of information that can be obtained from a single assay, microarrays are an extremely useful tool for candidate biomarker discovery. In addition to gene expression profiling, microarrays can also provide a powerful platform for high-throughput custom sequencing analysis. We designed an Affymetrix CustomSeq® microarray that reads over 300,000 base pairs spanning the entire coding regions of 81 genes. The genes selected for this CustomSeq® microarray regulate important tumor progression processes such as cell-cycle, apoptosis (intrinsic & extrinsic), drug-resistance, pro-survival, proliferation, metastasis, and angiogenesis. Also included in the microarray design are many of the molecular targets for the promising new anti-apoptotic therapies. To validate this new drug development microarray, we PCR amplified EGFR and members of the PI3K/AKT pathway from whole blood and HT29 colorectal tumor cells. After the PCR products were pooled and quantified, the samples were chemically fragmented, hybridized, washed, stained and scanned for fluorescent hybridization signatures indicating the sequence of the amplified genes. We demonstrated the ability of this array to perform high-throughput mutation and SNP screening. Information from this new molecular tool integrates well with gene expression profiling data and will help to implicate important genes, molecular pathways and host-drug interactions that influence cancer growth, development, disease susceptibility, drug resistance and drug response.

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p53-dependent repression of CHEK1 contributes to apoptosis in colorectal cancer cells: an in vitro and in vivo study

R. Schneider-Stock[1], C. Mawrin[2], D. Kuester[1], M. Ocker[3], C. Habold[4], C. Foltzer-Jourdaine[4], A. Diestel[1], P. Schönfeld[5], A. Roessner[1], H. Gali-Muhtasib[6]. *[1]Otto-von-Guericke University, Department of Pathology, Magdeburg, Germany; [2]Otto-von-Guericke University, Department of Neuropathology, Magdeburg, Germany; [3]University Hospital, Department of Medicine I, Erlangen, Germany; [4]INSERM, Strasbourg, France; [5]Otto-von-Guericke University, Department of Biochemistry, Magdeburg, Germany; [6]American University, Department of Biology, Beirut, Lebanon*

Background: There are only a few reports on the role of p53-dependent gene repression in apoptotic cell death. Here, we used the plant drug thymoquinone (TQ), to identify potential targets of p53.
Material and Methods: Human colon cancer cells HCT116 p53 +/+ and p53 −/− were treated with 60 μM TQ, and apoptosis-associated genes were analyzed by a cDNA microarray. Drug-induced DNA-damage was characterized by H2A.X foci and H_2O_2 formation. Apoptosis induction was analyzed by flow cytometry, caspase 3- and DASH assay. mRNA and protein expression were determined by *real-time* RT-PCR and Western Blotting, respectively. Using chromatin immunoprecipitation (ChIP), we studied p53 binding at the CHEK1 promoter. HCT116 p53 −/− cells were transfected with a wt-p53 vector in order to restore the p53 function and CHEK1 binding. Furthermore, we investigated the p53, CHEK1 and apoptosis status in a panel of human colon cancer tissues with known p53 mutation status.
Results: Only 17% of genes were dysregulated and might contribute to a significant portion of the TQ response. Strikingly, CHEK1 mRNA and protein were significantly induced in TQ-treated HCT-116 p53 (−/−) cells. Using ChIP, we verified a transcriptional repression of p53 at the CHEK1 promoter. Apoptosis was induced in response to TQ treatment in HCT-116 p53 (+/+) cells but to a much lower extent in HCT-116 p53 (−/−) cells, which fits with the drug-induced, significantly higher DNA damage signal in p53 +/+ cells. Transfection of p53 (−/−) cells with a p53-wt vector decreased the CHEK1 mRNA and protein levels and restored the apoptosis to the level of the p53 (+/+) cells. P53 (−/−) cells transplanted to nude mice intraperitoneally treated with 20 μM TQ also highly up-regulated CHEK1 expression and did not undergo apoptosis in contrast to p53 (+/+) cells. Colon carcinomas with p53 deletions resulting in a truncated non-functional p53 protein had significantly higher CHEK1 mRNA and protein expression levels which were accompanied by poor apoptosis compared to p53 wt-expressing tumors.
Conclusions: CHEK1 repression by p53 in HCT-116 p53 (+/+) cells could be responsible for drug-induced apoptosis, supporting recent findings that transcriptional repression by p53 rather than activation and selective blockade of p53-dependent gene repression accounts for DNA damage-induced apoptosis. In cancer therapy of colorectal cancer, the inactivation of CHEK1 might contribute to the anti-tumor activity of specific DNA-damage-inducing drugs.

