particularly, if inhibition of TGF-beta 2 by trabedersen also affects TGF-beta 1 levels.
Material and Methods: The human glioma cell line A-172 was used. Cells were treated with trabedersen or recombinant human TGF-beta 2 (rhTGF-beta 2). Cellular proliferation was assessed via DNA quantification. TGF-beta protein levels in cell culture supernatants were determined by ELISA, mRNA was quantified by qRT-PCR.
Results: While in cell culture medium containing serum, trabedersen potently inhibited TGF-beta 2 expression and barely affected TGF-beta 1 expression, a strong inhibition of both isoforms was observed under serum-free conditions. Serum contains considerable amounts of TGF-beta 1 and 2, which may affect TGF-beta expression by auto- and cross-regulatory loops. Treatment of cells with rhTGF-beta 2 strongly induced expression of TGF-beta 1 on the mRNA as well as on the protein level. The inhibitory effects of trabedersen on TGF-beta 1 expression could be reversed by addition of rhTGF-beta 2. Cellular proliferation of A-172 cells was not affected by trabedersen or rhTGF-beta 2, which might be explained by the fact that growth of A-172 cells is not affected by TGF-beta.
Conclusions: We have demonstrated that by cross-regulatory loops TGF-beta 2 induces the expression of TGF-beta 1 in human glioma cells. Specific inhibition of TGF-beta 2 expression by trabedersen interrupts this cross-regulatory loop and additionally down-regulates TGF-beta 1. This combined inhibition of different TGF-beta isoforms is assumed to contribute to the potency of trabedersen in tumors expressing several TGF-beta isoforms.

360 POSTER

BIO, the GSK3 beta blocker, is a potent inhibitor of cell proliferation and inducer of cell death of cervical carcinoma and rhabdomyosarcoma tumor cells

M. Majka[1], W. Bobela[1], K. Miekus[1]. [1]*Jagiellonian University Medical College, Department of Transplantation, Krakow, Poland*

Introduction: Since tumor disease is still an very important clinical problem new therapeutic strategies are needed. Cervical carcinoma (CC) is one of the major causes of death among women suffering from tumor. The highest mortality is observed in the group of patients with late diagnosis and with a metastatic disease. Rhabdomyosarcoma (RMS) is the most common soft tissue sarcoma among children. Also in this case the mortality is very high in patients with metastatic disease. The 5 year survival years in this group of patients is only about 20%.
Objectives: We have studied the influence of GSK3b inhibitor BIO on cervical carcinoma and rhabdomyosarcoma cell lines proliferation and survival.
Materials and Methods: CC cell lines (HeLa and HTB35) and RMS cell lines (RH30 and SMS) were used as a models. The influence of BIO on cell proliferation was studied using cell count assay and MTT assay. To check the influence of BIO on cell survival we used Hoechst 33342 staining and Annexin V and Caspase 3 staining. We also studied the influence of BIO on normal cells (MSC, HUVEC and myoblasts) and nonmalignant satellite cell lines (C2C12). The expression and activation of GSK3b was studied using western blot.
Results: We used three doses of BIO − 1, 5 and 10 uM. The lowest concentration was not able to inhibit proliferation of tumor cells or to induce apoptosis. Moreover, at this concentration in some instances proliferation was even increased. At higher doses the strong inhibitory effect on cell proliferation and survival was observed. We used two different rhabdomyosarcoma cell lines reflecting two RMS subtype. Interestingly, the alveolar subtype which is recognized as more malignant and highly metastatic was significantly more sensitive to the BIO. This phenomenon could be related to the different expression of GSK3b in this subtype in comparison to the embrional one (SMS). The cervical carcinoma cells were highly sensitive to the BIO inhibition as well. After 96 hours of incubation at the highest concentration of BIO more than 95% was inhibited in proliferation assay. At the same time profound cell death was observed. 5−10 uM BIO exerts massive cell death in HUVECs. On the other hand, mesenchymal stem cells and muscle cells were much more resistant to the action of BIO.
Summary: In the war with tumor new therapeutic strategies are still needed. In this study we showed for the first time that blocking of GSK3 function by specific small molecule inhibitors is able to block proliferation of cervical carcinoma and rhabdomyosarcoma cells and decrease their survival.

