to AG1478 but still very sensitive to C225. Other remarkable differences among DiFi-P, DiFi5 and DiFi-AG cells are the basal and EGF-stimulated phosphorylation levels of MAPK and Akt. The MAPK is constitutively activated (phosphorylated) and is insensitive to EGF stimulation in DiFi5 cells. In contrast, the basal levels of phosphorylated MAPK are low, and can be stimulated by EGF in DiFi-P and DiFi-AG cells. The basal levels of Akt phosphorylation are low in DiFi-P and the two sublines, and can be stimulated by EGF in DiFi-P and DiFi-5 cells, but not in DiFi-AG cells. Expression profile analysis with the Affymetrix microarray chips (U133A) showed that DiFi-P is clustered in the same group with DiFi-AG, however, principal component analysis (PCA) result shows that DiFi-P is distinct from DiFi-5 and DiFi-AG cells in the component-2 direction. There are 299 genes differentially expressed between DiFi-P and the two DiFi-resistant variants. We are currently validating and screening these differentially expressed genes using the "training and test" approaches, which may contribute to the acquired resistance.

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Characterization of the binding sites of P-glycoprotein by a functional flow cytometric assay

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Background: The overexpression of the MDR1 product P-glycoprotein (P-gp) is often responsible for limiting the success of cancer chemotherapy. P-gp is known to bind to and transport a wide variety of agents. Different drug binding sites have been proposed. Daunomycin and Hoechst 33342 have been shown to bind to different sites, which interact in a positively cooperative manner. We developed a functional flow cytometric assay searching not only for new modulators but focusing on the characterization of their binding sites as a basis for molecular modeling analysis aiming to understand the mode of action of P-gp.
Material and methods: P-gp activity was measured using a flow cytometry assay based on daunomycin influx. Measurement was gated to include only single, viable cells. Concentration-dependent effects of the P-gp modulators verapamil, imatinib, Hoechst 33342 and quercetin on daunomycin influx were determined in the P-gp expressing cell line A2780adr. Controls were incubated without modulator.
Results: Incubation of A2780adr with Hoechst 33342 stimulated P-gp activity and led to a decrease in daunomycin influx, whereas verapamil and imatinib inhibited P-gp activity significantly. Quercetin showed a biphasic effect. Lower concentrations of Quercetin decreased, concentrations above 10^{-6}M increased daunomycin influx, respectively.
Conclusions: Modulators interacting with the Hoechst binding site stimulate the daunomycin binding site in a positively cooperative manner and decrease daunomycin influx, whereas modulators of the daunomycin binding site, like e.g. verapamil and imatinib, increase daunomycin influx. The preliminary results of our study show that the developed assay is well suited for the characterization of the P-gp binding sites. Our data correlate well with the binding sites proposed in literature. The benefit of our method is the in situ measurement of P-gp activity using intact cells instead of membrane vesicles with reconstituted protein.

Radiation interactive agents

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Stat1 as mediator of acquired tumor radioresistance and potential target for anti-tumor therapy

