627 POSTER

The role of hypoxia-induced lysyl oxidase in cancer progression, tumor response to therapy and patient prognosis

J.T. Erler, A.J. Giaccia. *Stanford University School of Medicine, Radiation Oncology, Stanford, CA, USA*

All solid tumors have areas of hypoxia resulting from deregulated cell growth. Tumor hypoxia is associated with poor prognosis, tumor progression and resistance to therapy. The underlying molecular processes contributing to these events are poorly understood. Micro-array data generated in our lab has shown lysyl oxidase (LO) to be strongly induced under hypoxic conditions. LO plays a critical role in the formation and repair of the ECM, and has recently been shown to also function within the cell where it appears to be able to bind chromatin. Increased LO expression is associated with metastasis in breast cancer but little is known about its functions.

Human cancer cells of varying origin were incubated for 18 hours under normoxic (20%O_2), hypoxic (2% O_2) or anoxic (<0.1% O_2) conditions. LO expression levels were examined by RT-PCR and Northern blotting. For drug studies, cells were incubated for 16 hours under normoxic or oxygen deprived conditions then treated with chemotherapeutic agents for 2 hours with continued incubation. Short-term (apoptotic) response was assessed three days post-drug treatment. Long-term (clonogenic) response was assessed after 10 days. To examine the role of hypoxia-induced LO in metastasis, LO activity was inhibited prior to and during oxygen deprivation by chemical or genetic means, and cells subjected to *in vitro* invasion assays.

LO induction in response to oxygen deprivation was confirmed in SiHa cervical cancer cells and in MDA-235 breast cancer cells. Oxygen deprived MDA-235 cells showed significantly increased resistance to etoposide, tamoxifen and cisplatin-induced apoptosis compared with their normoxic counterparts. Inhibition of LO activity using the specific inhibitor beta-proprionitrile (BAPN) increased the sensitivity of oxygen deprived MDA-235 cells such that the levels of apoptosis were equal to or greater than the levels observed in the aerobic cells. Similar results were obtained using human RKO colon cancer cells and H1299 lung cancer cells. Furthermore, addition of BAPN dramatically reduced clonogenic survival of oxygen-deprived MDA-235 cells in response to drug treatment. Oxygen deprivation of MDA-235 cells resulted in significantly increased migration as assessed by *in vitro* invasion assays. This effect was blocked when LO function was impaired either through inhibition of LO activity by treatment with BAPN, or through decreased expression levels via transfection with specific antisense oligonucleotides.

The data suggest a role for LO in hypoxia-induced chemoresistance and metastasis. LO may be a valid target for drug design to increase treatment effectiveness and prevent disease spread.

628 POSTER

Targeting Bcr/Abl and Src family kinases reverses Imatinib mesylate resistance in CML

J.Y. Wu[1], M. Talpaz[1], J. Stapley[1], S. Lee[2], N. Donato[1]. [1]*M.D. Anderson Cancer Center, Bioimmunotherapy, Houston, Texas USA;* [2]*Bristol Myers Squibb Co, Oncology Drug Discovery, Princeton, NJ, USA*

Chronic myeloid leukemia (CML) is characterized by reciprocal chromosome translocation t(9:22) resulting in expression and constitutive activation of the Bcr/Abl oncoprotein and downstream signaling. Imatinib mesylate is a Bcr-Abl kinase inhibitor that successfully controls CML and other Bcr-Abl expressing leukemias. However both early and advanced phase CML patients express primary or acquired resistance to Imatinb-based therapy and elucidation of resistance mechanisms in these patients is clinically important. Based on multiple studies it is clear that Imatinib resistance is multi-factorial. To investigate possible drug resistance mechanisms, Bcr/Abl expressing blast crisis derived K562 CML cells were selected for resistance to Imatinib (K562R). K562R cells overexpressed the src-related kinase, Lyn, and SHP-1 tyrosine phosphatase, both of which play a role in hematopoesis. To determine the role of Lyn kinase in Imatinib resistant disease, Cos cells co-expressing Bcr/Abl and Lyn were examined for Imatinib sensitivity. Lyn kinase expression blocked Imatinib activity in both K562R and Cos transfectants. Treatment of Bcr/Abl/Lyn co-transfectants or K562R cells with a novel kinase inhibitor that inhibits both Abl and src kinases (BMS-354825) overcame resistance to Imatinib in both models. Based on this evaluation a role for src-related kinases in Imatinib resistant CML has emerged and may by operant in cells overexpressing both wild-type Bcr/Abl and src kinases. A clinical trial phase I of BMS-354825 is currently underway in Imatinib resistance CML patients.

