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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.

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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.

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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.

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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