phosphatidylinositol 3-kinase (PI3 kinase) activity. DNA from CYR61 transfectants treated with PTX displayed no signs of the classical DNA laddering pattern of apoptotic death. Inhibition of PTX-induced apoptosis in CYR61 transfectants was also demonstrated by TUNEL assay. Moreover, MCF-7/CYR61 cells were unable to induce p53 expression in response to PTX-induced damage. It is tempting to postulate that the angiogenic factor CYR61 -a downstream effector of HRG- might protect breast cancer cells from PTX-induced apoptosis by enhancing alphav beta3-PI3 kinase pro-survival signaling and inhibiting p53 pro-apoptotic functions. We suggest that new anti-HRG, anti-CYR61, and/or anti-integrin alphav beta3 strategies may prevent vessel growth simultaneously rendering tumor cells more sensitive to PTX-based chemotherapy in breast cancer.

367

NFkappaB repression by sulfasalazine sensitizes pancreatic carcinoma cells to cytostatic drugs *in vivo*: a new concept of combined chemotherapy

S. Müerköster[1], A. Arlt[1], M. Witt[1], A. Gehrz[1], S. Haye[2], C. March[2], F. Grohmann[1], H. Kalthoff[2], U.R. Fölsch[1], H. Schäfer[1]. [1]*Laboratory of Molecular Gastroenterology & Hepatol, 1st Department of Medicine, Kiel, Germany;* [2]*Research group of Molecular Oncology, Department of General Surgery, Kiel, Germany*

The pancreatic carcinoma is still characterized by a poor prognosis and ranks 5th among malignancy-associated deaths. Surgical resection is feasible only in a minority of patients and other therapy options based on chemotherapy are only palliative. One reason for this might be the resistance of tumor cells towards cytostatic drugs. To improve the therapy of pancreatic cancer it will be important to understand the mechanisms how tumor cells achieve chemoresistance and to define molecular targets for new therapeutic strategies. Some pancreatic carcinomas are characterized by a constitutively elevated NF-kappaB activity accounting for chemoresistance. In order to elucidate whether blockade of NF-kappaB activity with the anti-inflammatory drug sulfasalazine is suitable for overcoming this chemoresistance *in vivo*, we employed a mouse model with subcutaneously xenotransplanted human Capan-1 pancreatic carcinoma cells. Fourteen days upon tumor inoculation, animals were randomized in six groups, receiving no treatment, treatment with gemcitabine (2.5 mg/kg day, intraperitoneal), treatment with etoposide (6.5 mg/kg day, intraperitoneal), either alone, or in combination with sulfasalazine (70 mg/kg day, oral), or with sulfasalazine alone. Upon treatment with etoposide or gemcitabine alone, tumor sizes were moderately reduced to 50 % and 60-70 %, respectively, as compared to untreated tumors. The corresponding combination groups (etoposide: 20-25 %, gemcitabine: 45-50 %) showed significantly higher reduction in tumor sizes. TUNEL-staining revealed higher numbers of apoptotic cells in tumors from combination groups, and proliferation as indicated by Ki67 staining was strongly reduced. Furthermore, combined treatment of sulfasalazine with the cytostatic drugs led to a decreased blood vessel density. Immunohistochemical staining of the activated p65 subunit revealed that sulfasalazine treatment abolished the basal NF-kappaB activity in tumor cells. These data imply that NF-kappaB inhibition sensitizes pancreatic carcinoma cells to cytostatic drugs *in vivo*. In particular, a combined chemotherapy with the well established anti-inflammatory drug sulfasalazine offers great potential for improved anti-tumor responses in pancreatic carcinomas.

368

Validation of reliable assay methods for glutathione quantitation and glutathione s-transferase activity in cancer patients

E. Ferruzzi[1], R. Franceschini[1], G. Cazzolato[2], C. Geroni[3], C. Fowst[4], N. Tradati[5], U. Pastorino[6], J. Tursi[4], R. Dittadi[1], M. Gion[1]. [1]*Venice General Hospital, Regional Centre of Biological Markers, Venice, Italy;* [2]*Venice General Hospital, "P.Avogaro" Laboratory - Regional Centre for the St, Venice, Italy;* [3]*Pharmacia Corporation, Oncology - External Research, Nerviano (MI), Italy;* [4]*Pharmacia Corporation, Clinical Oncology, Nerviano (MI), Italy;* [5]*European Institute of Oncology, Thoracic Surgery, Milan, Italy;* [6]*European Institute of Oncology, Head and Neck Surgery, Milan, Italy*