629 POSTER

Acquired irofulven-resistance is mediated by a novel and drug-specific resistance mechanism associated with overexpression of ABCA12

F. Koeppel[1], J.-P. Annereau[2], A. Escargueil[1], V. Poindessous[1], G. Szakacs[2], E. Raymond[1], E.S. Van Laar[3], S.J. Waters[3], M. Gottesman[2], A.K. Larsen[1]. [1]*Institut Gustave-Roussy, CNRS UMR 8113, Villejuif, France;* [2]*Lab Cell Biology, NCI, NIH, Bethesda, USA;* [3]*MGI Pharma Inc., Bloomington MN, USA*

Irofulven (MGI-114) is a novel alkylating agent in active clinical development. To determine the molecular mechanisms associated with acquired irofulven resistance, we have selected irofulven-resistant cells by continuous exposure of HT-29 colon carcinoma cells to irofulven. The IF2 subline is about 90-fold resistant to irofulven and shows no notable differences in morphology or growth kinetics compared to the parental cells. Unexpectedly, IF2 cells showed basically unchanged sensitivity to 30 different anticancer agents including 10 alkylating agents as well as to UV and ionizing radiation, suggesting that acquired resistance to irofulven is mediated by a novel, drug-specific resistance mechanism. To characterize the transcriptional changes associated with acquired irofulven resistance, the transcriptional profiles of parental and IF2 cells were determined using a customized 18K human Oligo/cDNA hybrid chip. The expression profiles showed no marked alterations for proteins involved in glutathione metabolism or DNA repair. In contrast, significant changes were observed with genes coding for proteins involved in chromatin organization and in sterol metabolism. Drug accumulation studies with radiolabeled irofulven showed that resistance of IF2 is accompanied by decreased drug accumulation resulting in up to 12-fold reduced intracellular irofulven concentrations. Systematic screening of all known ABC proteins by quantitative RT-PCR analysis revealed equal expression of 48 ABC transporters and elevated expression of ABCA12 in IF2 cells. Further, analysis of sublines with intermediate acquired irofulven resistance showed a clear correlation between drug resistance, cellular irofulven accumulation and expression of ABCA12 mRNA. ABCA12 function has been implicated in lipid trafficking in keratinocytes, and mutations of the gene are associated with the rare skin disease lamellar ichthyosis (false leprosy), congenital cataract and insulin-dependent diabetes mellitus. To our knowledge, this is the first report to suggest a link between ABCA12 expression and drug resistance. In conclusion, we show that acquired irofulven resistance is associated with a unique resistance mechanism and unchanged sensitivity to other anticancer agents. Our results are consistent with a role for ABCA12 as an irofulven transporter and indicate that ABCA12 expression in human tumors might influence their natural sensitivity to irofulven.

630 POSTER

Molecular mechanisms of resistance against the ruthenium compound KP1019

P. Heffeter[1], M.A. Jakupec[2], M. Pongratz[2], P. Chiba[3], M. Micksche[1], W. Körner[4], M. Hauses[5], B. Marian[1], B.K. Keppler[2], W. Berger[1]. [1]*Institute of Cancer Research, Applied & Experimental Oncology, Vienna, Austria;* [2]*Institute of Inorganic Chemistry, Vienna, Austria;* [3]*Institute of Medical Chemistry, Vienna, Austria;* [4]*Institute of Geological Sciences, Vienna, Austria;* [5]*Faustus Forschung-Translational Cancer Research AG, Vienna, Austria*

Background: KP1019 (FFC 14a) is a new anticancer ruthenium compound currently evaluated in a clinical phase I trial. Since multidrug resistance (MDR) is a major obstacle for successful chemotherapy, the aim of our study was to investigate the impact of the drug exporter P-glycoprotein on the cytotoxic activity of KP1019.

Material and Methods: Cytotoxic activity of drugs was tested against KB 3–1 and HL60 cells in comparison with their P-gp-overexpressing sublines KB C-1 and HL60/vinc by MTT-based survival assays. The modulating activity of KP1019 on P-gp transport function and enzymatic activity was assessed by Rhodamine123 accumulation and ATPase assays, respectively. Cellular KP1019 accumulation was measured by ICP-MS and Zeeman AAS. Expression of various ABC-transporter proteins was determined by Western blotting

Results: HL60-vinc and KBC-1, both chemoresistant sublines overexpressing P-gp, displayed a moderate but significant resistance (1.8-fold and 3-fold) against KP1019 as compared to the parental lines. The resistance level against KP1019 was, however, very low as compared to other P-gp substrate drugs like anthracyclins and etoposide. Apoptosis rate induced by KP1019 was reduced in P-gp positive cell models. Co-incubation of cells with KP1019 and the P-gp inhibitors verapamil, tamoxifen, and dipyridamole resulted in a significantly enhanced sensitivity against KP1019, while the MRP1 inhibitor probenecid was ineffective in that respect. Drug accumulation data demonstrated significantly reduced