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Background. Mechanisms of acquired tumor radioresistance are an area of intense study. To approach understanding of these mechanisms we selected in vivo radioresistant tumors nu61 from the radiosensitive SCC-61. Expressional profiling revealed that nu61 tumors constitutively overexpressed sets of IFN-inducible genes and Stat1 compared with radiosensitive SCC61. We proposed that overexpression of the Stat1 may be critical for the radioresistance (Khodarev et al., PNAS, 2004, 101:1714). In the current report we investigated the effects of ionizing radiation on Stat1 expression in different cell lines and effects of Stat1 overexpression on clonogenic survival of transfected clones.
Materials and Methods. Selection of stably transfected clones and Western analysis are described in (Khodarev et al., PNAS, 2004, 101:1714). In the current report we used only β-1, α-16 and MT-4 clones. Clonogenic analysis was performed in the dose range between 0 and 10Gy. siRNA was synthesized with Silencer™ kit (Ambion, USA) and IFN measurements performed with R&D kits (R&D Systems, USA).
Results. Fractionated IR (3×5Gy) led to the up-regulation of Stat1 protein in 9 cell lines from breast, prostate, colon and head and neck cancer. Up-regulation varied from 1.2- to 5.1-fold at 24 hours after last dose. After single dose (5Gy) Stat1 up-regulation was detected at 30 min and reached a plateau at 8 hours. IR-induced up-regulation of Stat1 precedes the IR-induced production of IFNα. Clonogenic assays of β-1, α-16 and MT-4 clones revealed that β-1 and α-16 were significantly more radioresistent than mock-transfected clone MT-4 (6.9-fold and 9.7-fold respectively at 10Gy). Also, constitutive overexpression of Stat1 in β-1 clone led to the overexpression of IFN-inducible genes, previously detected in nu61 in vivo. Anti-Stat1 siRNA led to the 4.5-fold suppression of the cell growth of the radioresistent tumor cell line nu61.
Conclusions. 1. Ionizing radiation leads to the up-regulation of Stat1 common in tumor cell lines surveyed. 2. Stat1 mediates radioresistance and induction of IFN-inducible genes, which recapitulates radioresistant phenotype of nu61 tumor. Consistently suppression of Stat1 leads to the growth suppression of nu61. 3. IR-induced up-regulation of Stat1 is an early IFN-independent event. Data suggest that Stat1 is an important mediator of acquired tumor radioresistance and could be a potential target for pharmacological manipulations in the anti-cancer therapy.

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Inhibition of PDGF signaling attenuates radiation-induced pulmonary fibrosis

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Background: Pulmonary fibrosis is the consequence of a variety of diseases with often poor prognosis and no satisfactory treatment option. Fibrosis is also a common, delayed side effect of radiation therapy. Given new insights into cytokine signaling in the pathogenesis of fibrosis we sought to investigate the role of PDGF signaling in a radiation-induced lung fibrosis model.
Methods: The thoraces of C57BL/6 mice were irradiated, and the PDGF receptor (PDGFR) kinase inhibitor, SU9518, or vehicle were administered subcutaneously twice per week for 26 weeks. The progression of pulmonary fibrosis was monitored by high resolution CT and by histological examination. PDGFR phosphorylation status was demonstrated by IHC or IP/western blotting. A 2 chamber co-culture system was used to demonstrate radiation-induced endothelial cell PDGF expression.
Results: Administration of SU9518 potently inhibited the constitutive phosphorylation of PDGFR that was induced by total thoracic irradiation. Blockade of PDGF signaling markedly attenuated the development of pulmonary fibrosis and significantly increased survival of irradiated mice. We also demonstrated that radiation of endothelial cells stimulated sufficient PDGF expression to promote fibroblast proliferation, which was abrogated by SU9518.
Conclusions: Our data indicate that inhibition of fibrogenesis, rather than the anti-inflammatory response, is the key antifibrotic mechanism of the PDGFR kinase inhibitor. Our findings emphasize the pivotal role of PDGF signaling in the pathogenesis of pulmonary fibrosis. To our knowledge, this is the first report of an agent that can prolong survival in a pulmonary fibrosis model. The availability of new drugs which affect PDGF signaling makes these findings significant to current therapeutic approaches to pulmonary fibrosis and potentially to fibrosis in other organs and of various pathogenesis.

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Molecular targeting of epidermal growth factor receptor (EGFR) positive gliomas for neutron capture therapy using boronated bioconjugates

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Boron neutron capture therapy (BNCT) is based upon the nuclear capture and fission reactions that occur when non-radioactive ^{10}B is irradiated with low energy neutrons to produce high energy α particles (^{10}B[n,α]^{7}Li). In order for BNCT to be successful, a sufficient amount of ^{10}B (~20 μg/g tumor) and neutrons must be delivered to the tumor. The purpose of