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Inhibition of PDGFR-beta increases sarcoma cell sensitivity to tumor necrosis factor related apoptosis inducing ligand, TRAIL and promotes inhibition of tumor growth in dual therapy using imantanib (Gleevac) and TRAIL

A. Hayes-Jordan, Y. Wang, E. Kleinerman. *M.D. Anderson Cancer Center – Children's Cancer, Pediatrics, Houston, USA*

Background: The activation of the intrinsic and extrinsic apoptotic pathways have been found to sensitize cells to TRAIL. However, the interaction between antiangiogenic agents and TRAIL induced apoptosis has not been well studied. We hypothesize that inhibition of PDGFR-beta will sensitize sarcoma cells to TRAIL and therefore promote synergistic cytotoxicity, and inhibition of tumor growth using antiangiogenic and apoptotic therapy.
Methods: si RNA technology was used to 'knock down' PDGFR-beta in human Ewing's sarcoma cells, TC-71. More than 90% inhibition was achieved in the TC-71si cell line. The expression of death receptors-4 and 5 in the TC-71 versus TC-71si cell lines was then compared. TRAIL induced cytotoxicity in the 'knockdown' versus the wild type cell line was evaluated. The TC-71w cells were then injected into an orthotopic xenograft model of Ewing's sarcoma. An Imantanib (Gleevac) and TRAIL combination was then used to treat mice with either locally advanced chest wall Ewing's sarcoma, or spontaneous pulmonary metastasis secondary to Ewing's sarcoma. Combination treatment was compared to single therapy.
Results: The TC-71si cell line showed increased expression of DR-5 receptors (78% vs 48%) and DR-4 receptor expression (58% vs 35%) compared to the TC-71 cell line. Also, dose dependent TRAIL cytotoxicity was significantly more profound in the TC-71si cells (0% viability) compared to the TC-71 (35% viability) cells at 1500 ng/ml of TRAIL. In our locally advanced chest wall Ewing's model, maximal growth inhibition was seen using the Gleevac and IP TRAIL combination. Ewing's sarcoma chest wall tumors treated with Gleevac plus IP trail grew to an average of 100 mm^3 compared to an average of 1300 mm^3 in the control group without treatment. However, there was no significant difference in the inhibition of tumor growth seen in the the Gleevac and IP versus Gleevac and IN TRAIL. When using the Gleevac and IN TRAIL combination in mice with pulmonary metastasis, only 1 of 6 (16%) mice developed gross pulmonary metastasis versus 4 of 5 (80%) in the control group compared to Gleevac alone (3/6, 50%) versus IN TRAIL (2/6, 33%) or IP TRAIL alone 3/6 (50%).
Conclusion: A combination of TRAIL and Gleevac causes significant inhibition of pulmonary metastasis and primary tumor growth in Ewing's sarcoma. This is the first known report of synergy between apoptosis and antiangiogeneic therapy in a preclinical model.

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Involvement of the multi-ligand cell surface receptor RAGE in Tumor Necrosis Factor-induced cell death

S. Salliau, A. Cottyn, K. Vancompernolle. *University of Ghent and VIB, Medical Protein Research, Ghent, Belgium*

Tumor Necrosis Factor (TNF) is a potent anti-tumor agent and therefore an ideal tool to identify the signaling pathways that are required to kill a cancer cell efficiently. Our work is focused on a caspase-independent cell death pathway that is characterized by a necrosis phenotype and that requires the increased production of reactive oxygen species (ROS) in the mitochondria.
We have recently shown that the TNF induces increased concentrations of methylglyoxal, a cytotoxic metabolite derived from glycolysis that been considered for a long time as a natural anti-cancer agent. This, together with the TNF-induced phosphorylation of glyoxalase I, leads to the formation of specific methylglyoxal-derived Advanced Glycation End products (AGEs). The effect of AGEs are mediated via cell surface receptors of which the Receptor for AGEs (RAGE) is the best known. RAGE is a multi-ligand receptor involved in tumor growth, invasion and metastasis. Here we report that TNF-induced cell death is mediated via RAGE. Induced overexpression of the ligand-binding domain of RAGE [soluble (s)RAGE] strongly inhibits TNF-induced cell death, as did overexpression of WT RAGE but to a lesser extent than sRAGE. However, overexpression of a mutant of RAGE that lacks the intracellular domain has no significant effect on TNF-induced cell death. We found that TNF induces rapidly nucleocytoplasmic translocations of endogeneous full-length RAGE, which is followed by a considerable reduction in the amount of FL-RAGE and its higher molecular weight complexes. These TNF-induced nucleocytoplasmic translocations of endogeneous FL-RAGE are disrupted by overexpression of sRAGE. This implies that sRAGE may sequester a ligand that is required for TNF-induced nucleocytoplasmic translocations of RAGE and cell death. We further demonstrate that inhibition of a secretory pathway by Brefeldin A completely inhibits TNF-induced cell death as well as the nucleocytoplasmic translocation of FL-RAGE and the subsequent