KP1019 accumulation in P-gp positive cells which could be partly reversed by co-administration of P-gp modulators. KP1019 inhibited P-gp ATPase activity with an K_i of approximately 31 μM. Selection of KB-3−1 cells against increasing KP1019 concentrations for more than year led to only an around 2-fold resistance (KB-1019 cells), and unexpectedly no P-gp expression. Accordingly KB-1019 cells displayed no drug accumulation defect and a unique cross-resistance pattern, indicating an ABC-transporter-independent acquired resistance phenotype.
Conclusion: In summary P-gp has to be considered as significant but weak intrinsic resistance mechanism against KP1019. Acquisition of resistance against KP1019 during chemotherapy seems to be relatively unlikely and acquired resistance based on ABC-transporter overexpression has not to be expected.

631 POSTER

Tumor associated fibroblasts have a profound impact on drug sensitivity of gastric cancer cells

K. Mayer[1], L. Gaedtke[2], I. Funke[1], K. Jauch[1], B. Mayer[1]. [1]*University of Munich, Department of Surgery, Großhadern, Munich, Germany;* [2]*Universitiy of Munich, Department of Pharmacy, Munich, Germany*

Background: In most tumor types, including gastric cancer, one of the main obstacles in anti-cancer therapy is the development of drug resistance. Some of the molecular mechanisms involved in acquired drug resistance, such as MDR, are well-characterized. In contrast, cellular mechanisms, i.e. cell-to-cell interactions between the cancer cells and the surrounding stromal cells, are poorly understood. Therefore, the purpose of the present study was to investigate the impact of benign and tumor associated fibroblasts on drug sensitivity of gastric cancer cells. Material and Methods: Fibroblast cultures were originated from benign gastric mucosa and the corresponding primary gastric carcinomas obtained from eight gastric cancer patients. Characterization of the fibroblasts using a panel of cell type specific antibodies confirmed the connective tissue origin. Two gastric cancer cell lines, namely MKN-28 representing the intestinal type and Hs746T indicating the diffuse type of gastric cancer, were used for co-culture experiments using the multicellular spheroid model. Homotypic spheroids consisting of either cancer cells or fibroblasts and heterotypic spheroids consisting of both cell types were established and treated with a variety of clinically relevant drugs. Treatment effects were measured using apoptotic (TUNEL, nucleosome ELISA) and metabolic (MTS) assays. Changes in the protein profiling were identified using 2D-gel electrophoresis followed by MALDI-TOF analysis. Results: Homotypic and heterotypic multicellular spheroids imitated a number of features observed in gastric carcinomas, such as the original differentiation phenotype and a slow proliferation activity. In contrast to homotypic spheroids and heterotypic spheroids containing benign fibroblasts, heterotypic spheroids with tumor associated fibroblasts were less sensitive to most of the drugs tested. Two-dimensional gel electrophoresis revealed that the decreased drug sensitivity of the heterotypic spheroids was associated with changes in the protein expression profile detected in both the gastric cancer cells and the tumor related fibroblasts. Most often, quantitative changes of the proteins were found. In addition, de novo expression of distinct proteins also could be identified. Conclusion: Tumor related fibroblasts, but not their benign counterparts, modulate drug sensitivity of gastric cancer cells. This is associated with profound changes in the protein profile.

632 POSTER

Down-regulation of mitochondrial F1F0-ATP synthase in human colon cancer cells with induced 5-fluorouracil resistance

Y.K. Shin[1,2], B.C. Yoo[1], E.K. Jeon[1], S.H. Hong[1,2], M.S. Jung[1,2], J.G. Park[1,2]. [1]*Research Institute, National Cancer Center, Korea, Goyang, Republic of Korea;* [2]*Cancer Research Institute and Cancer Research Center, Seoul National University, Seoul, Republic of Korea*

5-Fluorouracil (5-FU) is widely used for treatment of advanced colorectal cancer. Unfortunately it is common for such patients to ultimately develop resistance to 5-FU, creating a major problem for chemotherapy. The mechanisms underlying this resistance are largely unknown. To screen for proteins possibly responsible for 5-FU resistance, cells resistant to 5-FU were derived from human colon cancer cell lines, and two-dimensional gel electrophoresis (2-DE)-based comparative proteomics was performed. 2-DE data showed there was lower expression of the alpha subunit of mitochondrial F_1F_0-ATP synthase (ATP synthase) in 5-FU-resistant cells compared to parent cells. Western blotting showed expression of other ATP synthase complex subunits was also lower in 5-FU-resistant cell lines, and that these resistant cells also showed decreased ATP synthase activity and reduced intracellular ATP content. The ATP synthase inhibitor, oligomycin A, strongly antagonized 5-FU-induced suppression of cell proliferation. W hen 5-FU sensitivity was compared to ATP synthase activity in six different human colon cancer cell lines, the positive correlation has been found. Bioenergetic dysfunction of mitochondria has been reported as a hallmark of many types of cancers, i.e., down-regulation of ATP synthase β-subunit expression in liver, kidney, colon, squamous oesophageal and lung carcinomas, as well as in breast and gastric adenocarcinomas. Our findings demonstrate that ATP synthase down-regulation may not only be a bioenergetic signature of colorectal carcinomas, but may also lead to cellular events responsible for 5-FU resistance.

