human integrin, inhibited the growth of established tumors by approximately 40%, confirming that inhibition of integrin alpha5beta1 can slow tumor growth by impeding angiogenesis in vivo. Importantly, targeting of both host vasculature and tumor integrin using 339.1 and volociximab together resulted in additive efficacy in multiple xenograft models, suggesting that in the clinic, volociximab may exert both anti-angiogenic and direct anti-tumor cell activity in vivo.

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Hsp90 inhibitor synergistically potentiates the growth inhibitory and pro-apoptotic effects of SN-38 in gastric carcinoma cells

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Background: Gastric cancer is the second most frequent cancer in the world. To date, we have few effective chemotherapeutic agents against it. A representative Hsp90 inhibitor, 17-(dimethylaminoethylamino)-17-demethoxygeldanamycin (17-DMAG) is a new anticancer agent for solid tumor currently in clinical trials. The aim of the current study was to determine the effects of combination treatment of 17-DMAG and CPT-11 on gastric cancer lines and investigate the mechanism responsible for this enhancement of CPT-11-induced cytotoxicity by 17-DMAG.
Methods: Human gastric cancer cells MKN-1, MKN-7 and MKN-45 were treated with 17-DMAG and SN-38, an active metabolite of CPT-11, alone and in combination, and their effect on growth and cell cycle distribution was evaluated using tetrazolium-based colorimetric assay (MTT) and flowcytometory, respectively. The possible synergism was analysed using median drug effect analysis resulting in combination indexes (CI), in which CI < 0.9 indicates synergism, CI = 0.9–1.1 indicates additivity and CI > 1.1 indicates antagonism when the two drugs were added in a 1:1 IC(50)-based molar ratio. Apoptosis was monitored by flow cytometrical analysis, DNA ladder fragmentation analysis and biochemical markers of apoptosis.
Results: We demonstrated that MKN45 (poorly differentiated adeno-carcinoma line) is sensitive to 17-DMAG, with an average IC (50) of 196.9+72.0 nmol/L although MKN1 (adeno-squamouscarcinoma line) and MKN7 (highly differentiated adenocarcinoma line) are less sensitive with an average IC (50) of 1309.7+275.2 and 2316.6+688.2 nmol/L respectively. Combination of 17-DMAG and SN-38 significantly induced cell death, and synergistically inhibited proliferative activity of all three cell lines. It resulted in enhanced accumulations of the sub-G1 phase population, occurrence of DNA fragmentation and a pronounced increase of active caspase-3, 8, 9 and poly (ADP) ribose polymerase cleavage.
Conclusion: These data suggest 17-DMAG could potentiate the cytotoxic effects of CPT-11 chemotherapy in patients with gastric cancer and underscore the need for rational design of human clinical trials.

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Doxorubicin cardiotoxicity and effectiveness in MCF-7 breast cancer cells could be mediated by polyprenol

