15

Peroxisome proliferator activated receptor γ (PPARγ) agonists for treatment of neoplastic diseases - in vitro data and early clinical observations in chronic myelomnocytic leukemia (CMML) and in liposarcoma. C. Denzlinger, A. Möhle, F. Machicao, R. Möhle, P. Brossart, H.-U. Häring, L. Kanz, K. Dittmann. University Clinic Tübingen, D-72076 Tübingen, Germany.

PPARγ is a nuclear hormone receptor which modulates gene expression upon agonist binding and heterodimeization with activated retinoid X receptor. PPARγ agonists comprise both natural (cyclopentenone prostanoids) and pharmaceutical compounds (e.g. thiazolidinediones) and have been shown to exert antiproliferative effects in several cell lines in vitro. We investigated whether the thiazolidinediones troglitazone (TRO) or rosiglitazone (ROS) have an effect on proliferation of mononuclear cells from healthy individuals and form patients with CMML in vitro. At a concentration of 50μM, TRO reduced [^{3}H]thymidine incorporation in mononuclear cells from healthy individuals (n=5) or from CMML patients (n=10) during 24 h of incubation to 57±18% (mean±SD) or to 9±7% of control. ROS had similar but less potent antiproliferative effects in CMML cells in vitro whereas cells from healthy individuals were not significantly affected. Our in vitro data prompted us to offer TRO to 3 selected CMML patients. Another 4 CMML patients were treated with ROS. Furthermore, 3 patients with metastasized liposarcoma were treated with TRO or ROS. Treatment with either drug was very well tolerated. Control of monocyte counts was transiently improved in 5 of the 7 CMML patients. In 2 of the 3 liposarcoma patients minor responses and transient disease stabilizations were noted. Our results suggest that PPARγ agonists may have therapeutic potential in CMML and in liposarcoma justifying further study of this non-genotoxic approach.

16

Multicellular gastric cancer spheroids represent a valid model for pre-clinical testing of new therapeutic strategies

B. Mayer, G. Klement, M. Kaneko, S. Jothy and R.S. Kerbel

Gastric carcinoma is largely unresponsive to current chemotherapy and better pre-clinical models to study therapeutic targets are urgently needed. An *in vitro* multicellular gastric cancer spheroid model was established, and compared to the subcutaneous xenografts in nu/nu mice as to: i) morphological and functional differentiation and ii) expression of cell adhesion molecules. Twelve out of 17 (71 %) gastric cancer cell lines grown in 3D reflected growth characteristics of their parental gastric carcinomas. Cell lines derived from peritoneal and pleural carcinomatosis grew as single cells (HSC-39, KATO-II, KATO-III) or cell aggregates (SNU-5, SNU-16). Adenosquamous (MKN-1) and tubularly differentiated (MKN-28, MKN-74, N87) cell lines formed partly compact multicellular spheroids and also reflected their parental architecture. Most importantly, differentiation was lost after s.c. implantation of these pre-formed spheroids. Levels of mucin and constitutive E-cadherin expression reflected the degree of morphological differentiation, but the changes of other adhesion molecules (EpCAM, $\alpha_2\beta_1$, CD44s, Le^x, sLe^x) were heterogeneous. In contrast, cell lines derived from poorly differentiated gastric carcinomas (Hs-746T, RF-1, RF-48) formed fully compact spheroids mimicking the poorly differentiated phenotype, were E-cadherin negative, and showed only CD44s upregulation. In summary, multicellular gastric cancer spheroid recapitulate the complexity of their *in vivo* counterparts, and represent a superior model for studying the biology of this cancer than s.c. xenografts. They embody a physiologically relevant model to test new therapeutic strategies such as differentiating and anti-adhesion agents.

17

Magnetic drug targeting: Biokinetic study and therapeutic efficacy

Ch. Alexiou, P. Hulin, R. Klein, A. Schmidt, Ch. Bergemann, F.G. Parak, W. Arnold, Technische Universität München, Deutschland.

Biocompatible ferrofluids (ff) are paramagnetic nanoparticles, that may be used as a delivery system for anticancer agents in locoregional tumor therapy, called „magnetic drug targeting". Tumor bearing rabbits (VX-2 SCC) in the area of the hind limb, were treated by a single i.a. injection (A. femoralis) of mitoxantrone bound ff, while focusing an external magnetic field (1,7 Tesla) onto the tumor for 60 min. Complete tumor remission could be achieved, without any negative side effects. Beside the clinical effects enrichment of ff in tissue focused by the external magnetic field was documented in vivo by histological analysis and MRI. Biodistribution was studied by the use of Iod^{123} and Fe^{59} labeled nanoparticles. The scintigraphically detected Iod^{123}- signal after i.a. application has been shown to be significantly higher in the magnetically focused region compared to the application without external magnetic field. Quantitative analysis by Fe^{59}-labeled ff showed more than 90% ff enrichment compared to other tissues after one and six hours by magnetic field. The enrichment of ff is dependent on the applied magnetic flux density, which was shown in vitro with Iod^{125}-labeled magnetic beats. By applying an external magnetic field of at least 0.4 Tesla ff could be seen intracellularly. These data demonstrate the accumulation of ff in tumor by focusing an external magnetic field. The therapeutic efficacy in tumor treatment with mitoxantrone bound ff may indicate that this system could be used as a delivery system for anticancer agents, like radionuclids, genes etc. Margarete-Ammon Stiftung, München und Technische Universität München. Dr. med. Ch. Alexiou, HNO-Klinik und Poliklinik des Klinikums rechts der Isar, Ismaninger str. 22, 81675München, Tel:089/41402370, C.Alexiou@lrz.tumd

18

APOPTOSIS RESISTANCE AND IMMUNE ESCAPE MECHANISMS OF BLADDER CANCER CELLS

Patricia L. Willert, Frank G.E. Perabo, Stefan Kamp, Doris H. Schmidt, Andreas Wirger[1], Peter Albers, Stefan C. Müller

Dept. of Urology, Bonn University, Medical University of Luebeck[1], Germany

Recent evidence indicates that tumor cells can exploit the Fas system system to their benefit in the dialogue with the host immune system. We investigated different mechanisms of immune evasion in bladder cancer cells of different grade RT4 (G1), RT112 (G1), T24 (G3) and SUP (G4). Fas, the receptor of Fas-ligand, is expressed and shedded by human transitional bladder carcinoma cell lines RT4, RT112, T24 and SUP. Cytotoxicity and apoptosis assays demonstrate that in spite of the Fas expression, poorly differentiated T24 and SUP cells are insensitive towards either recombinant Fas-ligand or agonistic apoptosis-inducing monoclonal antibody against Fas. In poorly differentiated T24 and TCCSUP cell lines we were able to detect marked Fas-ligand protein by flow cytometry and Western blot analysis. In grade 1 RT4 and RT112 cells only minor expression of Fas-ligand possibly because of proteinase action was found. Fas-ligand mRNA translation or post-translational processing seems to be regulated differentially in the cancer cell lines depending on malignant transformation. In co-culture experiments we show that poorly differentiated cells can induce apoptosis and cell death in Jurkat cells and activated peripheral blood mononuclear cells whereas immune cells had no effect on Fas-resistant cancer cells. This in vitro study suggests that bladder cancer cells can take advantage of different mechanisms of immune evasion and become more competent in avoiding immune surveillance during transformation to higher-grade malignant disease.