361 POSTER

HGF regulates the activity of GSK3 in rhabdomyosarcoma cells

M. Majka[1], K. Miekus[1], W. Bobela[1]. [1]*Jagiellonian University Medical College, Department of Transplantation, Krakow, Poland*

Introduction: Rhabdomyosarcoma (RMS) is the most common soft tissue sarcoma among children. Two major subtypes are recognized based on morphological and molecular features: alveolar (ARMS) and embryonal (ERMS). There are major differences between AMRS and EMRS e.g. presence of fusion proteins and higher rate of metastasis into distant organs like lungs and bone marrow in case of ARMS. Epithelial–mesenchymal-transition (EMT), is a phenomenon whereby epithelial cells temporarily or permanently acquire mesenchymal phenotype. EMT has been shown to play a key role during embryogenesis and wound healing. Latest reports have shown that EMT plays a crucial role in the development and progression of various tumors.
Aim: Dissecting the role of HGF in regulation of GSK3 activity and EMT related genes.
Materials and Methods: Cell lines used in experiments: RH30 (ARMS), SMS-CTR (ERMS). Real time RT-PCR and western blotting to evaluate gene expression and activation of various intracellular signaling pathways, respectively. Evaluation of protein activation in RMS cell lines was performed after stimulation with HGF.
Results: We observed the phosphorylation of GSK3b on serine 9 after HGF stimulation. To dissect the intracellular pathways responsible for GSK3b phosphorylation several inhibitors such as PI3K inhibitor − Ly294002, MEK inhibitor − U0126 and MET phosphorylation inhibitor were used. Use of both MET and PI3K inhibitors completely attenuated phosphorylation of GSK3b in HGF stimulated RMS cells. We observed also the accumulation of beta-catenin and Snail1 in the nucleus of RMS cells stimulated with HGF. This effect was father augmented when HGF was used together with BIO, small molecular inhibitor of GSK3b. When we study the expression of genes regulated by GSK3b, Snail1 and beta-catenin we noticed downregulation of E-cadherine expression and upregulation of cyclin D1 in cells stimulated with HGF.
Conclusion and future directions: Based on our data we can postulate that stimulation of GSK3b phosphorylation by HGF leads to stabilization and nuclear translocation of EMT activating proteins Snail1 and beta-catenin. This action subsequently causes the downregulation of E-cadherin and upregulation of cyclin D1. Finally, we think that GSK3b could be used as the new therapeutic target to block invasion and metastasis of RMS.
Future experiments will include (i) silencing of *GSK3b* (by viral transduction with shRNA), (ii) establishing the level of GSK3b expression at protein level in different RMS cell lines (by Western Blot) and RMS patients (by immunohistochemistry).

Molecular-targeted therapies − clinical trials

362 POSTER

First in human trial of a poly(ADP)-ribose polymerase (PARP) inhibitor MK-4827 in advanced cancer patients (p) with antitumor activity in BRCA-deficient and sporadic ovarian cancers

R.M. Wenham[1], S.K. Sandhu[2], G. Wilding[3], L. Sun[4], C. Toniatti[4], M. Stroh[4], C. Carpenter[4], J. de-Bono[2], R. Baird[2], W.R. Schelman[3]. [1]*Moffitt Cancer Center, Divisions of Gynecologic Oncology and Experimental Therapeutics, Tampa FL, USA;* [2]*Royal Marsden NHS Foundation Trust, Drug Development Unit, Sutton, United Kingdom;* [3]*University of Wisconsin, Carbone Cancer Center, Madison WI, USA;* [4]*Merck & Co. Inc., Merck Research Laboratories, Whitehouse Station NJ, USA*

Background: MK-4827 is a potent, selective, PARP1/2 inhibitor with IC_{50} of 3.8 nM. It induces selective synthetic lethality in homologous recombination (HR) repair deficient tumors with BRCA1/2 loss and in tumor cell lines with non-BRCA-related HR defects, supporting clinical utility in sporadic tumors.
Methods: MK4827 was administered orally once daily in cohorts of 3−6 p, enriched for BRCA-deficient and sporadic cancers associated with HR repair defects. Dose escalation was guided by toxicity, pharmacokinetic (PK) and pharmacodynamic (PD) data. Permission was obtained from the appropriate regulatory authorities and properly informed consent given.
Results: 59 p (M13, F46; median age 56 years; 23 BRCA-mutation carriers) were treated at 10 dose levels [30 mg (n = 6), 40 mg (n = 3), 60 mg (n = 7), 80 mg (n = 6), 110 mg (n = 5), 150 mg (n = 6), 210 mg (n = 6), 290 mg (n = 5), 300 mg (n = 9), 400 mg (n = 6)] on days 1−21 of a 28 day cycle (C) in C1, followed by continuous dosing. Prior systemic treatments were 1−2 (n = 5p), ⩾3 (n = 11 p), and ⩾4 (n = 40 p). Overall, dose-limiting toxicity was observed in 4 p: grade (G) 3 fatigue in 1/6 p at 30 mg, reversible G3