TARGETING APOPTOSIS PATHWAYS FOR CANCER THERAPY OF MALIGNANT BRAIN TUMORS

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Most cancer therapies, e.g. chemotherapy, radiotherapy or immunotherapy, primarily act by triggering apoptosis, the cell’s intrinsic death program, in cancer cells. Thus, defects in apoptosis programs may lead to cancer resistance. We investigated the role of various signaling pathways in determining sensitivity or resistance of malignant brain tumors. Most glioblastoma cell lines exhibited constitutive activity of the PI3-kinase/Akt pathway. Importantly, inhibition of PI3-kinase activity markedly sensitized resistant glioblastoma cells to TRAIL- or cytoxic drug-induced apoptosis. In contrast, inhibition of MAPK or mTOR signaling had no effect on apoptosis sensitivity in these cells. Treatment with the mitochondrial apoptotic cytoxic agent Betulinic acid resulted in activation of NF-kappa B through activation of the IKK complex, phosphorylation and downregulation of IkappaB-alpha followed by translocation of the NF-kappa B subunit p65 into the nucleus and NF-kappa B-mediated transcriptional activation. Surprisingly, specific inhibition of NF-kappa B by expression of an IkappaB-alpha superrepressor mutant, which suppressed NF-kappa B activation upon Betulinic acid treatment, also attenuated Betulinic acid-induced apoptosis pointing to a pro-apoptotic function of NF-kappa B in this context. Moreover, overexpression of mitochondrial or cytosolic Smac significantly increased irradiation-induced apoptosis of malignant glioma cells indicating that Smac agonists may be a useful tool to enhance radiosensitivity of malignant glioma. Thus, understanding the molecular mechanisms that regulate cell death programs including apoptosis, and how resistant forms of malignant brain tumors evade apoptotic events, may provide novel opportunities for cancer drug development.

IMPROVED SURVIVAL AFTER GROSS TOTAL RESECTION OF PRIMARY AND RELAPSED MALIGNANT GLIOMA IN PEDIATRIC PATIENTS

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The aim of the present study was to investigate the prognostic impact of tumor resection on survival in children and adolescents with primary and relapsed malignant gliomas. From the HHT-GBM databank (n=241) of the Society of Pediatric Oncology and Hematology (GPOH) in Germany, Austria, and Switzerland and from the Surveillance, Epidemiology, and End Result (SEER) US database (n=18), 295 pediatric patients aged 0-18 years with histologically proven WHO grade III (n=106) and IV (n=153) non-pontine gliomas were analyzed. In 120 patients, additional data on second surgery after tumor relapse was available. The extent of tumor surgery during first-line treatment represented the most prominent prognostic factor for overall (OS) and event-free survival (EFS) in univariate and Cox regression analysis. Five year survival after gross total tumor resection was 33.1±9.5% (OS) and 14.9±5.2% (EFS), after subtotal/partial resection 22.3±5.4% (OS) and 11.3±3.8% (EFS), after biopsy 13.2±6.3% (OS) and 0% (EFS). In Cox regression analysis, only histological grading showed a similar significance for OS and EFS whereas other clinical parameters either demonstrated significance only for OS (sex, tumor location) or no significance at all (radiotherapy, chemotherapy). In relapse patients, tumor reoperation appeared to represent an important part of a second-line treatment approach: Patients treated with second-line chemotherapy showed a significantly better 5 year OS with second gross total tumor resection (55.6±19.9%) than without additional tumor debulking (0%). In conclusion, gross total tumor resection improves survival in pediatric patients with primary high-grade gliomas. A second-line treatment approach including tumor reoperation and chemotherapy appears advantageous for relapse patients.

SEQUENTIAL CHEMOTHERAPY, HIGH-DOSE THIOTEPA, CIRCULATING PROGENITOR CELL RESCUE, AND RADIOTHERAPY FOR CHILDHOOD HIGH-GRADE GLIOMA: A MONO-INSTITUTIONAL STUDY

Maura Massimino, Lorenza Gandola, Roberto Luschi, Filippo Saffreto, Daria Riva, Carlo Solero, Felice Giagkousiklidis, Franco Locatelli, Maria Podd, Fabio Bozzi, Emanuele Pignoli, Paola Collini, Grazieda Cefalò, Marco Zeeca, Michela Casanova, Andrea Ferrari, Monica Tereziani, Cristina Meazza, Daniela Polastri, Davide Scarumuzza, Fernando Ravagnani, and Franca Fossati-Bellani

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FUTURE STRATEGIES IN RADIOTHERAPY OF CHILDHOOD HIGH GRADE GLIOMA

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Introduction: As an adjunct to surgery radiotherapy is the cornerstone in the management of high grade glioma. Recent developments in radiotherapeutic technologies opened the possibilities to perform 3 dimensional computer-assisted treatment planning in order to improve the therapeutic ratio in increasing tumor control while sparing normal tissue. Although data accumulated for adult high grade glioma in the last years, experiences in children are scarce.

