with SK-RC45 clear cell RCC and ACHN papillary RCC. In addition, 6x augmentation of apoptosis as assessed by TUNEL assay resulted for the combination of IFN-α 2 with 5-AZA-dC for ACHN cells. Caspase 3 activity increased 2.5x in ACHN cells with either IFN-α 2 or IFN-β. To further affirm specificity of the effects as being mediated through DNMT-1, transfection of ACHN cells with 40 nM of the antisense (MG98) was evaluated. The antisense for 8d suppressed DNMT-1 protein to undetectable levels for up to 48 hr after withdrawal, as did 5-AZA-dC. Reactivated was RASSF1A, a tumor suppressor gene that is silenced by DNA methylation in ACHN cells. MG98 treatment caused little or no apoptosis (<5% TUNEL positive) but sensitized ACHN cells to apoptosis in response to 50 U/ml of IFN-α 2 or IFN–β over 5 days (25% and 80% TUNEL positive, respectively). Mismatch oligonucleotide (MG207) treatment did not sensitize ACHN cells to IFN-induced apoptosis (< 5% TUNEL positive). To identify responsible genes, RNA of MG98 treated ACHN cells was harvested 16 hr after IFN-α 2b or IFN-β (24 hr after the 8th transfection) for analysis on Affymetrix U133A human genome arrays. Compared to MG207, MG98 reduced DNMT-1 signal by 94%. IFN-stimulated genes associated with apoptosis (TRAIL, XIAP, Caspases 1, 7, 10, IRF1, OAS1 and PKR) were minimally increased by the addition of MG98 to IFN-β. Up-regulated ⩾4-fold in response to MG98 compared to MG207 treated cells were 45 genes scored as present ($p<0.05$), 36 of which were absent ($p>0.06$) in all controls (MG207, Lipofectin, no treatment). Of the 45 MG98 induced genes, 24 had CpG islands (length ⩾200 bp, GC ⩾50%, observed/expected CpG ⩾0.6) within 200 bp 5′ of their transcription start. To confirm the validity of the array results, 8 genes with potential apoptotic activity and CpG islands were assessed by RT-PCR after either the antisense to DNMT-1 or 5-AZA-dC. Although there was variability in absolute effects, all were augmented by both methylation inhibitors but not the mismatch antisense. Results suggest different mechanisms may account for the augmentation by methylation inhibitors of IFN-induced apoptosis in melanoma and renal carcinoma cells.

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Global identification of genes involved in 5-flurouracil resistance

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5-fluorouracil (5-FU) and tomudex (TDX) are thymidylate synthase (TS) inhibitors widely used in colorectal, breast, head and neck and aerodigestive cancer treatment. Apart from inhibition of TS, 5-FU also induces DNA and RNA strand breaks and apoptosis by direct incorporation of fluorinated nucleotides into DNA and RNA. TS overexpression is a common feature of 5-FU and TDX resistant cells and has been widely accepted as a major molecular abnormality responsible for 5-FU and TDX resistance. The influence of TS overexpression on 5-FU and TDX sensitivity was studied in 6 TS-overexpressing resistant cancer cell lines (5-FU resistant: 3; TDX resistant: 3). Compared to relevant parental sensitive cell lines, the 5-FU resistant cell lines were >20000-fold cross-resistant to TDX. In contrast, TDX resistant cell lines were only slightly resistant to 5-FU (0.6- to 1.3-fold). Thymidine (20 μM) rescue induced TDX resistance in sensitive cell lines (>10000-fold) but only very mildly affected 5-FU sensitivity (1.2- to 2.3-fold). These data indicate other molecular events rather than TS overexpression may play more important role in 5-FU resistance.

To identify genes involved in 5-FU resistance, 5 pairs of 5-FU resistant and parental cancer cell lines were analyzed on Affymetrix HG-U133A microarrays. Ninety one 5-FU sensitivity phenotype associated genes were identified and subdivided into several biological pathways. Key genes involved in 5-FU activation were significantly down regulated (TK, 2.9-fold; OPRT, 2.3-fold; UMPK, 3.2-fold; PNP 3.6-fold) in resistant cells. 5-FU induced resistant cell lines manifested reduced expression of genes governing G1-S and S phase transition. Expression of genes involved in DNA replication was also down-regulated in resistant cell lines. These findings were highly consistent with the longer doubling time and S phase time, slower growth rate, higher proportion of G1 and lower proportion of S phase cells in the resistant cell lines. This phenotype may protect resistant cells from cell death induced by incorporation of 5-FU into DNA chains and allowing time to repair 5-FU induced damage.