633 POSTER

Genetic variation in P-glycoprotein gene (ABCB1) and tipifarnib exposure

V. Ozdemir, Q. Li, R. Favis, S. Francke, B. Fijal, J.J. Perez Ruixo, P. Zannikos, P. Palmer, A. Thibault, N. Cohen. *Johnson & Johnson Pharmaceutical Research and Development, Pharmacogenomics and Global Clinical Pharmacokinetics and Clinical Pharmacology, Raritan, NJ, USA*

Background: Farnesyl transferase inhibition is a novel approach to cancer chemotherapy in both solid and hematologic tumors. Tipifarnib (Zarnestra™; R115777) is a potent farnesyl transferase inhibitor currently under clinical development as a monotherapy or as a combination therapy with other antitumor agents. P-glycoprotein (P-gp) is an efflux transporter that contributes to transport of drugs from intracellular to extracellular compartments. Hence, interindividual variations in P-gp function may influence drug bioavailability, predisposition to treatment resistance as well as drug-drug interactions among compounds subject to drug efflux mediated by P-gp. The aim of the present study was to evaluate the influence of functional genetic variations in the P-gp gene (*ABCB1*) in relation to clinical pharmacokinetics of tipifarnib.
Material and Methods: 24 healthy volunteers who participated in a food-effect study with a single 100 mg oral dose of tipifarnib were included in the present study. Pharmacokinetic data from the unfed state were utilized for all association analyses. Three synonymous but functional single nucleotide polymorphisms (SNP) in the coding region of the *ABCB1* (C1236T, G2677T, C3435T) were genotyped. Additionally, the key functional C3435T SNP in exon 26 of the *ABCB1* was characterized in a patient sample (N= 29) with advanced solid tumors administered multiple oral doses of tipifarnib (200 mg b.i.d, 4 days).
Results: A high degree of linkage disequilibrium (LD) was observed among the three *ABCB1* SNPs with p-values for all pair-wise LD <0.002. There was no deviation from the Hardy-Weinberg equilibrium in the sample (p values: 0.23–0.69). No significant association was found between haplotypes consisting of any combination of one to three of the *ABCB1* SNPs and tipifarnib C_{max} and AUC_{0-72h} (p values: 0.26–0.99). These observations were consistent with the analysis of the C3435T SNP in relation to tipifarnib C_{max} and AUC_{0-10h} in the patient sample (p values: 0.28–0.57).
Conclusions: Tipifarnib plasma exposure is not appreciably influenced by common genetic variants in *ABCB1*. These preliminary data suggest that P-gp is not involved in tipifarnib absorption in humans.

634 POSTER

E2F-1 induction and MEK inactivation coordinates with p53-generated signals to switch chemotherapy-induced growth arrest to apoptosis in human colorectal HCT116 cancer cells

Y. Zhao, L. Zhuang, Y. Li, Q. Yu. *Genome Institute of Singapore, Molecular Pharmacology, Singapore*

Cancer chemotherapeutic agents exert their cytotoxic effect by inducing DNA damage and activating apoptosis. The tumour suppressor protein p53 is an important modulator of apoptosis and whose mutation often affects the sensitivity of cancer cells to chemotherapy. In human colon cancer cell HCT116, anti-metabolite anticancer drug 5-FU triggers a p53-dependent apoptosis, whereas DNA damaging agent adriamycin results in growth arrest albeit both agents are strong p53 activators. To investigate the molecular mechanisms leading to the differential outcomes of DNA damage, we compared the gene expression profiles induced by 5-FU and ADR. We found that 5-FU and ADR induced a similar expression profiles in p53 responsive genes, indicating that differential cellular response to 5-FU and ADR is not due to differential activation of p53 target genes but depends on additional molecular events. Further analysis revealed the activation of E2F-1 pathway in response to 5-FU treatment, which was not observed in ADR-treated cells. Over-expression of E2F-1 in HCT116 cells resulted in apoptosis and partially abrogated the G2/M arrest induced by ADR, which mimics a 5-FU-like phenotype. In addition, signaling pathway analysis indicates that 5-FU treatment results in inactivation of MEK/ERK pathway but ADR did not. Inhibition of this pathway by MEK inhibitor U0126 resulted in a significant apoptosis, suggesting that MEK/ERK pathway is required for the survival of HCT116 cells. Thus, our data suggest that