PROCEEDINGS

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(Arranged by Subject Category)

MDM2 positive specimens were also immunopositive for p53. Six p53 and MDM2 double positive specimens exhibited no mutations in exons 5–8 by PCR-SSCP analysis. MDM2 overexpression was associated with nuclear anaplasia ($p < 0.05$), previously linked with local recurrence of GCTs. The results indicate that MDM2 overexpression is a frequent abnormality in GCTs of bone and represents an alternative mechanism for inactivation of p53 in these tumors. The data suggest that MDM2 overexpression may contribute to the development of GCTs of bone.

#3974 MRP and LRP expression in rhabdomyosarcomas of children and adults. Plaat, B.E.C., Van der Graaf, W.T.A., Mastik, M.F., Hollema, H., Hoekstra, H.J., Molenaar, W.M. *Depts. of Pathology, Medical Oncology and Surgical Oncology. University Hospital Groningen, The Netherlands.*

Introduction: Adult patients with rhabdomyosarcoma (RMS) have a worse prognosis than children, in which chemotherapy has improved clinical outcome. Pgp associated multidrug resistance in RMS is controversial. Therefore, the expression of Multidrug Resistance associated Protein (MRP) and the major vault protein LRP was examined in RMS of children and adults.

Materials and Methods: On formalin fixed, paraffin embedded RMS tissue of 13 children (<18 yrs of age) and 10 adults, the expression of MRP (MRPr1 ab) and LRP (Transduction Lab) were assessed immunohistologically. Percentage of stained cells and staining intensity were estimated and combined in a histopath-score. Tumors <10% stained cells were considered negative. For statistics the Mann-Whitney U test and the Spearman correlation test were used.

Results: LRP positive RMS were found in 8/13 children and 7/10 adults. MRP positive RMS were found in 4/13 children and 2/10 adults. LRP expression in adult RMS (mean: 40% pos. cells) was higher as compared to childhood RMS (mean: 30%). The MRP expression was lower in adult RMS (mean: 3%) as compared to childhood RMS (mean 13%). The differences were not statistically significant. Both LRP and MRP expression did not correlate with age.

Conclusion: MRP and LRP are not clearly involved in the reported chemotherapeutic resistance of adult RMS, since their expression is not associated with age and does not clearly differ between pediatric and adult RMS. Supported by the Dutch Cancer Society—Grant 95-1085.

CLINICAL RESEARCH 16: CNS and Pediatric Malignancies

#3975 Blocking G-protein function accounts for farnesylation inhibitor-induced apoptosis in medulloblastoma cell lines. Wang, W. and Macaulay, R.J.B. *University of Saskatchewan, Saskatoon, SK, Canada S7N 5E5.*

We have previously shown that blocking isoprenylation with lovastatin, an inhibitor of HMG-CoA reductase, or manumycin A, an inhibitor of protein farnesyltransferase (FTase), inhibits medulloblastoma (MB) proliferation and induces apoptosis *in vitro*. We are investigating which of many intracellular farnesylated proteins may be involved in these phenomena. Since ras and other G-proteins are farnesylated, we used mycophenolic acid to deplete intracellular GTP, and thus block G-protein activation of downstream signalling cascades *in vitro*. We demonstrate that blocking of G-protein function inhibits MB proliferation and induces apoptosis in a dose and time dependent manner. About 40 hours treatment with 400 μM of mycophenolic acid induced the appearance of apoptotic DNA laddering in all tested cell lines (DAOY, UW228, D283 Med and D341 Med), while a higher concentration (700 μM) only needed 24 hours. Since lovastatin has been shown to downregulate ras gene expression in yeast, we assessed this possibility in MB cell lines. The expression levels of total ras, and the K-, N- and H-ras genes were evaluated using RT-PCR with their respective specific primers. Following exposure to lovastatin and manumycin A in concentrations sufficient to induce apoptosis, expression of the ras genes showed no significant change. Therefore, our results suggest that interference with G-protein function by blocking isoprenylation, rather than down-regulation of ras gene expression, contributes to lovastatin- and manumycin A-induced MB apoptosis. (Supported by the Brain Tumour Foundation of Canada).

#3976 ^{1}H NMR of primary brain tumor extracts could allow the discrimination between high-grade and low-grade gliomas. Sabatier, J., Gilard, V., Malet-Martino, M., Martino, R., Berry, I., and Tremoulet, M. *IMRCP Lab., Université Paul Sabatier and CHU Purpan, Toulouse, France.*

Several choline-containing compounds (choline (Cho), phosphocholine (PC), glycerophosphocholine (GPC)) contribute to the <<choline>> peak observed in vivo at 3.2 ppm, whose significance is not well understood. In ^{1}H NMR spectra of glioma extracts, the resonances of the 3 compounds are separated with GPC at 3.23 ppm, PC at 3.22 ppm and Cho at 3.21 ppm. Open surgery (6) or stereotactic biopsy (21) was conducted on 27 patients with brain tumors (20 high-grade and 7 low-grade gliomas). Tumor samples (6.0–98.5 mg) were extracted with perchloric acid. The absolute concentrations of choline-containing compounds (GPC + PC + Cho) in high-grade and low-grade gliomas were significantly different: 0.73 ± 0.37 vs 0.36 ± 0.34 μmole/g ($p < 0.025$). The relative contributions of each of the choline-containing compound to the total choline signal were also statistically different (except for Cho). For high-grade and low-grade gliomas, the percentages were: GPC, $30 \pm 11\%$ vs $68 \pm 13\%$ ($p < 0.0005$), PC, $58 \pm 14\%$ vs $25 \pm 8\%$ ($p < 0.0005$), and Cho, $12 \pm 9\%$ vs $7 \pm 6\%$ ($p > 0.05$). These data were corroborated by results obtained in 2 patients from samples of tissue from different regions (surface, tumor, core) of high-grade gliomas. Only the tumor tissue had a high-grade profile; the surrounding tissue had a profile of normal brain tissue and the core (necrosis) contained very low levels of metabolites. The differences in the concentration and the repartition of choline-containing compounds seem to be a marker of high-grade gliomas. They could also help to discriminate between high- and low-grade gliomas in some difficult cases specially when there is histological uncertainty between anaplastic astrocytomas and low-grade oligodendrogliomas. (Supported by ARC, grant 6635).

