

Immunoprecipitation and western analysis were conducted in order to detect the formation of each growth factor-integrin complex. Confocal microscopy was used in order to perform immunofluorescence, detecting the above mentioned complex formations.

Results: The application of both agents either alone or in combination showed significant reduction in proliferation and chemotacticism in both cell lines. There was also an induction of apoptosis in both cell lines. MMP levels were down regulated in M059K cells while there was no change of MMP levels in U87 cells. Lapatinib intercepted the formation of EGFR-integrin β 1 complex, in both cell lines while sunitinib intercepted VEGFR-integrin β 3 complex formation in U87 cells. Immunofluorescence revealed colocalisation of molecules in the above mentioned complexes and their disengagement after application of agents in a time course manner.

Conclusions: Lapatinib and Sunitinib have a strong inhibitory effect. Combinational dosing of these agents has a better and stronger effect in the above mentioned parameters than each one of them on its own. The current data showed an implication of the tested agents in the integrin – growth factor's pathway.

[511] Caveolin-1, TGF β /Smad2 and Alpha5 Beta1 integrins connection in human glioblastoma

E.C. Cosset¹, D. Bonnet², M. Döntenwill¹, S. Martin¹. ¹University of Strasbourg UMR CNRS 7213, Integrins and Cancer, Illkirch, France, ²University of Strasbourg UMR CNRS 7200, Innovations Thérapeutiques, Illkirch, France

Background: Caveolin-1 (cav1) plays a crucial role in cancer development and progression. Although caveolin-1 expression is increased in glioma, cav1 negative (cav1_{low}) and positive (cav1_{high}) cells coexist in glioblastoma (GBM). We reported that cav1_{low} GBM cells exerted a more aggressive phenotype than cav1_{high} GBM cells, suggesting that cav1 is a tumour suppressor in brain tumours. Transcriptomic analysis showed that cav1 represses integrins especially $\alpha_5\beta_1$ integrin so that cav1 and $\alpha_5\beta_1$ integrin expressions were inversely correlated. We identified $\alpha_5\beta_1$ integrin as the mediator of cav1's effect in GBM. This study focused on the mechanisms by which cav1 regulate $\alpha_5\beta_1$ integrin expression.

Material and Methods: 6 different GBM cell lines were used. Some were silenced (using si/shRNA_{cav1}) or forced to express (pEGFP_{cav1}) cav1. TGF β was quantified using a commercially available kit. Protein expression and activity was determined by western blot. Drugs used were SB431542, LY294002 and U0126 (inhibiting the TGF β receptor, PI3K and MEK1 respectively), K34c (a $\alpha_5\beta_1$ integrin antagonist), TGF β and activin. Surviving fraction after drug treatment was determined by clonogenic assays. Gene expression was studied by qPCR.

Results: Cav1 affects the TGF β /Smad2 pathway, previously identified as a regulator of integrin expression. Silencing cav1 increased the secretion of TGF β , the expression of TGF β receptor and the activity of its downstream effector Smad2. Conversely, forced expression of cav1 repressed the TGF β /Smad2 pathway so that cav1 expression and TGF β /Smad2 activity are inversely correlated. Using selective inhibitors, we showed that the TGF β /Smad2 pathway was involved in the regulation of $\alpha_5\beta_1$ integrin expression by cav1. Two Smad2-dependent signaling pathways were involved; one independent on the TGF β RI (cav1 $\rightarrow$ ERK $\rightarrow$ Smad2 $\rightarrow$ $\alpha_5\beta_1$ integrin) and one dependent on the TGF β RI (cav1 $\rightarrow$ TGF β RI $\rightarrow$ PI3K/Akt $\rightarrow$ Smad2 $\rightarrow$ $\alpha_5\beta_1$ integrin). Therefore, cav1_{low} cells exert high level of TGF β RI/Smad and $\alpha_5\beta_1$ integrin and vice versa. The reverse correlation between cav1 and $\alpha_5\beta_1$ /TGF β /Smad2 was confirmed in different GBM cell lines. Finally, we showed that cav1_{low}/ $\alpha_5\beta_1$ /TGF β /Smad_{high} cells (identified as being the most aggressive) are highly sensitive to SB431542 and K34c.

Conclusions: Cav1 controls $\alpha_5\beta_1$ integrin expression through the TGF β /Smad2 pathway. The status of cav1/ $\alpha_5\beta_1$ /TGF β /Smad2 might be a useful marker of the tumour behavior and a predictor of anti-TGF β or anti- $\alpha_5\beta_1$ integrin therapies.

[512] Tetraoxanes induced ROS production and activation of caspase 3 in HeLa cells

Z. Zizak¹, Z. Juranic¹, D. Opsenica², B.A. Solaja³, I. Besu¹. ¹Institute of Oncology & Radiology of Serbia, Experimental Oncology, Belgrade, Serbia, ²Institute of Chemistry Technology and Metallurgy, Chemistry, Belgrade, Serbia, ³Faculty of Chemistry University of Belgrade, Organic Chemistry, Belgrade, Serbia

Background: It was demonstrated that mixed steroidal tetraoxanes inhibit cancer cell proliferation at micromolar level through an apoptotic mechanism. It will be interesting to see if these compounds may possibly induce oxidative stress, which could lead to induction of apoptosis in tumour cells. As tumour cells contain more iron than other normal tissues it is reasonable to suggest that tetraoxanes could react with iron, generating alkoxy radicals or even highly reactive hydroxyl radicals in a Fenton-like reaction. To gain further insight into the mechanism of cell death induced by tetraoxane endoperoxides, we

tested production of reactive oxygen species (ROS) and level of activation of caspase 3 in tumour cells treated with several newly synthesized tetraoxanes.