Materials and Methods: Conventional fractionated postoperative radiotherapy (RT) of the tumor site alone yields a median survival of 12 months. Local dose escalating by using modern treatment techniques are promising. Conventional RT followed by radiosurgery in non-resectable tumors achieves survival times up to 72 months. After brachytherapy combined with conventional radiotherapy, tumor control rates of more than 80% at 4 years were observed. Reducing normal tissue volume included in the 95% isodose is a major aim to reduce the potential for late effects. Conformal radiotherapy in conjunction with modern functional imaging such as spectroscopy and PET opens up the possibility to include biological properties into treatment planning. In primary treatment and recurrent disease these tools provide delineation of high and low risk regions in order to achieve local adjustment of dose prescription.

Conclusions: Restricting the planning target volume by using conformal techniques appears to be feasible. The introduction of functional imaging may lead to a biological guided dose prescription. Local dose escalating by using modern techniques appear to improve local tumor control and warrant further investigation especially in incompletely resected high grade tumors. The use of modern treatment techniques achieves a reduction of normal tissue irradiation in order to reduce the potential for acute and long-term side effects. Generation of integral doses to target volumes and organs at risk allows the development of models to predict the risk of late effects.

Supported by Deutsche Kinderkrebsstiftung

TOWARD A SIOP HIGH-GRADE GLIOMA GROUP: TRYING A COMMON NEUROPATHOLOGY LANGUAGE

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Background. After the first meeting of the original core group in Augsburg (Nov. 2001), some proposals were done. In the neuropathology field we decided to launch the revision of just a few cases, belonging to different European Institutions among selected referral pathologists. The aim was to understand, on a selected sample, if there was a common background, i.e. common histopathology language, when dealing with “childhood malignant glioma”.

Methods. Thirteen sets of slides, each constituted by two HE slides renumbered, were collected by referral pediatric oncologists (France, Germany, Italy and Spain). Cases were to be re-classified according to WHO 2000 classification. Observers were unaware of original diagnosis and of those expressed by their colleagues. Five referral pathologists were involved. Each set was accompanied by a form filled by the referring pediatrician describing clinical history and outcome, MRI images, and clinical diagnosis. For 8 patients significant MRI pictures were available too. The pathologists had to fill an anonymous form that was sent back, together with slides, to the collecting centre, in Milan.

Results. The first pathologist received “the parcel” in April 2003, the second in November 2003, the third in January 2004, the forth in June 2004. Preliminary results are related to the diagnoses expressed by the first three pathologists. Hopefully the revision will be completed before the end of February 2005. A total of 37 (for November 2003, the third in January 2004, the forth in June 2004. Preliminary results are related to the diagnoses expressed by the first three pathologists. Hopefully the revision will be completed before the end of February 2005. A total of 37 (for 13 sets, revised by three pathologists) diagnoses were expressed because HE stain was not considered enough for diagnoses into two cases. Concordance was 89% for grade IV lesions and 58% for grade III lesions (chi square t, p < 0.04).

Conclusions. The revision through Europe was a quite hard and time consuming effort. Diagnostic reproducibility was satisfying as far as “extreme lesions” like glioblastoma, while it was less evident for grade III gliomas. Such a difference needs to be taken in mind when designing a common study.

EXPRESSON OF FASCIN, AN ACTIN-BUNDLING PROTEIN, IN ASTROCYTOMAS OF VARYING GRADES

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Malignant astrocytomas are highly infiltrative neoplasms which invade readily into regions of normal brain. On a cellular basis, the motility and invasiveness of human cancers can be assessed in part to complex rearrangements of the actin cytoskeleton that are governed by several actin-binding proteins. One such actin-binding protein that has been linked to the invasive behaviour of carcinomas is fascin which serves aggregate F actin into bundles. In this study, we examined the expression of fascin in a series of human malignant astrocytomas (WHO grades I – IV). Five Grade I, 5 Grade II, 10 Grade III, and 26 Grade IV human astrocytomas were examined for fascin and glial fibrillary acidic protein (GFAP) expression by double immunofluorescence confocal microscopy. Expression of fascin and GFAP was also determined by western blot analysis. Fascin expression increased with increasing WHO grade astrocytoma. This is in marked contrast to GFAP expression which decreased with increasing WHO grade. In Grade I and II neoplasms, and within non-neoplastic brain, fascin and GFAP were expressed diffusely within regions examined. However, in the higher grade astrocytomas (Grade III and IV), fascin and GFAP were expressed regionally in distinctly separate tumor cell populations. This is the first study to demonstrate the expression of fascin in human astrocytic neoplasms. The role that fascin plays in contributing to the invasive phenotype of anaplastic astrocytomas awaits further study and investigation.

