15% of A431 cells vs. 2% of the MDA-468 cells were stained positive by AnnexinV, which is a marker for apoptosis. The induction of apoptosis appeared to correlate with loss of TK1 expression, as assessed by western blotting and film densitometry. Thus, we reasoned that caspase-mediated degradation of TK1 could be at least partially responsible for the drug-induced suppression of FLT uptake. To test this, A431 cells were treated simultaneously with the caspase inhibitor ZVAD-fmk and erlotinib. ZVAD-fmk treatment reduced the drug-induced cell death, and this was associated with a corresponding attenuation of erlotinib-induced suppression of FLT uptake (Table 1). The effects of ZVAD-fmk on TK1 expression and apoptosis induction will be reported.

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Signaling mediators of bystander response are potential therapeutic targets for attenuating tumor relapse

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Background: Our objective is to understand whether radiation used to treat local tumor after surgery can, trigger a positive feed back signaling mechanism. This may cause the cells to have a memory of the initial irradiation insult for an extended period of time that may result in local/regional tumor recurrence at later time. In this study we determined whether exposure to radiation could initiate NF-kB and TNF-a signaling and maintain NF-kB >TNF-a > NF-kB feed back cycle in human epithelial cells.
Materials and Methods: To examine whether cells at normal tissue at the radiation-exposed treatment site could undergo these alterations, normal human epithelial cells were exposed to radiation at doses used in fractionated radiotherapy and examined for (a) the dose and time dependent activation of NF-kB by electrophoretic mobility shift assay (EMSA), (b) the kinetics of TNF-a expression by RT-PCR (mRNA expression) and ELISA (secreted protein expression), and (c) the involvement of reactive oxygen species (ROS) in TNF-a mediated NF-kB activation by FACS analysis. Blocking experiments were performed using specific inhibitors.
Results: EMSA of nuclear extracts from cells exposed to clinical doses of radiation revealed a bi-phasic time-dependent expression of NF-kB, reaching a first maximum at 3h and a second maximum at 48 h. The functional integrity of the radiation-induced NF-kB, determined by transient transfection with pNF-kB-Luc that expresses the luciferase reporter gene in an NF-kB-dependent manner showed a 3.8-folds compared to mock irradiated control indicating that NF-kB DNA-binding activity triggered by radiation exposure could initiate transcriptional activation of NF-kB–dependent genes. Cells either incubated with TNF-a soluble receptor or TNF-a neutralizing antibody blocked the second phase (24 & 48 h) induction of NF-kB activation. Similarly, TNF-a mRNA expression was observed at 8h and protein expression at 16 and 24 h post-exposure. The TNF-a expression both at mRNA and protein level were inhibited to constitutive level by pre-incubating the cells with NF-kB inhibitor NF-kB SN50 cell permeable inhibitory peptide (100 μg/ml) 1 h prior to radiation exposure. These results clearly indicated the occurrence of a positive feedback cycle initiated by the activation of NF-kB upon radiation exposure. This activated NF-kB signaling mechanism triggers the TNF-a production, which in turn induces the activation of NF-kB through generation of ROS in primary endothelial cells.
Conclusions: Reappearance of a local or regional tumor after treatment is a major limitation in achieving disease-free survival. Identifying and intervening the mediators involved in this mechanism may help to achieve a prolonged disease free survival. This research was supported by the Office of Science (BER), U.S. Department of Energy, Grant No. DE-FG03–02ER63449.

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A phase I/II trial of erlotinib and bexarotene in aerodigestive tract cancers