Material and Methods: Stock solutions of investigated tetraoxanes, were prepared in DMSO at concentrations of 10 mM and afterwards they were diluted with complete nutrient medium to various final concentrations. Target cells used were malignant cervix carcinoma HeLa cells.

Production of intracellular ROS was measured using the fluorescent dye 2',7'-dichlorofluorescein diacetate, a nonpolar compound that readily diffuses into cells, where it is hydrolyzed to 2',7'-dichlorofluorescein (H₂DCFDA), a nonfluorescent polar compound. In the presence of an oxidizing compound, H₂DCFDA is converted into highly fluorescent 2',7'-dichlorofluorescein.

Level of active caspase 3 is measured using the caspase 3 fluorimetric assay kit (Sigma Chemicals), based on the hydrolysis of the peptide substrate by caspase 3, resulting in the release of the fluorescent 7-amino-4-methylcoumarin moiety.

Results: After treatment with investigated tetraoxanes ROS level in HeLa cells significantly increased indicating possible oxidative stress, maybe as a result of the production of reactive alkoxy or hydroxyl radicals. Significant increase in activity of caspase 3 was observed after incubation of HeLa cells with all investigated tetraoxanes.

Conclusion: Taken together, these results demonstrate that tetraoxanes potentially generates ROS, and strongly inhibits the growth of HeLa cells throughout apoptosis. Although the mechanisms by which mixed tetraoxanes activates caspase 3 in HeLa cells remains unclear, those results provide correlation between ROS production, caspase 3 activity and tetraoxanes-induced apoptosis.

[513] DNA copy number changes in radiation-induced mammary carcinoma of (SD x COP) F1 hybrid rats

M. Nishimura¹, T. Imaoka¹, T. Takabatake¹, K. Daino¹, S. Kakinuma¹, T. Okutani¹, M. Takabatake¹, Y. Shimada¹. ¹National Institute of Radiological Sciences, Experimental Radiobiology for Children's Health Research Group, Chiba, Japan

Background: Epidemiological studies indicate that breast is one of the most susceptible organs to radiation-induced carcinogenesis. Most studies, however, have failed in identifying clear genetic alterations in radiation-induced breast/mammary cancers. The Copenhagen (COP) rats are completely resistant, whereas Sprague-Dawley (SD) rats are highly susceptible, to chemically-induced mammary carcinogenesis, and this resistance of the COP background is regarded as a dominant trait.

Material and Methods: Mammary cancer-prone SD, -resistant COP, and their hybrid (SD x COP) F1 rats were irradiated with gamma-rays at 4 Gy and underwent autopsy at the time of spontaneous death or at 1.5 years post-irradiation. Genomic DNA was extracted from mammary cancers and ear skins of corresponding individuals. Genome-wide DNA copy number was analyzed by array comparative genomic hybridization (aCGH).

Results: COP rats were resistant to radiation-induced mammary carcinogenesis. Interestingly, F1 rats showed a relatively susceptible trait, suggesting recessive inheritance of the resistance to radiation-induced mammary carcinogenesis. The preliminary results of aCGH analysis indicated that partial deletions of the proximal region of chromosome 2, where Mcs-1 (mammary cancer susceptibility gene-1 for chemical carcinogenesis) is mapped, were occasionally observed, suggesting a tumour suppressive role of Mcs-1 in radiation carcinogenesis. Other aberrations including small deletions and aneuploidy were also frequent but scattered throughout the genome.

Conclusions: These findings implicate that radiation-induced rat mammary cancers are rather heterogeneous with regard to copy number changes. The frequent deletion of COP alleles suggests that these tumour suppressive alleles may be readily targeted by radiation but not carcinogenic chemicals.

[514] Co-expression of E- and P-cadherin in breast cancer: role as an invasion suppressor or as an invasion promoter?

A.S. Ribeiro¹, L.C. Carreto², A. Albergaria¹, B. Sousa¹, S. Ricardo¹, F. Milanezi¹, R. Seruca¹, M.A. Santos², F. Schmitt¹, J. Paredes¹. ¹IPATIMUP, Cancer Genetics, Oporto, Portugal, ²University of Aveiro, Department of Biology and CESAM, Aveiro, Portugal

Background: Cadherins are cell-cell adhesion molecules. During tumour progression, their expression and/or function are frequently altered. E-cadherin down-regulation is often associated with tumour initiation and progression in breast cancer, whereas P-cadherin overexpression is associated with a worse patient survival, as well as with invasive breast cancer cells. In this study, we aimed to understand if P-cadherin overexpression could interfere with E-cadherin invasion suppressor role in breast cancer.

Materials and Methods: E- and P-cadherin expression was evaluated in a series of invasive breast carcinomas. The results were correlated with prognosis and clinico-pathological parameters. To study the functional value of E- and P-cadherin co-expression, we silenced the transcription of both cadherins in BT-20 breast cancer cells, and investigated the *in vitro* effects