Results and Conclusions: In contrast to HCT-116, $CD133^{-/low}$ HT29 cells showed a lower clonogenic survival and reduced spheroid formation capacity than their $CD133^{+}$ counterparts. HT29 cell survival decreased in a lactate-enriched milieu, an effect that was more pronounced in the $CD133^{-/low}$ population indicating that $CD133^{+}$ cells may better survive in a pathophysiological environment. All differences were significant but not as pronounced as expected. Also, no difference in response to treatment was observed for the different populations, and tumour formation capacity was 100% for as low as 500 cells injected s.c. per animal. We therefore analyzed CD133 expression after sorting and found a clear, yet unexpected rapid increase of the $CD133^{+}$ fraction in the $CD133^{-/low}$ sorted HT29 population in 2-D and 3-D culture under serum-supplemented conditions. The mechanisms of CD133 expression control have to be elucidated to verify if CD133 in CRC cell lines and tissue may be an epiphenomenon of environmental conditions.
Supported by the DFG (KU 971/7-1 / GR 3376/2-1 and KFO179).

448 Targeting the p53 tumour suppressor activity in Glioblastomas using small molecule MDM2-inhibitor

P. Monfared[1], D. Rudan[1], L. Franken[1], G. Schneider[1], T. Viel[1], M. Hadamitzky[1], Y. Waerzeggers[1], A.H. Jacobs[1]. [1]*Max Planck Institute for Neurological Research, Neurology, Cologne, Germany*

Introduction: Targeted therapies that inhibit the MDM2-p53 interaction and the downstream Rb-E2F signalling pathway have shown promising anticancer activity, but their efficacies in human glioma have not been investigated. Recently, small-molecule antagonists of MDM2, the MDM2-inhibitiors, have been developed to inhibit the MDM2-p53 interaction and to activate p53 signalling serving possible anti-cancer activity.
Aim: To investigate the therapeutic potential of disrupting the MDM2-p53 interaction in human glioma cells with various p53 status. We particularly followed whether MDM2-inhibition would sensitize gliomas to additional chemotherapy.
Methods: We investigated the activity of MDM2-inhibitor alone and in combination with chemotherapy on cell cycle regulating proteins by Western blot and *in vivo* by employing imaging sensing vectors.
Results: MDM2-inhibior alone and in combination with BCNU results in a dose- and time-dependent reduction in cell viability and proliferation. Western blot studies showed that MDM2-inhibition modifies expression of several genes and results in cell cycle arrest and induction of apoptosis. Moreover, we found consistent and robust accumulation of p53 protein and downregulation of E2F-1 protein triggered by MDM2-inhibition alone and in combination with BCNU in all glioma cells as well as primary glioma samples. The MDM2-inhibitor and BCNU mediated alteration of p53 and E2F1 activities could be quantified in vivo by bioluminescence imaging and correlated to our results in culture.
Conclusions: Our results demonstrate that MDM2 inhibition elicits a dose- and time-dependent antiproliferative effect of glioma growth and potentiates the effects of BCNU via p53-dependent and p53-independent mechanisms and multiple genes seem to be involved in this process. MDM2 inhibitors with broad spectrum of antitumour activities in human cancers regardless of p53 status, may provide novel approaches to the therapy of malignant brain tumours.
Acknowledgement: This work is supported in part by the EC-FP6 Clinigene, LSHB-CT-2006–018933 and the EC-FP6-project DiMi, LSHB-CT-2005–512146.

Reference(s)

[1] Berg, Curr opin Drug Discov Devel. 2008
[2] Monfared P. et al, *Cancer Res*. 2008

449 Inhibition of vascular-like network formation of highly aggressive melanoma

O. Itzhaki[1], E. Greenberg[1,2], M.J. Besser[1], Y. Nemlich[1], R. Ortenberg[1,2], J. Schachter[1], A. Treves[1], G. Markel[1,2,3]. [1]*Ella Institute of Melanoma, Sheba Cancer Research Center, Tel Aviv, Israel,* [2]*Departments of Clinical Microbiology and Immunology, Sackler Faculty of Medicine, Tel Aviv University, Israel,* [3]*Talpiot Medical Leadership Program, Sheba Medical Center, Tel Aviv University, Israel*

