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Elevated expression of IL-8 and IL-8 receptors in prostate cancer cells correlates with disease progression and resistance to oxaliplatin

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Animal models demonstrate that interleukin-8 (IL-8) expression correlates with the angiogenesis and metastasis of prostate cancer, however, its expression in human disease is poorly characterized. Therefore, we studied the expression of IL-8 and IL-8 receptors (CXCR1 and CXCR2) in normal epithelium and neoplastic prostate tissue from patients with increasing stages of disease. Weak to moderate IL-8 expression was localized strictly to the apical membrane of normal prostate epithelium in all cases examined. In contrast, increased expression of IL-8, CXCR1 and CXCR2 was detected on the apical and basal membranes and also within the cytoplasm of cancer cells in all Basal membrane expression and cytoplasmic expression of IL-8 but not apical membrane expression correlated with markers of cell proliferation (Ki67 and cyclin D1 expression) and microvessel density (CD34 staining), confirming the relationship of IL-8 expression with disease progression in human prostate cancer. In vitro models using the androgen-independent metastatic prostate cancer cell line PC3 were established to study the significance of constitutive IL-8 signaling in prostate cancer cells. Stimulation of PC3 cells with IL-8 (i) potentiated the phosphorylation of Akt in immunoblotting experiments (ii) increased NF-κ B binding to DNA and (iii) increased the expression of the anti-apoptotic NF-κ B-regulated genes IAP and Bcl2. To test the hypothesis that IL-8 signaling may confer a survival advantage, PC3 cells were treated with full concentration-response curves to oxaliplatin in the absence and presence of (i) a small molecule inhibitor of CXCR2 and (ii) a monoclonal anti-human IL-8 antibody. Inhibition of IL-8 signaling by either strategy had a marked effect on oxaliplatin cytotoxicity increasing both the sensitivity of PC3 cells to this agent and the potency of the response in these cells. FACS analysis confirmed an increase in the percentage of apoptotic cells detected following combination of oxaliplatin with inhibitors of IL-8 signaling when compared to oxaliplatin treated or untreated PC3 cells. Therefore, attenuation of constitutive IL-8 signaling in prostate cancer cells increases their sensitivity to oxaliplatin and suggests that the elevated expression of IL-8 contributes to the high intrinsic resistance of prostate cancer to chemotherapy.

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Disruption of the serine kinase SKY1 gene in yeast results in hypersensitivity to 5-fluorouracil (5-FU) and low expression of the human SKY1 ortholog SRPK1, in colorectal cancers correlates with prolonged overall survival after 5-FU treatment

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The fluoropyrimidine 5-fluorouracil (5-FU) is one of the most commonly used chemotherapeutic drugs. However, the therapeutic potential of 5-FU is severely limited by the widespread occurrence of intrinsic and acquired resistance. Insight in the molecular mechanisms governing the 5-FU induced cytotoxicity may ultimately lead to ways to overcome unresponsiveness and give rise to better treatment results, especially in colorectal cancer. We initially used the budding yeast Saccharomyces cerevisiae as a model organism to identify and characterize novel genes/pathways involved in cell kill provoked by the anticancer agent, cisplatin, and found that disruption of SKY – a serine kinase that specifically phosphorylates serine/arginine (SR) rich protein domains – conferred resistance to cisplatin (Cancer Res. 61: 6982–6986, 2001; Mol. Pharmacol. 61: 659–666, 2002). In cross-resistance studies, we found that the Δsky1 knockout strain was also resistant to the cisplatin analog carboplatin (but not oxaliplatin) and the anthracyclines doxorubicin and daunorubicin. In contrast the Δsky1 cells were found to be hypersensitive to 5-FU. The 5-FU hypersensitivity phenotype was associated with an increased incorporation of 5-FU metabolites into DNA and RNA. In addition we observed that Δsky1 cells display an increased retention of 5-FU in their DNA suggesting an impaired DNA repair. Currently we are investigating the hypothesis that the Δsky1 phenotype (that is a mutator phenotype in combination with resistance to cisplatin and sensitivity to 5-FU and oxaliplatin) is associated with increased activity of an error-prone DNA polymerase with translesion synthesis activity. Because of the similarity of the Δsky1 phenotype with a MMR-deficient colorectal cancer phenotype, we performed a large-scale immuno-histochemistry study on colorectal cancer tissue arrays from patients treated with 5-FU as adjuvant chemotherapy. In patients with

low expression of SRPK1, the human ortholog of SKY1, 5-FU adjuvant chemotherapy led to a significant increase (about 20%) in overall survival. In contrast, patients with a high SRPK1 expression did not benefit from 5-FU treatment. These data suggest that low SRPK1 expression levels might be predictive for a survival benefit of colorectal cancer patients treated with 5-FU.