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Background: Doxorubicin (Dox) is an important and effective anticancer drug widely used for the treatment of various types of cancer but its clinical use is limited by dose-dependent cardiotoxicity. The investigations reveals that Dox toxicity and multidrug resistance (MDR) correlates with concentration of P-glycoprotein (P-gp) in plasma membrane. Poliprenol (Pol) have been proved to be a rate limiting factor in membrane glycoprotein synthesis in Dolichyl Phosphate Cycle (DPC). The purpose of this study was to investigate the role of Pol in cardiotoxicity Dox and its effectivness in MDR MCF-7 breast cancer cells.
Methods: Pol concentration in the culture medium with neonatal rat ventricular myocytes (NRVM) made up 10^{-3}–10^{-8} M. Cell viability was evaluated. Breast cancer cell lines, MCF-7 and MCF-7 cells with induced resistance to Doxorubicin (MCF-7/ADR) were used. Pol concentration in the culture medium made up 10^{-2}–10^{-6} M. MDR1 expression was assessed by an immunohistochemical technique. Intermediates of DPC and Pgp fractions were analysed by HPLC methods.
Results: Pol in concentration 10^{-5}–10^{-6} M could increase the viability of NRVM that were treated with Dox. Pol in concentration 10^{-2}–10^{-3} M induced apoptosis in MCF-7/ADR cells within 3–4 hours. It is confirmed that plasmatic membranes of MCF-7 cells contain 5.6–6.4% of P-gp (the total protein amount) as a resistance marker. Resistant MCF-7/ADR cells differ from sensitive ones MCF-7 in Pgp content by 10–12 times. The study showed 8.5-fold DPC intermediates decrease in MCF-7/ADR cells. The investigations demonstrate that the situation can be changed by treatment with Pol. The DPC concentration in MCF-7/ADR cells was returned to the normal level. It is established that Pol in the concentration 10^{-4} M aid 7–9-fold reducing P-gp in membranes of MCF-7/ADR cells. The MCF-7/ADR cells cultivation in medium with Pol proceeded to give lowered P-gp content in membranes no over 0.4–0.6%, which amount was consistent with the level of Pgp in MCF-7 cells. NRVM cells cultivation in medium with Pol proceeded to give P-gp content in membranes about 18–2.5%, which amount could reduce the toxicity concentration of Dox in myocytes.
Conclusions: These results indicate that noncontrollable accumulation of P-gp, which cause MDR and Dox cardiotoxicity can be overcomed using stimulation of DPC with Pol, which provides a DPC substitute in regulation of P-gp. Pol is a promising new drug which clinical usage can open up possibilities to tackling the problem of cardiotoxicity and resistance in breast cancer chemotherapy.

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Ectopic expression of PIK3CD in human cancer cell lines and human lung carcinoma

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Class I phosphoinositide-3-kinase (PI3K) consists of four isoforms of the catalytic subunit, p110a, -β, -d and -? generated from the gene PIK3CA, -B, -D and -G, respectively. These isoforms show different tissue distribution and some specific and indispensable functions in various biological pathways such as development, inflammation and cancer. In human cancers, frequent genomic amplification, over expression and gain-of-function mutations of PIK3CA were reported, which suggests its oncogenic potential. However, the connections of other three isoforms containing PIK3CD to human cancers remain unclear. We previously established a panel of 39 human cancer cell lines (JFCR39). JFCR39 has been well characterized in the profiles of gene expression [1], protein expression [2] and sensitivity to various types of pathway inhibitors including PI3K inhibitors [3]. Therefore, JFCR39 is considered to be a good model for studying the PI3K pathway and its implication in cancer. To get more information on non-a isoforms in human cancers, we herein have established an absolute-quantification system of all four isoforms by real-time RT-PCR using isoform-specific primers. This system revealed that, in JFCR39, not only PIK3CA or -B but also PIK3CD was expressed ubiquitously, while PIK3CG expression was restricted in several cell lines. PIK3CD expression was confirmed by semi-quantitative RT-PCR technique and by sequencing the resulting PCR products. Next we examined 30 human lung carcinoma tissues for the expression of the four isoforms and revealed that PIK3CD, not only PIK3CA or -B, was also expressed in most of the cases, while PIK3CG was expressed only in several cases. It has been considered that PIK3CD is expressed predominantly in leukocytes. However, by measuring the expression of all four isoforms at a time, we demonstrated for the first time the ectopic expression of PIK3CD in human cancer cell lines as well as in clinical specimens of lung carcinoma. Biological implications of the ectopic PIK3CD expression remain to be solved.

