8710 POSTER
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.
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8711 POSTER
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*.

8712 POSTER
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.

8713 POSTER
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

based on: 1) Absent NPAS3 expression is predominant in high grade astrocytomas, in comparison to low grade astrocytomas suggestive of an expression pattern typical of tumour suppressive-late stage progression factors. 2) Loss of function mutations of NPAS3, which are associated with a loss of heterozygosity of the NPAS3 locus are identified in glioblastomas. 3) Absent NPAS3 expression is predominant in >60% of malignant human glioma cell lines. 4) An over-expressed NPAS3 in malignant glioma cell lines suppresses the transformation potential, while the converse reduced expression promotes an increase in transformation potential. 5) A reduced NPAS3 expression (efficiency >90%) in concert with gliomagenesis pre-disposition genes transforms a well characterized TERT immortalized human astrocyte cell line and promotes the growth of malignant astrocytomas, while an over-expressed NPAS3 suppresses the transformation. 6) NPAS3 drives tumour progression by the control of cell cycle, proliferation, apoptosis, migration/invasion and angiogenesis.

Conclusions: Our data provide findings of NPAS3 as a novel gene which drives the progression of malignant astrocytomas, with tumour suppressive roles. We believe that this body of work is highly significant in our quest to better understand the biology of astrocytomas, and with prospects for improved targeted therapies in the treatment of this currently and relatively incurable disease.

8714   POSTER

Tamoxifen Interaction With Protein Kinase C Delta

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Background: Tamoxifen, selective estrogen receptor modulator, is efficient in the treatment of some brain cancer tumours. This effect is thought to be related with tamoxifen influence on protein kinase C (PKC) and mechanism of antiestrogen influence on the enzyme is still unclear. We have supposed that direct tamoxifen interaction with PKC could be one of the reasons of the antiestrogen therapeutic activity in brain tumours.

Materials and Methods: A549 human non-small-cell lung cancer cells were studied by flow cytometry and fluorescent microscopy. A549 cells were incubated with Abcam antibodies: primary (PKC delta, EP1484Y or rabbit IgG) for 1.5h and with secondary FITC-conjugated antibody (ab6108) for 1.5 h. The important feature of the primary anti-PKC antibody is that the epitope resides near the C-terminus of the protein, where catalytic domain of enzyme is located.

Results:
1. It was revealed PKC delta expression in about 70% of the A549 cells in culture.
2. Tamoxifen pretreatment decreased significantly both the mean cell specific fluorescence (in about 1.6 times) and the specific stained cells number (up to 40%) after incubation with anti-PKC delta antibody.
3. In the microscopic visualization of the tamoxifen effect on specific antibody interaction with the A549 cells the result was the same: the cell specific fluorescence intensity as well as specific stained cells number after tamoxifen pretreatment were decreased significantly.
4. Tamoxifen didn't influence the isotypic antibodies interaction with the cells and didn't change the cell autofluorescence.

Conclusion: Decrease in the mean cell fluorescence intensity under tamoxifen pretreatment shows disturbed availability of the PKC delta catalytic domain to antibody binding, most likely because of the antiestrogen interaction with the PKC delta molecule. Because specific triphenylethylene-binding site is located near the catalytic domain of the PKC, there is high probability that the tamoxifen interaction with the PKC delta results in the modification of the enzyme catalytic activity and changes regulation in downstream signaling pathways of the PKC delta. We believe that tamoxifen interaction with the PKC delta could be one of the reasons of the antiestrogen therapeutic activity in some brain cancer tumours.

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8715   POSTER

Comparison of Instruments for Quality of Life Measurement in Bulgarian Patients With Malignant Glial Tumours

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Background: Measuring the quality of life (QoL) has become an integral part of initial examination, choosing a therapeutic approach and for the follow-up of the neurosurgical treatment. Most commonly used questionnaires for QoL are SF-36 (Short-Form Health Survey), QLQ-C30 of the European Organization for Research and Treatment of Cancer (EORTC) and World Health Organization Quality of Life-100 (WHOQOL-100). Sofia Self-Assessment Scale (SoS-SAT) is a newly developed in Bulgaria questionnaire for QoL assessment. SoS-SAT is self-directed, mainly to patients with supratentorial processes and consists of 12 questions (5 related to physical functioning and the presence of neurological symptoms, 7 related to QoL and symptoms of depression). The objective of our study was to evaluated applicability of aforementioned instruments for measuring QoL in Bulgarian patients with malignant gliomas.

Material and Methods: In the present study 100 patients with malignant gliomas treated in the period 2003–2005 were included. QoL was measured through the SF-36 (n = 95), SoS-SAT (n = 45), QLQ-C30 (n = 40) and WHOQOL-100 (n = 35) before and after treatment. All patients were asked to answer 5 questions related to ease of completion, clartiry and comprehensiveness of each used in this study QoL questionnaires.

Results: The studied questionnaires demonstrate that all of them are clearly formulated and relevant to the health of patients with malignant gliomas. 60% of patients found WHOQOL-100 instrument more difficult to complete than others. Averaged results of this study revealed that SF-36, QLQ-C30 and SoS-SAT seems to be more appropriate for measuring QoL in patients with malignant gliomas − 80% of the patients did not have any difficulties with questions clarity or questionnaires completion. Also SoS-SAT is the instrument to which most patients show positive attitude in comparison of other.

Conclusions: The SF-36, QLQ-30 and SoS-SAT questionnaires are the most suitable existing instruments for QoL assessment in Bulgarian patients with malignant gliomas.

8716   POSTER

Novel Features That Impact the Outcome of Patients With Glioblastoma Multiforme − Multivariate Analysis From a Comprehensive Dataset

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Background: Glioblastoma multiforme (GBM) is the most common malignant adult brain tumour. While the impact of variables such as age and performance status on outcome is well known, the potential impact of other variables such as socio-econonomic status and clinical trial participation to date have not been well explored.

Materials and Methods: Data on patients (pts) with GBM were accessed from a prospective neuro-oncology dataset collected over 12 years (1998–2010) at two institutions (one public, one private). Death data were obtained from the state Cancer Registry. Data linkage and analyses were performed by BioGrid Australia. Both univariate and multivariate logistic regression analyses were performed to look for relationships between clinical and socio-demographic variables and overall survival.

Results: In total 541 pts were evaluated; the median age was 60 years. Sixty-five patients (12%) were enrolled in a clinical trial. In univariable analysis, positive predictors for longer survival were: clinical trial participation (HR for death 0.43, 95% CI 0.33–0.60, p < 0.0001), higher IRSAD (index of relative socio-economic advantage and disadvantage) score (socioeconomically advantaged) (HR 0.85, 95% CI 0.8–0.95, p = 0.0093), macroscopic resection versus biopsy alone (HR 0.5, 95% CI 0.4−0.6, p < 0.001), and more than one operation (HR 0.5, 95% CI 0.4−0.6). Older age (HR for death 3.0, 95% CI 2.4−3.7, p < 0.0001), worse ECOG performance status (HR 1.5, 95% CI 1.3−1.7, p < 0.0001) and multifocal disease (HR 1.5, 95% CI 1.2–1.97, p = 0.001) were significantly associated