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Resistance to cytotoxic drugs is associated with changes in telomere length

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We have previously shown that testicular cancer cell lines and tissues resistant to cisplatin have elevated telomerase activity and we found that breast cancer cells expressing the dominant negative catalytic subunit of human telomerase reverse transcriptase (hTERT) were more sensitive to certain cytotoxic agents. This study was therefore aimed to investigate whether changes in telomerase activity and telomere length are a general phenomenon of development of drug resistance to standard agents in tumor cell lines and whether this would have implications for selecting treatment options.
We generated a total of 4 renal, lung and ovarian tumor lines sensitive and resistant to either cisplatin, gemcitabine, or vindesine and determined telomerase activity and telomere length upon occurrence of resistance. Parental cell lines A2780 (ovarian), RXF944L (renal), LXFL529L (lung) and LXFA526L (lung) were maintained for 15–32 passages under continuous exposure to cisplatin, vindesine, or gemcitabine at increasing concentrations and development of resistance was monitored by sulforhodamine B proliferation tests. Resistance factors (RF) obtained for the 3 agents ranged between 25–100.000. Telomerase activity was measured using the telomeric repeat amplification protocol (TRAP assay); mean telomere restriction fragment (TRF) length was analyzed by Southern blotting. We found that cell lines with longer telomeres such as RXF944L (9.1 kb) expressed up to 2-fold higher telomerase levels than those with short TRF length e.g. LXFA526L (3.5 kb). In the isogenic ovarian cancer lines A2780, ADDP (cisplatin resistant) and AG6000 (gemcitabine resistant), appearance of drug resistance led to induction of telomerase activity and marked increase in telomere length (from 4.2 to 9.0 and 6.1 kb respectively). In contrast, gemcitabine treatment of RXF944L renal and LXFL529L lung cancer cells resulted in marked telomere shortening (from 9.1 kb to 3.3 kb and 4.0 to 3.6 kb). Both, LXFL529L and LXFA526L lung cancer lines resistant to vindesine (RF = 25) had also reduced telomere length (from 4 kb to 2.7 and 3.5 to 3.3 kb).
Our findings indicate that telomere length maintenance and telomere dynamics are altered under treatment with cytotoxic drugs, the induction of telomere lengthening or shortening seems to be dependent on the molecular mechanism of a particular agent and tumor type. An arsenal of telomerase inhibitory and telomere interactive compounds are currently in advanced preclinical development, they have been found to be more effective in tumors with short telomeres. The observation that some gemcitabine and vindesine resistant cell lines have shorter telomeres indicates that gemcitabine and vindesine refractory tumors might be more sensitive to telomerase inhibition and this might aid the selection of patients that would qualify for treatment with anti-telomerases in phase I/II clinical trials.

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Gene expression enhancement by inhibitors of methylation: potential role in augmentation of apoptosis induced by interferons (IFNs) in melanoma and renal cell carcinoma (RCC)

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To determine whether methylation-mediated silencing of genes plays a role in resistance of melanoma and RCC to IFNs, the effects of 5-aza-deoxycytidine (5-Aza-dC) and an antisense phosphorothioate oligonucleotide selective for DNMT1 were assessed. In melanoma cells, 5-AZA-dC (1uM) overcame resistance to antiproliferative effects of IFN-β, reducing the ID50 from 1500 to 300 U/ml for A375 melanoma cells and from 900 to 100 U/ml for Minors melanoma, synergistic by combination index analysis. When assessed by RNase protection assay, expression of the interferon-stimulated genes (ISG54 and IRF-1) was augmented in melanoma cells. Similar potentiation of antiproliferative effects occurred