945 cohort. *J Neurosurg* 2006; **105**: 418–424.
- Jeuken J, van den Broecke C, Gijzen S, Boots-Sprenger S, Wesseling P. RAS/RAF pathway activation in gliomas: the result of copy number gains rather than