Drug resistance and modifiers

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Novel bifunctional alkylating agents overcome multidrug resistant cancer cells

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Several bifunctional alkylating derivatives of 3a-aza-cyclopenta[*a*]inden (BO-1012, BO-1005, BO-1099, and BO-1101) were synthesized in our laboratory. In the present study, we explored their anticancer activity against tumor cells with multidrug resistance (MDR). Our results showed that 4 MDR cell lines, KBvin10, KBtax50, CEM/VBL, and MCF7/Adr, were more susceptible to these derivatives than their parental cell lines. By using xenograft model, we confirmed that BO-1012 significantly suppressed the growth of MDR KBvin10 cells in nude mice as compared to the parental KB cells. We have also investigated the mechanisms of action on the collateral sensitivity of BO-1012 to KBvin10 cells. We revealed that BO-1012 induced higher levels of autophagy in KBvin cells than KB cells. Furthermore, by using gH2AX as a marker of DNA double strand breaks, we demonstrated that the repair efficiency of BO-1012-induced DNA damage in KBvin10 cells was significantly lower than that in KB cells. Our present results showed that lower repair activity in KBvin10 cells was likely due to its defective in translocation of DNA-PK, a component of repair machinery of non-homologous end-joining, from cytosol into nucleus. By aid of DNA-PK inhibitor, we confirmed the roles of DNA-PK on repair of DNA damage induced by BO-1012 in KB cells. Taken together, we have revealed that the collateral sensitivity of MDR cancer cells to DNA damage agents, such as BO-1012, may be due to their impaired DNA-PK repair pathway. These results suggest that bifunctional alkylating derivatives of 3a-aza-cyclopenta[*a*]inden may serve as a promised anticancer agent against human cancer cells with MDR.

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Silencing of TCF7L2 sensitizes colorectal cancer cells to radiation therapy

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Background: The clinical response of locally advanced rectal cancers to preoperative chemoradiotherapy is very heterogeneous. To determine the molecular characteristics associated with this heterogeneity, we recently profiled a series of responsive and resistant rectal adenocarcinomas using gene expression microarrays, and identified a set of differentially expressed genes. One gene that was significantly overexpressed in the resistant tumors was TCF7L2, the main downstream effector of the Wnt signaling pathway. The aim of this study was to evaluate if RNAi-mediated silencing of TCF7L2 sensitizes tumor cells to radiation.
Material and Methods: We transfected three colorectal cancer cell lines (SW480, SW837 and HT-29) with two different shRNA-vectors targeting TCF7L2, and a non-silencing control, and subsequently established stable single cell clones. TCF7L2 protein levels after RNAi-mediated silencing were analyzed by Western blotting. For each vector, single cell clones were irradiated at 0, 1, 2, 4, 6 and 8 Gy, and survival fractions were calculated.
Results: The decrease in TCF7L2 protein levels ranged from ~60% to ~90%. RNAi-mediated silencing of TCF7L2 significantly reduced colony-formation after radiation: We observed dose reduction factors of ~1.55 and ~1.49 at 37% survival for SW480 and SW837, respectively. Interestingly, colony formation of HT-29 cells was only scarcely reduced.
Conclusions: TCF7L2 was overexpressed in resistant rectal carcinomas, and its RNAi-mediated silencing caused a significant radiosensitization in SW480 and SW837 cells, but not in HT-29 cells. Preliminary experimental evidence suggests that this diversity is based on differences in Wnt/beta-catenin signaling activity. This is now being investigated. Importantly, these data suggest TCF7L2 as a potential molecular target to sensitize a priori resistant tumor cells.

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Sulforaphane enhances effects of quercetin, sorafenib, and chemotherapy towards pancreatic cancer stem-like cells

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Background: Despite intense efforts to develop treatments against pancreatic cancer, agents that cure this highly resistant and metastasizing disease are not available. Considerable attention has focused on broccoli compound sulforaphane, which is suggested as combination therapy for targeting of pancreatic cancer stem cells. However, there are concerns that anti-oxidative agents such as sulforaphane may interfere with cytotoxic therapy − as suggested e.g. for vitamins.
Material and Methods: The effects of sulforaphane upon combination with various standard chemotherapeutics, the dietary agent quercetin and the multi kinase inhibitor sorafenib were evaluated using in vitro and in vivo models of pancreatic tumor cells with stem-like phenotype. CSC-marker expression, ALDH1 activity, self-renewal potential, Notch signaling, migratory activity, apoptosis induction, viability, proliferation, NF-kB-signaling, and angiogenesis were analyzed.
Results: While each therapeutic agent alone diminished the stem-like characteristics, elimination of highly aggressive stem-like cells was not complete. However, combination with sulforaphane led to an additive effect of each single agent. This was due to inhibition of self-renewal activity and sensitization to apoptosis by inhibition of Notch, NF-kB, caspases, clonogenicity, spheroid-forming, migratory activity and downregulation of anti-apoptotic and EMT-related proteins. *In vivo,* combination treatment was most effective and totally abolished growth of cancer stem-like xenografts. No pronounced side effects were observed in mice. Our data suggest that sulforaphane increases the effectiveness of various cytotoxic drugs, sorafenib and quercetin against cancer stem cells without inducing additional toxicity in mice.
Conclusions: Our data suggest the combination sulforaphane with conventional or novel cancer therapeutics is safe and a promising new concept for targeting of pancreatic cancer stem-like phenotype.