#3977 *In vitro* evaluation of rapamycin sensitivity and synergy with cisplatin and camptothecin in medulloblastoma. Kerr, Karol H., Sutton, Leslie N. and Phillips, Peter C. *Division of Oncology, Children's Hospital of Philadelphia, Philadelphia, PA 19104.*

Medulloblastoma is the most common malignant Pediatric brain tumor. Although recent advances have been made in clinical trials with combination chemotherapy and radiation therapy, 20–40% of children will die secondary to relapsed disease and lack of adequate salvage therapy. Rapamycin (RAP), a immuno-suppressant, is an agent with significant therapeutic potential for embryonal solid tumors. Seven medulloblastoma cell lines and one glioma cell line were tested for RAP sensitivity in an *in vitro* cellular proliferation assay. Cells demonstrated either exquisite RAP sensitivity (IC50 < 5 ng/ml) or high resistance (IC 50 > 800 ng/ml). A RAP sensitive cell line, DAOY, and a RAP resistant cell line, D283, were tested in drug synergy experiments. Rapamycin's cytotoxic effects were assayed with cisplatin (CDDP) or camptothecin (CPT) alone and in combination with RAP. RAP demonstrated a significant additive effect for both CDDP and CPT in the sensitive cell line only. Preliminary molecular studies identified potential signal transduction intermediaries involved in the RAP-dependent pathway in medulloblastoma. These finding suggest that rapamycin may be an outstanding candidate for chemotheraputic clinical trials in medulloblastoma either alone or in combination with other agents. In addition, rapamycin represents a valuable probe to elucidate the tyrosine kinase - receptor linked pathways of signal transduction in medulloblastoma.

#3978 Rapamycin analog CCI 779 inhibits growth of human medulloblastoma xenografts. Geoerger, B., Kerr, K., Janss, A.J., Sutton, L.N. and Phillips, P.C. *Children's Hospital of Philadelphia, Philadelphia, PA 19104.*

Rapamycin and other immunophilins with demonstrable antitumor activity have specific advantages for brain tumor therapy: relative lipophilicity; unique mechanisms of cytotoxicity; absence of cross-resistance to other effective drugs; and minimal systemic toxicity. To evaluate the role of CCI 779, a rapamycin analog, in the treatment of medulloblastoma and other embryonal nervous system tumors, we examined *in vitro* cytotoxicity of CCI 779 in human medulloblastoma, neuroblastoma, and glioblastoma cell lines and *in vivo* activity of CCI 779 in athymic nude mice bearing DAOY medulloblastoma subcutaneous flank xenografts. In vitro cytotoxicity studies demonstrated ID50 ≤ 10 ng/ml in 4/7 medulloblastoma and 1/2 neuroblastoma cell lines. By contrast, a glioblastoma cell line (U251) was highly resistant (ID50 > 1000 ng/ml). To evaluate in vivo cytotoxicity in athymic mice bearing DAOY medulloblastoma flank xenografts, CCI 779 was administered i.p., daily × 5 for 1 or 2 weeks. Tumor volumes were measured serially and growth delay endpoints were defined as the post-treatment interval at which tumor volume increased by 5-fold. CCI 779 1-or 2-week treatments yielded significant tumor growth delays; i.e., the time to 5× initial tumor volume increased by 160% and 240%, respectively, compared to controls. Prolonged growth delay (> 50 days) was observed in 20% of the 2-week group but not in the 1-week group. Retreatment of large tumors with CCI 779 restored growth inhibition but did not yield tumor regression. Our results indicate that prolonged treatment with CCI 779 causes significant growth delay in DAOY medulloblastoma and suggests that this class of immunophilins has major cytotoxic activity in neuroectodermal tumors.

#3979 Intratumoral immunotoxin treatment of human malignant brain tumors in nude animal models. Engebraaten, O., Hjortland, G.-O., Juell, S., Fodstad, Ø. *Department of Tumour Biology, Institute for Cancer Research, The Norwegian Radium Hospital, Oslo, Norway.*

Treatment of malignant brain tumors remains a clinical challenge. New treatment modalities have been introduced, and among these are infusion of immunotoxin molecules, recognizing specific cell surface epitopes on the tumor cells. We have compared the efficacy of two immunotoxins, Tfn-CRM107 and 425.3-PE targeted to the transferrin receptor and EGF receptor, respectively, in the treatment of subcutaneous and intracranial glioma models in nude animals. Intratumoral administration of 1 μg Tfn-CRM107 into subcutaneously growing U87Mg cells efficiently inhibited tumor growth, whereas injection of 1 μg 425.3-PE were moderately effective. Treatment of intracerebral U87Mg tumors with Tfn-CRM107 proved ineffective, as doses above 20 ng/animal were lethal to the tumor bearing nude rats. In contrast, intratumoral administration of 425.3-PE delayed the onset