Glutathione s-transferases (GST) are members of a superfamily of enzymes that catalyze the reaction of electrophilic compounds with glutathione (GSH) to form inactive conjugates. These enzymes are of great importance in cancer biology since their levels have been correlated with resistance to cytotoxic drugs such as alkylating agents, platinum compounds and anthracyclines. Conversely, recently has been published that a DNA minor groove binder is activated by the GST/GSH systems (Geroni et al, Cancer Res. 2002). GST distribution in cancer patients has been extensively investigated but mixed data have been reported. The objectives of this study were primarily to validate robust and reliable assays for GSH/GST detection, suitable for routine clinical use and to explore the correlation between blood and tissue levels for both. Matched blood and tissue samples (normal and malignant) from 52 cancer patients (NSCLC and SCCHN) were investigated. GSH concentration and GST activity were measured by an enzymatic assay. GST content was also analysed by HPLC. Moreover, since the existence of regions of tissue heterogeneity is well documented within the tumor, multiple samples from seven cancer specimens have been analysed. Data were evaluated for either intra- and inter-patient variability to verify whether GSH/GST exhibit heterogeneity in samples from different areas of the same specimen. Both GST activity and GSH levels were higher in cancer than in normal tissue. The difference was statistically significant in NSCLC ($p=0.0004$ and $p=0.0002$, respectively for GSH and GST) and borderline in SCCHN ($p=0.03$ and $p=0.02$, respectively for GSH and GST). Moreover GSH levels in whole blood showed a highly significant correlation with GST activity in matched cancer samples in both malignancies ($p=0.003$, $r=0.53$ in NSCLC, $p<0.0001$, $r=0.89$ in SCCHN). The strong correlation found between GST activity in cancer tissue and GSH level in whole blood indicates that GSH could have a clinical relevance as a surrogate marker of GST activity in tumor tissue and should be further investigated. However, tissue heterogeneity analysis suggested that GSH and GST levels could be linked to tissue variability in both normal and tumour tissues. Since statistical analysis indicates that heterogeneity is a true biological fact rather than an analytical artefact, it is recommended to have a larger sample of tumor tissue for GSH and GST biochemical analysis to confirm the validity of GSH as biomarker.

369

Activation of wt p53 protein in normal and tumor cells by a novel anti-cancer drug CHS 828

J. Wesierska-Gadek[1], J. Wojciechowski[1], R. Larsson[2], H. Lövborg[2]. [1]*Institute of Cancer Research, Cell Cycle Regulation, VIENNA, Austria;* [2]*Uppsala University Hospital, Dept. of Clinical Pharmacology, UPPSALA, Swedon*

CHS 828, a novel cyanoguanidine, represents a new class of drugs for cancer treatment, with an unknown primary mechanism of action. It is known that anti-cancer drugs induce p53 response thereby triggering the cell cycle arrest or apoptosis We investigated the effect of anti-cancer drug CHS 828 on p53 induction in normal and tumor cells. We observed dose-dependent upregulation of wt p53 by CHS 828 in MCF-7 cells as well as in human and mouse fibroblasts. The drug, however, failed to induce p53 protein in PARP-1 deficient cells even at a 30-fold higher dose and after prolonged treatment. Combined treatment of PARP-1 -/- cells by CHS 828 and MDR modulators did not alter p53 expression. CHS 828 inhibited cell proliferation and DNA replication in tested cells. Interestingly, the DNA synthesis as well as proliferation of PARP-1 -/- cells was inhibited by about three-fold lower drug concentration then their normal counterparts. The treatment of mouse cells by CHS 828 for 48h impaired the integrity of plasma membrane in PARP-1 deficient mouse cells as evidenced by Trypan blue dye exclusion test. The effect of CHS 828 on p53 in normal cells seems to be cell cycle dependent. Treatment of quiescent cells resulted in downregulation of p53 protein. These results show that the drug is able to activate p53 response depending on cell cycle and PARP-1 functional status. The inactivation of PARP-1 sensitizes cells to the novel anti-cancer drug CHS 828.

370

PPARgamma ligands modulate PPARgamma and RARbeta expression in human glioblastoma cell lines

R. Morosetti[1], T. Servidei[1], G. Cusano[1], G. Segni[2], H.P. Koeffler[3], R. Mastrangelo[1]. [1]*Catholic University of Rome, Division of Pediatric Oncology, Rome, Italy;* [2]*Catholic University of Rome, Institute of Pediatrics, Rome, Italy;* [3]*Cedars-Sinai Medical Center, Division of Hematology/Oncology, Los Angeles, USA*