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Effects of a neutrophil elastase inhibitor on the reduction of radiation pneumonitis

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Background: As the cause of radiation-induced lung injury, experimental studies have shown that the immediate release of pro-inflammatory cytokines such as TNF-α, IL-1 and IL-6 after lung irradiation is closely related with lung toxicity. The increase in these cytokines activates neutrophils, resulting in an accumulation of the activated neutrophils in the lung and the release of elastase. Neutrophil elastase (NE) is deeply involved in the non-specific phylaxis of neutrophils. When neutrophils are activated by stimulation, NE is released from the granules to the extracellular, thereby accelerating permeability of the vascular endothelial

and alveolar cells of the lung. NE disintegrates extracellular matrices and impairs tissue, thereby causing lung injury. In this study, we irradiated the murine lung and analyzed the inhibitory effects of Sivelestat, an NE inhibitor, on lung injury in mice.
Materials and Methods: Twelve-week-old female C57BL/6J mice were used. A dose of 12 Gy, with a 4MV photon beam was delivered to the whole lung in a single fraction via a posterior field with a linear accelerator under Nembutal anesthesia. Sivelestat (3 mg/kg) was administered through intraperitoneal injection immediately, 3 hrs, 6 hrs, and 12 hrs after irradiation in groups RE-0, RE-3, RE-6, and RE-12, respectively. A control group (group C) and a group receiving radiation without sivelestat (group R) were also used. NE activity was measured 24 h and 48 h after irradiation. The lungs were simultaneously extirpated and stained with hematoxylin-eosin. Histopathological features of these cross-sections were analyzed under an optical microscopy.
Results: 1. NE activity: NE activity increased in the groups in which murine lungs were irradiated. There was no increase in NE activity in group C. Among the sivelestat-administered groups, NE activity was slightly elevated in the group RE-0 and was suppressed compared to the group R in groups the RE-3, RE-6, and RE-12 at 24 hours after irradiation. 2. Histopathological features: In the irradiated groups, intra-alveolar neutrophil infiltration, perivascular edema, and alveolar wall thickness were found, but these changes were mild in the sivelestat-administered groups.
Conclusion: NE plays an important role in the development of radiation-induced lung injury. Sivelestat is thus expected to decrease radiation-induced lung toxicity by suppressing NE release from neutrophils.

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Docosahexaenoic acid (DHA) enhances the effect of docetaxel in prostate cancer cells:Modulation of apoptotic pathways

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Background: Prostate cancer is one of the most common male cancers. The chemotherapeutic agent, docetaxel, is currently treating hormone refractory prostate cancer. However there are a proportion of patients who cannot tolerate docetaxel either due to toxicity or due to premorbid conditions. Recently, omega-3 fatty acids have been shown to enhance the anti-tumour effect of docetaxel against human cancers. We aim to assess the effect of adding an omega-3 fatty acid, docosahexaenoic acid (DHA), along with docetaxel in prostate cancer cell lines on cell viability and apoptosis.
Materials and Methods: LNCaP and PC3 prostate cancer cells were treated with DHA and docetaxel, alone or in combination. We studied the drug interaction concurrently and sequentially at a range of drug concentrations. Drug response was determined by standard MTT assay to measure cell viability and drug interaction was analyzed by combination index (CI) method. To assess apoptosis and cell cycle, flow cytometry was performed using PI, Annexin V and JC-1 staining protocols.
Results: DHA enhanced, in a synergistic manner, the anti-tumour effect of docetaxel in LNCaP cells but not in PC3 cells. The IC50 of docetaxel showed a 3-fold decrease upon concurrent treatment with 25micromolar DHA and a 2-fold decrease upon sequential treatment with 100micromolar DHA. Flow cytometry analysis by JC-1 staining showed significant increase ($p < 0.018$) in apoptosis and PI staining showed increase in sub-G1 population, but did not reach statistical significance. Annexin V staining did not show a significant increase in apoptosis, however the results showed increased cells with necrosis upon concurrent treatment.
Conclusion: DHA enhances synergistically the anti-tumour effect of docetaxel in LNCaP cells but not in PC3 cells. This may suggest that the response may be cell specific or may be related to androgen-sensitivity. DHA may act with docetaxel through the apoptotic pathway.