NFκB p65 mRNA and protein over-expression and high DNA binding activity were detected in resistant cells. NF-κB transfected MCF-7 and p53 knockout HCT116 cells were resistant to 5-FU (5.9- and 2.2-fold respectively) but not to TDX. The TS protein expression in NF-κB transfected and p53 knockout cell lines was comparable to the relevant parental cell lines. Thus, p53 mutations and NF-κB overexpression may be critical TS-independent molecular events mediating 5-FU resistance in cancer cells. Our findings may provide novel targets for tackling 5-FU resistance.

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Targeting Mcl-1 exhibits a strong single agent activity in hepatocellular carcinoma

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Background: Advanced hepatocellular carcinoma (HCC) is highly treatment resistant to various systemic therapeutic modalities. The Bcl-2 family members Bcl-2 and Bcl-xl have been previously implicated to contribute to treatment resistance of HCC. Recently, the antiapoptotic bcl-2 family member Mcl-1 was reported as an even more important treatment resistance factor in various type of cancers. However, there is no data about the significance of Mcl-1 in HCC. In the present study we evaluated the biological role of Mcl-1 as a molecular drug target in HCC by an antisense oligonucleotide (ASO) strategy.

Methods: ASO targeting Mcl-1 were evaluated as single agent and in combination with cisplatin or doxorubicin in the HCC cell lines HepG2 and SNU398. Protein regulation, cell viability and apoptosis were assessed by western blotting, cell count and FACS analysis, respectively.

Results: HCC cell lines display strong endogenous Mcl-1 protein expression. ASO targeting Mcl-1 specifically downregulated Mcl-1 protein expression by up to 80% and decreased cell viability by about 60% in a dose- and time-dependent manner. A moderate increase of apoptosis was observed. Notably, no significant target regulation or cell growth inhibition was observed for control oligonucleotide treatment. Combination of ASO with cisplatin or doxorubicin showed an additive, but not synergistic effect on cell viability and apoptosis.

Conclusions: Mcl-1 protein is expressed in HCC cell lines and appears to be an attractive molecular target. ASO targeting Mcl-1 revealed a powerful single agent activity against HCC *in vitro*. Given the hepatotropic pharmacokinetic properties and low toxicity of ASO *in vivo*, targeting Mcl-1 by ASO might become a promising novel approach in HCC therapy.

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Comparison of gene expression profiles in breast cancer cells treated with 5-fluorouracil, cisplatin or etoposide using cDNA microarray

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Background: Drug resistance in cancer is a major limitation for successful chemotherapy. Mechanisms of development of intrinsic drug resistance are not thoroughly understood and may involve the expression of multiple genes during tumor progression and also the emergence of acquired resistance may be associated with drug selection during chemotherapy. 5-fluorouracil(5-FU), cisplatin, and etoposide are commonly used in the treatment of breast cancer. To identify downstream mediators of tumor cell response to each chemotherapeutic agent, we used cDNA microarray technology to elucidate genes that are regulated by different chemotherapeutic agent treatment in the MCF-7 breast cancer cell line.

Methods: Breast cancer cells, MCF-7 were treated with IC50 concentration of 5-fluorouracil, cisplatin, and etoposide for 24 hour exposure, respectively. The use of cDNA microarrays containing 13,000 genes in our analysis provides a global view of the response of breast cancer cells to each chemotherapeutic agent at the genomic levels.

Results: Of 13,000 genes, 31, 108 and 6 genes were up-regulated (>2-fold) in breast cancer cells treated with 5-FU, cisplatin, or etoposide, respectively. Sixty-two, 137, and 11 genes were down-regulated (>2-fold) in breast cancer cells treated with 5-FU, cisplatin or etoposide, respectively. Thirty-eight and 8 genes were commonly up- or down-regulated by 5-FU and cisplatin. However, only 15 and 6 genes were commonly up- or down-regulated by 5-FU, cisplatin, and etoposide.

Conclusion: Our studies demonstrate that the downstream mediators of tumor cell response to chemotherapeutic agents may be variable according to the individual chemotherapeutic agents. However, several commonly regulated genes may be useful biomarkers of resistance. Our results suggest that cDNA microarray has a potential to identify genes involved in mediating the response of breast cancer cells to chemotherapy.