A recent approach to cancer therapy is the control of cell growth and induction of apoptosis via ligands of nuclear hormone receptors (NHR). Among NHR, much attention has been focused on peroxisome proliferator-activated receptor gamma (PPARgamma), that has been involved in the control of growth and/or differentiation of several malignant cell types. Currently, we are evaluating whether PPARgamma may be a molecular target for novel therapies in glioblastoma. We have already reported that PPARgamma is expressed in glioblastoma tumors and cell lines (A172, U87-MG,

M059K, M059J) and that the activation of the PPARgamma pathway by its ligands - namely, 15-deoxy-delta12,14-prostaglandin J2 (PGJ2) and rosiglitazone - induces cell growth inhibition in these cell lines, with PGJ_2 being more effective. In this report, we further characterized the mechanisms of action of these two PPARgamma ligands by looking at induction of apoptosis, cell cycle arrest, and gene modulation. Treatment of glioblastoma cells with PGJ_2 and rosiglitazone resulted in a dose-dependent induction of apoptosis, which correlated with the sensitivity of the cells to each ligand. In the most responsive of our glioblastoma cell lines, namely M059K, we observed a reversible (with rosiglitazone) and irreversible (with PGJ_2) arrest in the G_2/M phase, accompanied by p21 up-regulation only with PGJ_2. After treatment with PPARgamma ligands, a consistent amount of M059K cells detach in a short time. Far more than the attached cells, the floating population is growth-arrested and undergoing apoptosis, as shown by cell cycle analysis and apoptosis detection through PARP cleavage and annexin-V positivity. Interestingly, in the floating population of the treated samples, we observed a dramatic down-regulation of PPARgamma and a concomitant up-regulation of RXRalpha and RARbeta, while in the attached population no substantial modulation of these genes was observed. PPARgamma down-regulation may be due to proteasome-dependent degradation following activation of PPARgamma by its ligands. Our results indicate that PPARgamma ligands exert an antiproliferative effect in glioblastoma cells through induction of apoptosis and cell cycle arrest in G_2/M phase. In addition, PPARgamma ligands might induce glioblastoma cells to differentiate, since RARbeta up-regulation is a marker of this phenomenon.This work was supported by Fondazione per l'Oncologia Pediatrica, Roma

371

Establishment of a chemotherapeutic drug/gene expression database for the molecular pharmacology of cancer

S. Johnson, S. Biade, M. Marinucci, J. Schick, E. Lynch, B. Campling, J. Stevenson, P. O'Dwyer. *University of Pennsylvania, Pharmacology, Philadelphia, USA*

The potential of microarray technology for the identification of new drug targets is at an exploratory phase. Studies using the 60-cell line screen of the National Cancer Institute have revealed its usefulness in predicting mechanism of drug action. We have studied a panel of 42 cell lines of colon, lung and ovarian origin. The sensitivity of these cell lines to a variety of chemotherapeutic agents (N>30) has been measured using the MTT cytotoxicity assay and IC_{50} values determined. Gene expression analysis in the same cell lines was done using a 7000-element cDNA microarray platform. Relationships were established between genes and drugs across the entire panel of cell lines using a variety of statistical algorithms. Cluster analysis grouped the cell lines with respect to their tissue of origin and arranged the drugs with respect to their individual classes (e.g. antimicrotubule agents, topoisomerase inhibitors). Relationships between classes of chemotherapeutic drugs and gene expression patterns were established so groups of drug sensitivity or resistance genes could be identified. The expression of several potential drug targets was validated by quantitative "real time" RT-PCR. This approach has the potential to allow *in vitro* data from cancer cell lines to guide selection of candidate drugs within a class for clinical development and to identify new therapeutic targets.

372

Growth suppressive effect and apoptosis induction by N-(4-hydroxy phenyl) retinamide in human osteosarcoma cells *in vitro*

A. Gasperi-Campani[1,2], D. Baiocchi[1], I. Brognara[1], S. Cocchi[1], L. Roncuzzi[1,3]. *1) University Of Bologna, Department Of Experimental Pathology, Bologna, Italy; [2]University Of Bologna, Interdepartmental Center For The Research On Cancer, Bologna, Italy; [3]University Of Bologna, Biotechnology Unit Of The Ist-Ge In The Department, Bologna, Italy*