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Proliferation of human lung cancer in orthotopic transplantation model, comparing with in subcutaneous transplantation model of nude mice

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Purpose: Previously we have established an orthotopic transplantation model of lung cancer in nude mice. The objective of this study is to analyze the proliferation of human lung cancer growing in mouse lung tissue and compare it with tumors implanted in subcutaneous (s.c.).
Experimental design: Human lung adenocarcinoma A549 cell line and squamous cell carcinoma SQ5 cell line were used. In orthotopic lung cancer model tumor suspensions were directly injected into the main bronchi of anesthetized athymic nude mice (7−9 week old) with simultaneous administration of 0.01 M EDTA. In s.c. model tumor suspensions were injected into the flank. To label the proliferation tumor cells mice were intraperitoneal injected with 60 mg/kg of body weight bromodeoxyuridine (BrdUrd) in PBS at 20 min before sacrifice. Lung tissue with tumor nodules and s.c. tumor were fixed with formalin and conformed by histology. Proliferation tumor cells were stained by anti-BrdUrd and labeling index (LI) were counted.
Results: Tumor formation rate in mouse lung of A549 cell line and SQ5 cell line were 80% and 100%, respectively. Tumor nodules occurred more frequently in the right lung than the left lung, and in the frequency order of the upper, lower, and middle lobes. The nodular xenografts were numerous, of various sizes. Histological expression showed that adenocarcinoma A549 tumor nodules were distributed primarily in alveoli, showing an abortive glandular arrangement. The squamous cell carcinoma SQ5 tumor nodules mainly invaded the bronchioles and terminal bronchioles. LI in orthotopic implanted lung cancer was 28% per field. Proliferation cells distributed as several groups. LI in s.c. implanted lung cancer was 22% per field. Proliferation cells distributed separately.
Conclusion: Our results showed that in orthotopic lung cancer model, tumor grew in a suitable position which resembles the lung cancer position in humans. Proliferation of tumor cells in orthotopic transplantation model showed different pattern with s.c. transplantation model. These data suggest that this orthotopic lung cancer model may be suitable for analyzing biological behavior of lung cancer.

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Mitochondrial effects of combination cetuximab and ionizing radiation in head and neck squamous carcinoma cells

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The combination of Epidermal Growth Factor Receptor (EGFR) inhibitors and conventional cancer therapies has become the subject of intensive investigations. Overexpression of EGFR is involved in carcinogenesis of several types of cancer (specially in head and neck squamous cell cancer). Recently, a phase III clinical trial had shown that the treatment of locoregionally advanced head and neck cancer with concomitant high-dose radiotherapy plus cetuximab improves locoregional control and reduces mortality without increasing the common toxic effects.
The aim of this study is to explain radiosensitizing properties of anti-EGFR treatments and to clarify the molecular mechanisms involved in the combination.
To explore this question, we focused our attention on cell death pathways and especially on mitochondrial effects. The combination of cetuximab and ionizing radiation has been studied on different head and neck cell lines (CAL27, CAL33, SQ20B). Several conditions of treatment were tested. Standard proliferation studies were performed. Cell cycle analysis, mitochondrial changes (mitochondrial membrane potential and mitochondrial mass) and cellular modifications were performed by flow cytometry, confocal and electronic microsopies.
Our data shown that CAL27 cell line is sensitive to ionizing radiation and cetuximab and the combination increases markedly this sensitivity of cells in vitro. Cell cycle analysis shows characteristics modifications and apoptotic cell population induced by ionizing radiation. Conversely, cetuximab with or without ionizing radiation has no effect on cell cycle. Cell cycle perturbations can be connected with changes in mitochondrial membrane potential. Ionizing radiation induces marked G2 arrest and concomitantly provokes a collapse in mitochondrial membrane potential, the depolarized population increases with the dose of irradiation, cetuximab alone induces a stronger depolarization and the combination increases proportionally the depolarized population cells. In a very interesting way, an increase of the mitochondrial membrane potential was also observed on cells treated with cetuximab with or without ionizing radiation. This apparent increase corresponds to an increase of mitochondrial mass which is not observed with ionizing radiation and is amplified with the combination of cetuximab and ionizing radiation.
These findings provide insight into a specific role of the mitochondria and may help explain the potency of the combination.