intracellular hydrogen peroxide (H2O2) and by a low expression of the major H2O2-detoxifying enzymes, catalase and peroxiredoxin 3, compared to MCF-7 cells. Suppression of MnSOD expression by antisense RNA was associated with a decrease of H2O2 content and caused a stimulation of growth with a reduced cell doubling time but induced a decrease of colony formation. Furthermore, treatment of MDA-MB231 cells with H2O2 scavengers markedly reduced tumor cell growth and colony formation. In addition, MnSOD suppression or treatment with H2O2 scavengers reduced the invasive properties of MDA-MB231 cells up to 43%, with a concomitant decrease of metalloproteinase-9 activity.
Conclusions: We conclude that MnSOD plays a role in regulating tumor cell growth and invasive properties of estrogen-independent metastatic breast cancer cells. These action are mediated by MnSOD-dependent H2O2 production. In addition, these results suggest that MnSOD up-regulation may be one mechanism that contributes to the development of metastatic breast cancers.

374 POSTER

Synergic antiproliferative effect of Hsp90 inhibitor in combination with cisplatin in gastric carcinoma cell lines

H. Dote, S. Hato, R. Koshimune, H. Ino, M. Naito, H. Date. *Okayama University graduate school of medicine, Cancer and thoracic surgery, Okayama, Japan*

Background: 17-(dimethylaminoethylamino)-17-demethoxygeldanamycin (17-DMAG) is a new anticancer agent currently in clinical trials. The ability of 17-DMAG to abrogate the function of heat-shock protein Hsp90 and modulate cellular sensitivity to anticancer agents has prompted recent research to use this compound in drug combination therapy. In this study, we determined whether 17-DMAG potentiates the cytotoxic effect of cisplatin (CDDP) on gastric cancer lines and investigate the mechanism underlying this enhancement of CDDP-induced cytotoxicity by 17-DMAG.
Methods: In a panel of four gastric cancer cell lines (MKN-1, MKN-7, MKN-45, and NUGC-4) we investigated the antiproliferative and cytotoxic effects of 17-DMAG, CDDP alone or a simultaneous combination of two drugs using in vitro tetrazolium-based colorimetric assay (MTT). The combination treatment was evaluated for synergism, additivity, or antagonism with a quantitative method based on the median-effect principle of Chou and Talalay. Cell cycle alterations were analysed by flow cytometry, while apoptosis was assessed by the occurrence of DNA internucleosomal fragmentation. Along with Western blotting experiments were performed to determine whether this synergistic combination of two drugs has significant effect on MAPK signaling and biochemical markers of apoptosis.
Results: In all four gastric cancer cells, 17-DMAG synergistically potentiated the inhibitory effect of CDDP on cell growth. Western blot analysis showed that phosphorylation of JNK1 and c-Jun induced by CDDP was down-regulated by the combination treatment. Mechanistic studies showed enhanced accumulations of the sub-G1 phase population in cells treated by the combination, which indicate the induction of apoptosis. Moreover, this correlated with enhanced activation of caspases 3, 8 and 9 and poly (ADP-ribose) polymerase cleavage. Evidence of synergy was formally demonstrated and occurred across a wide range of drug concentrations.
Conclusion: 17-DMAG synergistically augments the growth inhibition inserted by CDDP in gastric cancer cells. The synergistic effect was mediated through inhibition of activation of JNK1-cJun and induction of apoptosis. These studies provide the basis for potential clinical evaluation of this combination treatment for gastric cancer patients.

375 POSTER

Increased expression of EphA2 correlates with adverse outcome in primary and recurrent glioblastoma multiforme patients

E. Fokas[1], L. Wang[1], M. Bieker[1], P. Rexin[2], A. Pagenstecher[2], R. Engenhart-Cabillic[1], H. An[1]. *[1]Philipps University, Radiotherapy and Radiation Oncology, Marburg, Germany; [2]Philipps University, Pathology, Marburg, Germany*

