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Boldine Exerts Anti-glioma Activity in Vitro and in Vivo

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Background: The aporphines alkaloids represent a potential category for the development of new anticancer agents. Boldine, one of these alkaloids, occurs in leaves and bark of *Peumus boldus*. Considering that the anticancer properties of this compound have not been well characterized, the aim of this study was to investigate the effect of boldine on glioma lineages *in vitro* and *in vivo*.
Methods: Cultures (C6 and U138 lineages) were treated with boldine and percentage growth was assessed by Sulforhodamine B assay. Propidium Iodide incorporation was used to determine cell death. For Western blot analysis of phospho-AKT, AKT, phospho-GSK-3β and GSK-3β proteins, cultures were treated for 1, 3 and 24 h. Intracellular reactive oxygen species (ROS) were detected using a fluorescent probe, 2',7'-dichlorodihydrofluorescein diacetate (DCFH-DA). Total reactive antioxidant potential (TRAP) was used to estimate the antioxidant capacity of boldine. *In vivo* antitumoral activity was assessed in a model of intracranial tumours (C6 cells) implanted in *Wistar* rats following i.p. injections of 50 mg/kg boldine for 10 days. Hematoxylin and Eosin (H&E) sections from each animal were analyzed by a pathologist and tumour size was quantified. All procedures were approved by local Ethical Committee.
Results: A significant growth inhibition effect was observed in C6 glioma lineage 72 h after treatment. We also observed necrotic cells. Western blot analysis revealed a decrease in Akt and GSK-3β phosphorylation. Treatment with boldine did not result in ROS production, and additionally was capable to prevent the increase of ROS induced by H_2O_2. This effect is probably related to its high reactive antioxidant potencial. Similar results were obtained with U138 cells. Preliminary results using a model of intracranial tumour implantation, suggest that the treatment with boldine for 10 days reduced tumour size in the rat brain. Pathological analysis demonstrated that tumours of rats treated with boldine present lesser malignant characteristics typical of glioblastomas than tumours of untreated rats.
Conclusion: According to these results, boldine appear to induce antitumoral effect in glioma cell lines. This effect could be mediated by activation of GSK-3β and inhibition of Akt, and is independent of ROS production. Our results also suggest that boldine has the ability to affect the growth of intracranial tumours. Considering these, we suggest that boldine could be a promising drug for anticancer agent development.
Supported by CNPq, FIPE.

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Addition of Erlotinib Changes Gene Expression in Glioblastoma Cell Lines Treated With Vorinostat

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This study investigated the effects of the epidermal growth factor receptor tyrosine kinase inhibitor (EGFR TKI) erlotinib on proliferation, cell viability and gene expression of glioblastoma cell lines treated with the histone deacetylase (HDAC) inhibitor vorinostat.
Five human glioblastoma cell lines (U-87MG, U-138MG, A-172, LN-18, and U-251MG) were treated with 4 combinations of vorinostat and erlotinib (vorinostat 1 μM with erlotinib 4 μM and 1 μM, and vorinostat 0.25 μM with erlotinib 4 μM and 1 μM), as well as with single agents vorinostat and erlotinib in the above concentrations. After 3 days of drug exposure, cells were counted and collected for total RNA extraction. Real-time quantitative RT-PCR was performed on a panel of 25 genes known to play a role in progression of glioblastoma. In a separate experiment, metabolic activity as an indicator of cell viability was measured daily for 3 days of drug exposure. Addition of erlotinib 4 μM to vorinostat 1 μM led to >20% reduction of cell proliferation in U-87MG and LN-18, and was associated with transcriptional repression of *PDGFRA* (platelet-derived growth factor receptor A) and activation of *MIIP* (migration and invasion inhibitor protein) and *TIMP1* (tissue inhibitor of metalloproteinase 1) in comparison with single agent vorinostat. When added to vorinostat 0.25 μM, erlotinib 4 μM inhibited proliferation by >20% only in LN-18, the cell line with the highest *EGFR* baseline expression.
In all investigated cell lines, addition of erlotinib to vorinostat in all 4 combinations was associated with up-regulation of the invasion-enhancing gene, *IGFBP2* (insulin-like growth factor binding protein 2). This *IGFBP2* activation did not correlate with an alteration in cell proliferation.
In the cell viability assay, the strongest inhibiting activity (when compared with untreated control) was seen with combination vorinostat 1 μM and erlotinib 4 μM in the cell line LN-18, while the strongest additive effect of erlotinib was observed when erlotinib 4 μM was combined with vorinostat 0.25 μM again in LN-18.
This study has shown that addition of an EGFR TKI, erlotinib, to an HDAC inhibitor, vorinostat, is effective in treatment of glioblastoma cells, particularly those with high levels of *EGFR* expression, and is associated with reduced expression of *PDGFRA* and activation of *MIIP*.

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Validation of Differential mRNA Expression of Genes in Astrocytoma

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Background: Malignant gliomas remain a poorly understood form of cancer associated with high rates of mortality. Although histopathological features are the basis for glioma diagnosis and grading, recent findings indicate that response to treatment correlates better with some particular genetic or epigenetic characteristics of the tumours than to their morphological features. In an attempt to identify genes differentially expressed between low and high grade astrocytic gliomas, in a previous study we compared five publicly available microarray datasets using Gen Set Enrichment Analysis (GSEA). As a result, we obtained differentially expressed functions and identified genes associated to high or low grade astrocytoma. In the present work we show the validation of differential expression of two genes (ARF4 and EZH2) that were associated *in silico* to high grade astrocytoma.
Materials and Methods: Total RNA was extracted from high and low grade astrocytic glioma frozen samples obtained from biobanks (Red Regional de Castilla y León and Hospital Central de Asturias). Tumour classification was performed by two pathologists. Differential expression was assessed by quantitative real-time PCR using the 2^{-DDCT} method. The EGFR amplification status was analysed by FISH.
Results: ARF4 and EZH2 are upregulated in high grade gliomas. As refers to EZH2 our data agrees with recently published results. No significant association was found between the expression of the studied genes and the EGFR status. As EGFR amplification is frequently associated to primary glioblastoma, our observations suggest that the expression of the analysed genes is not related to the slow or rapid pathways of tumour evolution.
Conclusions: Bioinformatics tools as Gene Set Enrichment analysis are useful in the selection of candidate differentially expressed genes. The differential expression of ARF4 and EZH2 was validated in our subset of astrocytoma samples. Further research is warranted to assess whether mRNA expression correlates with protein expression and to evaluate potential usefulness of the studied genes as glioma markers.

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NPAS3 Demonstrates Features of a Tumour Suppressive Role in the Progression of Astrocytomas

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Background: Primary brain tumours are among the top 5 causes of cancer-related deaths, with astrocytomas being the most common. Despite current therapies, patients with glioblastoma unfortunately still succumb to a median survival of <2 years. In our effort to better comprehend the genetic basis of glioblastomas, we explored the prospects for new therapeutic targets. We previously cloned and characterized the function of NPAS3, a transcription factor which maps to human chromosome 14. In our pursuit to understand the role of Neuronal PAS 3 (NPAS3) in human diseases, we investigated it as a candidate for astrocytomagenesis based on the presence of aberrant protein expression in >70% of our human astrocytoma panel, and most notably in surgically resected high grade lesions.
Methods and Results: After undertaking extensive functional analyses of NPAS3 using human surgical astrocytoma specimens, glioma cell line and human astrocyte cell line models, we now have novel and strong evidence supporting NPAS3 as an astrocytoma tumour suppressor involved in tumour progression. Our data in support of this discovery are