ERUCYLPHOSPHOLCHOLINE INCREASES SENSITIVITY OF GIBLOSTOMA CELL LINES TO RADIATION-INDUCED APOPTOSIS

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The membrane targeted synthetic phospholipid derivative erucylphosphocholine (ErPC) induces apoptosis in highly resistant glioblastoma cell lines. The aim of the present study was to analyse putative sensitizing effects of ErPC on radiation induced cell death in human glioblastoma cell lines in vitro. To this end, induction of apoptosis was evaluated in U87MG, A172 and T98G cells upon irradiation (1-10 Gy) with 6 MV photons from a linear accelerator and/or subsequent ErPC-treatment (0.50mM T98G/A172 cells) or 0-100 μM (U87MG cells)). Cell death was quantified 24-72h after treatment by fluorescence microscopy using combined staining with Hoechst 33342 and propidium iodide. The biomathematical evaluation of putative additive or synergistic effects was performed by isobologram analysis.

While all glioblastoma cell lines showed high intrinsic resistance against radiation induced apoptosis, treatment with ErPC strongly increased sensitivity of the cells to radiation induced cell death. T98G were most responsive to the combined treatment revealing highly synergistic effects while A172 showed additive or synergistic effects and U87MG cells maximum additive effects depending on radiation and ErPC doses used.

In conclusion, ErPC strongly increases sensitivity of glioblastoma cell lines to radiation induced cell death. The impact of the combined treatment on radiation sensitivity as determined by clonony forming assays is under current investigation.

Supported by the Deutsche Krebshilfe (10-1970 Be-III) to V.J. and the fortune-program Universität Tübingen (126-0-0) to V.J.
MUTUAL CYTOKINE PRODUCTION BY IMMATURE DC CO-CULTURED WITH GliOBLASTOMA MULTIFORME TUMOR CELLS

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Glioblastoma Multiforme (GBM) is the most common primary central nervous system (CNS) neoplasm. Little is known about the interaction between GBM cells and antigen-presenting cells in the CNS. To mimic the micro-environment of the tumor, we co-cultured GBM cells with immature DC (DCi), which closely resemble microglia cells. DCi were generated in vitro by culture of adherent monocytes in the presence of rIL-4/rGM-CSF, after which they were co-cultured with GBM cells for 2 to 6 days. The production of IL-6, IL-10, IL-12, TNF-α and IFN-γ in the supernatant was measured with ELISA, and a stimulation index (co-culture compared to separate cultures) was calculated. DCi alone produced moderate amounts of IL-6. GBM cells alone produced IL-6 and in some experiments IL-12 (p70), the latter being confirmed also at the mRNA level. We found a significant induction of IL-6, IL-10, TNF-α and IFN-γ production in the co-cultures, which was totally dependent on direct cell-cell contact between tumor cells and DCi. DCi cultured in the presence of lysates of the GBM cells also produced IL-10, TNF-α and IFN-γ, but no IL-12. The data point to GBM cell-mediated stimulation of DCi resulting in the production of anti-tumoral factors (TNF-α, IFN-γ), but also tumor-promoting factors via angiogenesis (IL-6) and immune suppressive factors (IL-10), and possibly reflect the intratumoral micro-environment.