Background: Glioblastoma multiforme (GBM) is the most aggressive form of brain tumor characterized by exuberant angiogenesis. The dismal prognosis of patients with GBM warrants the development of new targeting therapies based on novel molecular markers. The EphA2 receptor tyrosine kinase plays a pivotal role in tumor angiogenesis and increased expression in glioma patients has been recently reported. In this study, we investigated the expression of EphA2 in primary and recurrent GBM and correlated it with clinical pathological parameters and patient's outcome.
Materials: The immunohistochemical expression of EphA2 receptor tyrosine kinase was analysed in a series of 32 formalin-fixed, paraffin embedded primary and recurrent GBM previously treated with surgery and radiation therapy. In addition, tumor microvascular density (MVD) was quantified by immunostaining for endothelial cell marker, von Willebrand factor (vWF). The correlation between expression of EphA2 and MVD as well as the prognostic relevance of EphA2 and MVD for long-term survival were investigated using the Kaplan-Meier statistical test.
Results: Different intensity of membranous and cytoplasmic expression of EphA2 were observed in GBM samples analysed while a strong expression of EphA2 was demonstrated in 24 (60%) of these primary and recurrent GBM. Additionally, no strong association between EphA2 expression and MVD was found ($P > 0.05$). No close correlation was noted between the expression levels of EphA2 or MVD and clinical pathological parameters such as age, and gender of patients. Increased expression of EphA2 protein was significantly associated with adverse outcome of GBM patients ($p < 0.01$ for overall survival) but was not prognostic for disease-free survival ($P > 0.05$).
Conclusions: The data presented in this study define for the first time the expression pattern of EphA2 in primary and recurrent glioblastoma and suggest the involvement of EphA2 in the development of GBM. The EphA2 might be used as surrogate marker to screen patients for tyrosine kinase inhibitor therapy.

376 POSTER

Effects of magnetic field exposure in the mammary gland tissue of female F344 rats and the impact of amylase

M. Fedrowitz, W. Löscher. *University of Veterinary Medicine, Dept. of Pharmacology Toxicology and Pharmacy, Hannover, Germany*

Epidemiological data have raised concerns about the relationship between exposure to power frequency magnetic fields (MFs) and breast cancer. We have shown previously in in vivo animal experiments that the effect of MF exposure on the rat mammary gland differs depending on the different rat strain or substrain that is used. Comparison of different rat strains indicated that the genetic background plays a pivotal role in the MF effects. Among several rat strains, only Fischer 344 (F344) rats showed an enhanced proliferative activity in the mammary epithelium exposed to MF for 2 weeks. Prolonged MF exposure significantly increased tumor development and growth in the dimethylbenz[a]anthracene (DMBA) breast cancer model in F344 rats. These results indicate that the F344 inbred rat serves as a MF-sensitive rat strain.
Recently, we investigated the gene expression in the breast tissue of F344 rats and compared the results with Lewis rats (Lew) that are considered MF-insensitive. Unexpectedly, the most striking result was a marked decrease of amylase gene expression in MF-exposed F344, but not in Lew. Because of this finding, we now determined amylase enzyme activity in the breast tissue of juvenile F344 rats that were exposed to MFs or to the synthetic estrogen diethylstilbestrol (DES). F344 were MF-exposed over different periods, the insensitive Lew rats only over 2 weeks. DES was administered to F344 rats at different dosages and time points. Enzyme activity was measured colorimetrically with Starch Azure as a substrate. The grade of differentiation of the breast tissue was checked up by whole mount analysis.
DES application increased the appearance of more differentiated structures in the breast tissue in a dose-dependent manner. No alteration was observed in MF-exposed whole mounts of mammary glands. DES significantly increased amylase activity at the highest dosage (30 μg, 6 times). MF exposure also significantly increased enzyme activity in F344 rats (2 or 4 weeks of exposure) and in Lewis rats (2 weeks).
These data demonstrate that MF exposure and DES altered amylase activity in the rat mammary gland tissue. In literature, few associations between amylase and tumor development are described, but the underlying mechanisms are not known. Future cell culture experiments from breast tissue and breast tumors might be able to reveal the amylase effect in the tissue.
This work is funded by a grant (Lo 274/6−3) from the Deutsche Forschungsgemeinschaft.

377 POSTER

Papaverine derivatives – new telomerase inhibitors

B. Rubis[1], B. Juskowiak[2], T. Hermann[3], E. Galezowska[2], A. Czyrski[3], M. Rybczynska[1]. *[1]K. Marcinkowski University of Medical Sciences Poznan, Division of Clinical Chemistry Department of Pharmaceutical Biochemistry, Poznan, Poland; [2]Adam Mickiewicz University Poznan, Faculty of Chemistry Laboratory of Analytical Chemistry, Poznan, Poland; [3]K. Marcinkowski University of Medical Sciences, Physical Pharmacy and Pharmacokinetics, Poznan, Poland*

One of the cancer cell growth inhibiting strategy is application of drugs poisoning or inhibiting activity of enzymes engaged in DNA processing including topoisomerases and telomerase. The effectiveness of these enzymes inhibition results from the ability of drugs to intercalate DNA