Melanoma cells display substantial plasticity, demonstrated by directly forming tube-like structures composed of tumour cells but not of endothelial cells, which conduct blood cells and fluid. This phenomenon was termed Vasculogenic Mimicry (VM). Recently, it was shown that the presence of VM in melanoma masses predicts poor prognosis. Noteworthy, several anti-angiogenic lines of therapy seem ineffective against melanoma. It could be speculated that this alternative vascularization pathway might be of importance for advancement of melanoma.
We examined the ability of two agents to abrogate VM: IFNalpha, an immunomodulator with an antiangiogenic effect, and nicotinamide, the amide form of vitamin B3 (niacin).
The in vitro effects of the agents were examined using the highly aggressive melanoma cells (C8161). VM was tested as formation of tubular networks when grown in three-dimensional (3D) culture. In addition, cell proliferation (measured with XTT), cell cycle analysis (DNA content) and invasion capacity through matrigel were tested concomitantly.
IFNalpha affected *in vitro* VM formation in a dose-depended manner (at concentrations of 5×10^4 and 5×10^5 IU). Further, IFNalpha significantly inhibited the proliferation of C8161 cells. Cell cycle analysis reveled a significantly increased proportion of apoptotic cells. Moreover, the invasion ability was decreased in the treated cells. Nicotinamide (at concentrations of 1 and 5 mM) significantly inhibited the proliferation of the melanoma cells, but had no effect on their invasion capacity. According to cell cycle analysis, nicotinamide treated cells showed no significant changes in their respective apoptotic indices. Nicotinamide inhibited VM formation, but the effect was inconsistent. All effects were compared to control treatments with carrier only.
Due to the fact that both IFNalpha and nicotinamide hold a wide range of biological activity, the dose for optimal results may differ greatly as different effects are mediated by different concentrations. Nevertheless, both demonstrated anti-melanoma properties, including an effect on VM formation. Targeting VM could be of great importance, especially in combination with anti-angiogenic strategies. This combination is expected to be synergistic and yield substantial anti neoplastic effect.

450 Leptin and estrogen receptor expression in breast cancer patients with different clinical characteristics

A. Lushnikova[1], E. Peredereeva[1], L. Lyubchenko[2], A. Parokonnaya[3], E. Polevaya[4]. [1]*N.N. Blokhin Russian Cancer Research Center, Oncovirus Immunology Laboratory, Moscow, Russian Federation,* [2]*N.N. Blokhin Russian Cancer Research Center, Clinical Genetics Laboratory, Moscow, Russian Federation,* [3]*N.N. Blokhin Russian Cancer Research Center, Radiosurgery Department, Moscow, Russian Federation,* [4]*N.N. Blokhin Russian Cancer Research Center, Mammary Tumours Department, Moscow, Russian Federation*

Introduction: Leptin is a multifunctional hormone produced by adipocytes. It plays important role in angiogenesis. Induction of cell proliferation, survival and anchorage-independent growth.
These leptin activities are mediated through leptin receptor (ObR) that binds leptin molecule and stimulates Jak/STAT 3, ERK 1/2, cyclin D1 expression and other signal pathways. A recent data show that targeting leptin signaling may reduce mammary carcinogenesis and breast cancer (BC) progression. However, the link between obesity and leptin expression in serum/breast tumour as well as its role in modulation of estrogen receptors (EsR) and HER2/neu expression is not clear.
Material and Methods: We studied leptin, ObR, EsR, HER2/neu expression in patients with sporadic, familial and pregnancy-associated BC by RT PCR using BC fresh tissue and primers for genes encoding leptin, ObR, EsR-α, β. Leptin level in the patient sera was estimated also by ELISA (Leptin Sandwich DRG, DRG Diagnostics, Germany) followed by comassie staining. The data on routine immunohistochemical staining of BC paraffin embeded section for HER2/neu, EsR and PrR were also obtained. In control group were patients with benign fibroadenoma (BFa) and healthy women of comparable age.
Results: RT PCR results and immunohistochemistry method are mainly concordant: only 5% (5/29) of data were different. In triplonegative tumours (n = 40) leptin overexpression was significantly higher than in other tumour types. Blood sera leptin level was correlates positively with ObR expression. Leptin expression in tumour tissue also correlates with HER2/neu over expression in all BC groups except triplonegative ones: Esr (−), PrR(−), HER2/neu(−). Serum leptin levels in BFa patients was higher (100–300 ng/ml) than in healthy women of comparable age and body weight. Moreover, leptin serum level was positively correlates with ObR expression in tumours on I-III BC stages and with high body weight index (obesity).
Conclusions: The data obtained indicate that leptin/ObR may involved in BC progression. It indicates that ObR suppression is the possible way for target BC therapy, especially of triplonegative tumours which do not express HER2/neu, so hormone therapy is not effective for these neoplasia.

451 Progesterone regulation of breast cancer cell coagulative and invasive potential is dependent on the distinct membrane localization of tissue factor

G. Owen[1], M.L. Bravo[1], M. Quezada[1], S. Henriquez[1]. [1]*Pontificia Universidad Catolica de Chile, Physiology, Santiago, Chile*

Background: The oncogene Tissue Factor (TF) is over-expressed in breast cancers and is correlated to metastasis and thus poor prognosis. The usage of exogenous progestins is associated with increased breast cancer incidence. We previously reported that TF is transiently regulated by progesterone at the level of transcription and that the blocking of TF activity by antibodies eliminates the progesterone-mediated coagulative and invasive potential of the breast cancer cell lines ZR-75 and T47D.
Material and Methods: Coagulation was measured in whole cells by the generation of FXa in the presence of FX and FVIIa. Invasion was measured