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Hypoxia-inducible factor 1a promotes gastric cancer chemoresistance via modulation of p53 and NF-κB

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Background: Reduced chemosensitivity of solid cancer cells represents a pivotal obstacle in clinical oncology. Hence, the molecular characterization of pathways regulating chemosensitivity is a central prerequisite to improve cancer therapy. The hypoxia-inducible factor HIF-1α has been linked to chemosensitivity while the underlying molecular mechanisms remain largely elusive. Therefore, we comprehensively analysed HIF-1α's role in determining chemosensitivity focussing on responsible molecular pathways.
Material and Methods: RNA interference was applied to inactivate HIF-1α or p53 in the human gastric cancer cell lines AGS and MKN28. The chemotherapeutic agents 5-fluorouracil and cisplatin were used and chemosensitivity was assessed by cell proliferation assays as well as determination of cell cycle distribution and apoptosis. Expression of p53 and p53 target proteins was analyzed by western blot. NF-κB activity was characterized by means of electrophoretic mobility shift assay.
Results: Inactivation of HIF-1α in gastric cancer cells resulted in robust elevation of chemosensitivity. Accordingly, HIF-1α-competent cells displayed a significant reduction of chemotherapy-induced senescence and apoptosis. Remarkably, this phenotype was completely absent in *p53* mutant cells while inactivation of p53 *per se* did not affect chemosensitivity. HIF-1α markedly suppressed chemotherapy-induced activation of p53 and p21 as well as the retinoblastoma protein, eventually resulting in cell cycle arrest. Reduced formation of reactive oxygen species in HIF-1α-competent cells was identified as the molecular mechanism of HIF-1α-mediated inhibition of p53. Furthermore, loss of HIF-1α abrogated, in a p53-dependent manner, chemotherapy-induced DNA-binding of NF-κB and expression of anti-apoptotic NF-κB target genes. Accordingly, reconstitution

of the NF-κB subunit p65 reversed the increased chemosensitivity of HIF-1α-deficient cells.
Conclusions: In summary, we identified HIF-1α as a potent regulator of p53 and NF-κBactivity under conditions of genotoxic stress. We conclude that *p53* mutations in human tumors hold the potential to confound the efficacy of HIF-1-inhibitors in cancer therapy.

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Anti-angiogenic therapy improves response rate in erlotinib resistant NSCLC xenografts

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Non small cell lung cancer (NSCLC) is one of the leading causes of cancer deaths in the Western world. Combination chemotherapy is the therapeutic option for advanced diseases but with limited efficacy. Targeted therapies entered the clinics and led to a minor improvement of survival. However, many patients do not benefit from cytotoxic agents or targeted therapies. Therefore, reliable markers to select treatments for patients most likely to respond are in urgent need.
In our study, 25 patient-derived NSCLC xenografts were established and characterized. They revealed a high degree of similarity with the original tumor concerning histology, immunohistochemistry as well as gene profiling. The responsiveness to four cytostatics drugs (etoposide, carboplatin, gemcitabine, paclitaxel) and two epidermal growth factor receptor inhibitors (erlotinib and cetuximab) was evaluated in these xenografts according to clinical criteria.
The RNA expression profile of the xenografts was analyzed with the GeneChip HGU133Plus2.0. The data were evaluated statistically with the help of GeneSpring GX 11.0.
Within a class comparison, more than 2500 probe sets were found to be differentially expressed between erlotinib responders (2 xenografts) and non-responders (23 xenografts).
Differentially expressed genes were vascular endothelial growth factor a (VEGFA) and neuropilin and tolloid-like 2 (NETO2). VEGFA was higher expressed in the erlotinib resistant tumors. It may cause a better vascularization and thus result in a better survival of the tumor. The results were validated with TaqMan-PCR. With a combination therapy of erlotinib and bevacizumab the response rates could substantially be improved in the erlotinib resistant tumors.
In conclusion, a differential gene expression pattern was found in our patient derived xenografts allowing the identification of rational combination therapies. The patient-derived xenograft system offers a valuable tool to investigate targeted therapies and biomarker regulations in a clinically related way.