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Background: We have previously reported the overexpression of the epidermal growth factor receptor (EGFR) and cyclin D1 as early events in lung carcinogenesis. Classical retinoids prevent the carcinogenic transformation of human bronchial epithelial (HBE) cells at least partly through repression of these proteins. Clinical activity of classical retinoids is limited by the frequent repression of the critical retinoic acid receptor, RAR-beta. Non-classical retinoids such as the rexinoid, bexarotene, exist that repress cyclin D1 and EGFR expression but can signal independent of RAR-beta. We found that combining an EGFR tyrosine kinase inhibitor, erlotinib, with bexarotene induced at least additive suppression of growth and cyclin D1 expression in HBE cells which had silenced RAR beta. Based on these and other pre-clinical findings we performed a phase **I/II** clinical trial of this combination in patients with advanced aerodigestive tract cancers.
Materials and Methods: Patients with advanced aerodigestive tract cancers who had failed prior chemotherapy were enrolled onto this dose-escalation study. Three dose levels were utilized and at least three patients were enrolled at each level. Primary objectives were to determine the maximum tolerated dose. Secondary objectives were to determine activity, toxicity, and surrogate markers of response in buccal epithelial cells.
Results: Twenty patients were enrolled and sixteen are evaluable. Toxicities have generally been mild with asymptomatic elevations of cholesterol and triglycerides occurring frequently. No cases of pancreatitis were observed. One case of dose-limiting rash (grade 3) and one case of dose-limiting diarrhea (grade 3) were observed. To date, three patients have had radiographic responses (2 PR, 1MR), one of which has lasted more than 1 year. Four additional patients remain on study with stable disease. Two have had stable disease for more than three months. Changes in surrogate markers of response in buccal epithelial cells are being determined.
Conclusions: The combination of erlotinib and bexarotene is well tolerated and appears active for the treatment of advanced aerodigestive tract cancer resistant to chemotherapy. A confirmatory trial comparing this regimen to erlotinib alone is warranted to determine the efficacy of this regimen.

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p38/JTV-1 is a novel modulator of TGF-beta required for the downregulation of c-myc and lung cell differentiation: its functional association with lung cancer formation

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p38/JTV-1 is known to be an essential scaffold protein for the formation of macromolecular tRNA synthetase complex (ref1). Interestingly. The mice lacking the gene encoding p38 were developed with severe hyperplasia of the alveolar epithelial cells in lung that caused immediate death after birth due to respiratory dysplasia (ref2). Molecular screening revealed that p38 interacts with FBP (FUSE-binding protein) that is a transcriptional activator of protooncogene, c-myc. The binding of p38 enhanced the ubiquitin-mediated degradation of FBP, resulting in downregulation of c-myc, which is required for the functional differentiation of alveolar type **II** cells. The ectopic expression of p38 suppressed proliferation and restored the differentiation markers in lung carcinoma cells. The cellular level of p38 was increased by TGF-beta to mediate cell growth arrest. In reverse, the loss of p38 led to resistance to TGF-induced cell cycle inhibition. The mice with reduced expression of p38 showed higher susceptibility to tumorigenesis, and the severe reduction of p38 level was frequently found in lung cancer cell lines and clinical cancer tissues. The working mechanism and association with tumor formation in animal model and human cancer patients strongly suggest p38/JTV-1 as a novel tumor suppressor.

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Inhibition of PI3K/AKT pathway by rhabdastrellic acid-A induced caspase-3-dependent apoptosis in leukemia cells

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Background: It has been demonstrated that PI3K/AKT signaling is aberrantly activated in AML cells. It is a promising strategy to target PI3K/AKT pathway for cancer treatment. Rhabdastrellic acid-A, an isomalabaricane triterpenoid, isolated from the sponge Rhabdastrella globostellata. The aim of this study is to explore effect of Rhabdastrellic acid-A on PI3K/AKT pathway and apoptosis.
Methods: Cytotoxicity was determined by MTT assay. Immunoblot analysis was employed to detect protein expression. DNA fragmentation was analyzed using agrose gel electrophoresis.
Results: Our investigation indicated that Rhabdastrellic acid-A inhibited proliferation of HL-60 cells with IC_{50} value of 0.64 μg/ml and induced apoptosis of HL-60 cells. Also, Rhabdastrellic acid-A induced cleavage of the death substrate poly (ADP-ribose) polymerase (PARP) and caspase-3. Pretreatment of HL-60 cells with caspase-3 specific inhibitor DEVD-CHO prevented Rhabdastrellic acid-A-induced DNA fragmentation, PARP cleavage. The expression levels of protein bcl-2, bax have no change in response to Rhabdastrellic acid-A treatment in HL-60 cells, whereas activated PI3K had significantly a decrease after treatment with