Osteosarcomas are the most common primary bone tumors and occurr mainly in children and young people between 10 and 20 years of age. Despite intensive treatment, including adjuvant chemotherapy, wide excision of tumors and amputation of the affected limbs, approximately half patients die within 5 years. The optimal schedule of therapy is still being investigated, as is the acquisition of novel active agents. Retinoids, the naturally occurring and synthetic analogues of vitamin A, have demonstrated therapeutic and chemopreventive activities. Among these, N-(4-hydroxyphenyl)-retinamide (4-HPR) (fenretinide) is a synthetic derivative and is being evaluated clinically in the prevention of the development of second primary tumors of breast and aerodigestive tract. The primary cellular target of this drug is unidentified as is the mechanism through which it kills tumor cells, by necrosis or apoptosis, and in this case by p53 or caspases dependent or independent pathways. In this study we analysed the growth suppressive effect of 4-HPR on human HOS (p53-positive) and MG-63 (p53-negative) osteosarcoma cell lines *in vitro* and the molecular mechanism of this response. Results show for the first time that 4-HPR (0.125 - 25μM) is active against human osteosarcoma *in vitro*, causing in it a significant dose- and time- dependent inhibition of cell survival, as determined by SRB and Trypan Blue exclusion tests, in each cell line. IC 50% was about 10 μM after 24hr of treatment in the HOS cells, which lowered to 6 μM after 72 hr of treatment, whereas the MG-63 cells were less sensitive to the drug, the IC 50% being about 10 μM only after 48 hr of treatment with 4-HPR. Programmed cell death by the drug was definitively documented here by the internucleosomal DNA fragmentation shown by the evidence of ladder after 48 hr of treatment in each cell line, independent of p53, the role of which as an apoptotic marker is not relevant here. By Western blot it was evident the dephosphorylation of pRb in each cell line treated with 4-HPR for 48 hr and up. This is the first report indicating that pRb may represent the cellular target for the molecular pathway carried out by fenretinide for the suppression of cell proliferation in osteosarcoma. Work supported by grants from Carisbo Foundation, Bologna, MIUR, CNR and University of Bologna (Funds for Selected Topics).

373

Prognostic value of nuclear survivin expression in oesophageal squamous cell carcinoma

P. Grabowski[1], T. Kuehnel[1], F. Muehr-Wilkenshoff[1], B. Heine[2], H. Stein[2], M. Hoepfner[1], C. Germer[3], M. Zeitz[1], H. Scheruebl[1]. *[1]Benjamin Franklin University Hospital, Medical Clinic I, Gastroenterology; [2]Benjamin Franklin University Hospital, Institute of Pathology, Berlin, Germany; [3]Benjamin Franklin University Hospital, Department of Surgery, Berlin, Germany*

Purpose: Survivin, a new member of the family of apoptosis inhibitors, is expressed almost exclusively in proliferating cells, above all in cancers. Subcellular localization and prognostic implications of the survivin protein have not yet been determined in oesophageal squamous cell carcinoma.
Patients and methods: Survival of 84 patients with oesophageal squamous cell carcinomas was correlated with the extent of immunohistochemical survivin expression in tumour cell nuclei. Tumours were scored positive when >5% cells stained positive. Patients were followed up for at least 5 years or until death.
Results: In normal oesophageal squamous cell epithelium some cytoplasmic survivin expression was detected in the basal cells, whereas proliferating cells showed nuclear staining of survivin. Nuclear expression of survivin was also detected in 67 cancers (80%). The mean survival for patients of this group (28 months, range 20-36) was significantly less than that for patients without survivin expression in the tumour cell nuclei (108 months, range 62-154, p=0.003). Using univariate analysis nuclear survivin expression (p=0.003), tumour depth (p=0.001), lymph node metastasis (p=0.003) and stage (p< 0.001) were the best predictors of survival. In contrast, cytoplasmic survivin staining was noted in 53 (63%) tumours and had no prognostic relevance.
Conclusion: The analysis of nuclear survivin expression identifies subgroups in oesophageal squamous cell cancer with favorable (survivin-) or with poor prognosis (survivin+). We suggest that the determination of nuclear survivin expression could be used to individualize therapeutic strategies in oesophageal squamous cell cancer in the future.

374

Carbohydrate mimics bind to tumour cell surfaces and inhibit cell adhesion

E. Frei[1], C. Gronewold[1], E.Y.-L. Kim[1], A. Chatterjee[2], C. Kliem[1], M. Wiessler[1]. *[1]Deutsches Krebsforschungszentrum, Molekulare Toxikologie, Heidelberg, Germany; [2]Chittaranjan National Cancer Institute, Biochemistry, Kolkata, India*

Lectin-carbohydrate interactions mediate cell recognition and are involved in cell adhesion. The binding avidity of branched, i.e. multiantennary oligosaccharides to lectins has been shown to be greater than of linear saccharides. We synthesised di- and triantennary galactosides and fucosides with a carbohydrate mimic as core; either bishydroxymethyl-furan (**1**) or trishydroxymethyl-cyclohexane (TMH, **2**).
To visualise cell surface carbohydrate-binding proteins, the diantennary carbohydrates were coupled via a Diels-Alder reaction (**1**) or directly (**2**) to