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Targeting glioblastoma stem cells: overcoming temozolomide resistance by ALDH1 inhibition

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Glioblastoma (GBM), the most frequent brain tumor of adults is still associated with a poor prognosis. Despite various efforts to improve postoperative therapeutic regimens in recent times, relapse occurs regularly. The chemoresistance of malignant gliomas might be caused by a barely characterized tumor stem cell subpopulation residing in a specific tumor microenvironment. Besides others, hypoxia may play a critical role in inducing a resistant tumor cell type. While normal brain tissue shows an oxygen partial pressure (pO_2) of 24−27 mmHg, gliomas feature an average pO_2 of only 13 mmHg.
So far, temozolomide (TMZ) is the gold standard of care for newly diagnosed glioblastoma. Recently we could show that aldehyde dehydrogenase 1 (ALDH1) positive glioblastoma cells show brain tumor stem cell capacity. In the current investigation we examined the impact of ALDH1 expression on GBM temozolomide resistance. In vitro cytotoxicity was evaluated by colorimetric MTT- and colony formation assays. Flow cytometry was used to analyze the amount of apoptotic cells. Neurosphere formation in neurobasal medium and differentiation experiments were applied to identify tumor stem cells. Furthermore, ALDH1 expression and temozolomide resistance was correlated with the MGMT status of various established and primary cell lines.
Sensitivity to temozolomide in resistant established and primary glioma cell lines was achieved by inhibition of ALDH1 with 4-diethylaminobenzaldehyde (DEAB). A specific knock down of ALDH1 by siRNA confirmed these findings. Under hypoxic conditions the cytotoxic effect of temozolomide was strongly attenuated but could be restored by ALDH1 inhibition. In the present study we show that ALDH1 is strikingly upregulated under hypoxic conditions, potentially leading to an increase of chemoresistance in gliomas. Hypoxia inducible factors (HIF1 alpha, HIF2 alpha) are involved in the regulation of ALDH1. Since post-therapeutic relapse is most probably due to a stem cell subfraction within the tumor bulk, special interest should be drawn to these cells. After combination therapy of temozolomide and DEAB, glioma cells were no longer able to proliferate in stem cell promoting medium or to form neurospheres; the remaining cells lost their undifferentiated stem cell-like phenotype.
In conclusion we suggest ALDH1 as an important prognostic factor in glioma care. Combination therapy of temozolomide with ALDH1 inhibitors might strongly improve clinical outcome of GBM patients.

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The selective reduction in the production of Bmi-1 protein leads to tumor growth control in multiple tumor models

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Background: Elevated expression of Bmi-1, a polycomb protein (also called PCGF4), is correlated with the chemo- and radio-resistance of a sub-fraction of tumor cells that also demonstrate stem cell characteristics. These stem-like cells are thought to be responsible for tumor recurrence leading to treatment failures in many cancer types. Bmi-1 has been shown to play a significant role in many neoplasias, particularly in glioblastoma where there is compelling evidence that Bmi-1 over-expression in glioblastoma multiforme (GBM) is a key event for tumor growth and intrinsic chemo-resistance.
Results: PTC has identified low molecular weight compounds that potently and selectively inhibit the production of Bmi-1 protein. A subset of these compounds act by targeting the post-transcriptional regulation of Bmi-1 synthesis, which reduces the translation rate of Bmi-1. This occurs both in cancer cells in culture and in various xenograft tumor models. The loss of Bmi-1 expression induced by these molecules leads to the reduction in global levels of mono-ubiquitinated histone 2A and causes either apoptosis or senescence in tumor cells. In murine xenograft tumor models, these compounds reduce intratumor Bmi-1 protein levels by up to 70% and tumor growth by up to 50% when administered orally as single agent therapy. The in vivo evaluation of activity is in progress where lead molecules are used in combination with standard-of-care cytotoxics.
Conclusions: Results from our studies support the hypothesis that targeting the production of the stem cell protein Bmi-1, known to be important for tumor cell survival and resistance, may enhance treatment success and improve patient outcomes.
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An antisense molecule to HER3 sustains growth inhibitory effects in gefitinib resistant cells that are independent of MET overexpression

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Although HER3 is not typically amplified or overexpressed in many tumor cells lines like EGFR or HER2 family members, HER3 is emerging as a critical family member since (1) it is a key link to the PI3K pathway for HER family members, (2) it can heterodimerize with HER1 and HER2, and (3) it can be activated via autocrine signaling by binding its cognate ligand, heregulin. These features help explain why increased activation of HER3 can mediate resistance to HER1 and HER2 inhibitors such as gefitinib, lapatanib, or Herceptin.
We have been attempting to understand the basis of unusual sensitivity of the lung carcinoma cell line, HCC827 to gefitinib (IC_{50} ~10 nM) and acquired resistance mechanisms after the cells were chronically exposed to increasing concentrations of gefitinib in vitro. The resistant cell lines were independently selected and are distinct from that reported to be driven by HER3 hyperactivation associated with MET amplification (Engelman et al., Science. 2007 316: 1039). The cell lines were highly resistant to gefinitib (IC_{50} >10 μM) but unlike past reports, the intracellular pEGFR levels were dramatically reduced while pHER3 and HER3 levels were unchanged when compared to HCC827 parental cells. Furthermore, no alteration in MET expression has been detected. Despite the lack of a HER3 activation signature, the resistant clones were equally or more sensitive to the treatment of an antisense molecule against HER3, designated EZN-3920. The parental HCC827, as well as the resistant cell lines were the most sensitive cells to EZN-3920 compared with 20 other cell lines. Furthermore,