IMPROVEMENT OF DENDRITIC CELL VACCINATION STRATEGY FOR PATIENTS WITH RELAPSED HIGH GRADE GLIOMA: AN INTERIM ANALYSIS


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The results of the first cohort of the HGG-IMMUNO-2003 protocol show that an immune response against high grade glioma (HGG) can be induced by injecting autologous DC loaded with tumor proteins. With the schedule used, the induction of immunity takes about 2 months. CCR is obtained in patients with complete tumor resection prior to vaccination. However, tumor control was sufficient for patients with residual disease. In order to induce immunization over a shorter time after resection, the next cohort of patients was scheduled for vaccinations each 2 weeks for V1 to V5, and later on each 4 weeks. When tumor resection was undoubtedly incomplete, patients were scheduled to receive in parallel weekly IV vinblastine (VBL) 6 mg/m2, aimed to control tumor growth based on anti-angiogenesis during the period of immunization. Ten patients were treated thusfar (mean age: 41; range: 19-60). One patient had immediate and massive relapse and received only one vaccination. Three patients are clearly progressive. Other patients can be evaluated yet. One girl of 19y showed response of a lesion and is now in CCR. A boy of 22y had a complex history (Childs Nerv Syst 2004:20:114). He received vaccination, prepared out of one of 2 remaining tumors in the spinal axis, in combination with VBL. We demonstrated normal immunity against recall antigens in spite of VBL treatment. The residual tumor lesion remained stable for 6 months, and was removed afterwards. Because all material was used for further DC loading, pathology assessment of an immune response was not done. Three further vaccinations have been scheduled for him. Finally, a girl of 19 received also vaccination + VBL. She showed response of a small lesion and is now in CCR. We could perform skin test, and observed prominent delayed type hypersensitivity response in general. There was no vaccination-related toxicity. Because of mild leukopenia, both VBL-treated patients had to reduce the dose to 4 mg/m2. Although far too early to draw any conclusion, vaccination each 2 weeks is feasible. VBL seems not to counteract DC vaccination-based immunotherapy. Combining DC vaccination with 4 mg/m2 VBL should be further investigated.

PALLIATIVE VALUE OF AGGRESSIVE TREATMENT IN HIGH GRADE GLIOMA AND DIFFUSE INTRINSIC PONTINE GLIOMA: PRELIMINARY RESULTS OF HIT-GBM-C


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Background: Despite significant efforts, most patients with diffuse intrinsic pontine glioma and high grade glioma die from their disease. A major debate continues, if the treatment with the inevitable toxicity is worth the benefit. For the individual patient, this benefit has three elements: The increased chance to survive, the temporary improvement of symptoms and signs, and the psychological effect of being cared for. In particular in diffuse intrinsic pontine glioma, the temporary improvement of neurological signs is retrospectively frequently valued very high by patents of deceased patients. But this is influenced by many factors and might not reflect the patients views. In order to add scientific data to this discussion, an attempt is ongoing to measure health related quality of life in the HIT-GBM-C protocol.

Methods: Physicians participating in the HIT-GBM-C study were asked to fill in the FMH at various time points of the protocol. The FMH is a questionnaire to measure health related quality of life with simple objective questions focusing on the independence in daily life. 56 item are to be answered with Yes or No. The number of Yes answers is converted to a percent level using an age dependent percentile table (Klin Pädiatirie, 208:294-8.1996).

Results: In the total HIT-GBM database, 161 patients (93 male, average age 10 years) were documented as treated with the HIT-GBM-C protocol between 1999 and 2003. The most frequent tumor locations were pons (58), cerebral cortex (50) and basal ganglia (18). WHO-grading was IV: 65; III: 55, the others were enrolled based upon the MRI morphology of diffuse intrinsic pontine glioma. The primary surgical resection was complete in 28, subtotal or partial in 58, with only biopsy or no surgery in the others. Only 88 patients received the simultaneous radiochemotherapy as prescribed in the protocol. FMH questionnaires were received from 49 patients (32 male, age 3-17, average 10.6 years). The diagnoses were: HGG WHO grade IV: 20; grade III: 17, diagnosis based upon MRI only: 12. The result of the resection was: complete 12, partial or subtotal: 13, biopsy or no surgery: 24. The average percentile FMH result from patients with German first language appeared higher (32±18.5 SD, n=43) when compared to others (20.5 ± 6) and valproic acid maintenance (24.9 ± 11). At the time of recurrence the average FMH percentile was low (7.5 ± 1.5). In 10 patients, more than one FMH questionnaire was received giving a very small basis to address the main question. Of those, 5 improved, 2 remained unchanged, and 3 deteriorated. Two of the three, which deteriorated had a tumor progression at that time, and one suffered from treatment side effects at the time of measurement. In the others the health related quality remained unchanged (2) or improved (5) despite aggressive treatment.

Conclusion: The improvement of health related quality of life during treatment of pediatric high grade glioma and diffuse intrinsic pontine glioma patients is measurable using the FMH. The data are too sparse to judge the treatment protocol HIT-GBM-C. We hope this report will encourage participation in the ongoing HIT-GBM-D protocol.