derivatives were obtained by changing the lipophilic or metoxy groups on the different positions of the phenyl ring.
Results: The results showed all compounds are inserted into a hydrophobic pocket in the active site region of ABCB2. The ki values has confirmed a good hydrophobic interaction of the designed compounds. The most potent compound was found to have two Cl groups on Meta- positions of the phenyl ring. The orientation of this derivative in the active site of P-gp 3D model was examined by a docking experiment. The molecular modeling shows that the NO_2 substituent forms a hydrogen bond interaction with the H of THR318. These observations and experimental results provide a good explanation for the potent and selective activity of these compounds.
Conclusion: The interactions of DHP derivatives showed that they can be considered as possible MDR reversing agents. In order to achieve better potency, it is better to keep the main structure and alternatively change the phenyl ring with a heterocyclic ring (eg. isoxazole) and add a lipophilic group (eg. Cl) to heterocyclic ring in Ortho- or Meta- positions. We hope the results of the present study are useful for the design of more effective compounds against cancer.

31LBA LATE BREAKING ABSTRACT

β-arrestin-dependent signaling by IGF-1R regulates the Ras induced transformation of mammalian cells

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Background: The receptors tyrosine kinase (RTKs) is a related family of cell surface receptors with similar structural and functional characteristics. Among them, the insulin-like growth factor receptor (IGF-1R) is one of the most important players in cancer development. IGF-1R is responsible for the transformation and proliferation of malignant cells, in prevention of apoptosis and in maintenance of the malignant phenotype of tumor cells. IGF-1R expression is a requirement for transformation by oncogenes. Mouse embryo fibroblasts with a disruption of the IGF-IR genes (R− cells), have been found to be resistant to transformation by a variety of viral and cellular oncogenes, except v-src and a mutant of Gq13. Recently, we provide evidence that b-arrestin1, which is better known to be involved in the regulation of GPCR, serves as an adaptor to bring the oncoprotein MDM2 to the IGF-1R leading to both the ubiquitination of the receptor and activation of MAPK/ERK signaling pathway.
Here we aim to investigate whether the β-arrestins mediated signals of IGF-1R is necessary for tumor transformation.
Material and Methods: We used mouse embryonic fibroblast cells (MEF) lacking β-arrestin1 (KO cells) and control MEFs stably transfected either with H-RasV12 (MEF-Ras; KO-Ras), PyMT (MEF-MT; KO-MT) or v-Src (MEF-Src; KO-Src). The transfection efficiency was verified by Western blot. To evaluate transformation we tested the cells for proliferation under serum free conditions and the ability to form colonies during anchorage independent growth.
Results: Our results suggest that oncogenic H-Ras is unable to transform immortalized mouse embryonic fibroblasts in the absence of β-arrestin1. The direct explanation of H-Ras inability to transform cells devoid to beta-arrestin is the impaired IGF-1R signaling and insufficient activation of the PI3K/Akt and ERK pathways.
Conclusions: The present results propose a more generalized, alternative mechanism for transformation by Ras and, implicitly, another possible way for targeting Ras in tumor cells.

32LBA LATE BREAKING ABSTRACT

High level gene amplification of MYC characterizes radiation-induced angiosarcomas

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Angiosarcomas (AS) are rare vascular malignancies that arise either *de novo* as primary tumors or secondary to irradiation or less often to chronic lymph edema. The cytogenetics of angiosarcomas are poorly characterized.
We applied array-CGH as a screening method to identify and FISH to confirm recurrent alterations in 33 secondary angiosarcomas (31 tumors secondary to irradiation, 2 tumors secondary to chronic lymph edema) and compared the results with 28 primary angiosarcomas. Recurrent genetic alterations were identified only in secondary but not in primary cancers.
The most frequent alterations were high level amplifications on chromosome 8q24.21 (50%), 10p12.33 (33 %) and 5q35.3 (11 %). FISH analysis confirmed high level amplification of *c-myc* on chr. 8q24.21 as a recurrent genetic alteration found exclusively in AS secondary to irradiation or chronic lymph edema. Amplification of *c-myc* was not predisposing to high grade morphology or increased cell turn over.
In conclusion, in spite of their identical morphology, secondary AS are genetically different from primary AS and are characterized by a high frequency of high level amplifications of *c-myc*. These findings may have implications both for the diagnosis and treatment of these tumors. Therapeutics targeting MYC and MYC-dependent signalling could be of major interest.
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33LBA LATE BREAKING ABSTRACT

Synergistic augmentation of arsenic trioxide-induced cytotoxicity by BCNU through reactive oxygen species-related autophagic pathway in human solid tumors

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Background: Arsenic trioxide (ATO) is an effective cancer therapeutic drug for acute promyelocytic leukemia and has potential anticancer activity against a wide range of solid tumors. To improve therapeutic efficacy of ATO in solid tumors, we systematically investigated the combinatory interaction of this drug with other chemotherapeutic agents.
Material and Methods: Growth inhibition was determined using the methylene blue staining method and MTT assay. Two agents were combined at equitoxic ratios based on the IC_{50} of each drug. Efficacy improvement was evaluated using isobologram at 50% inhibition level. Western blot, flow cytometry, immunohistochemistry, enzymatic activity assay were used to reveal molecular events of synergistic interaction of two drugs in this study.
Results: Isobologram analysis revealed that BCNU exhibited synergistic interaction with ATO in human nasopharyngeal carcinoma (HONE-1), melanoma (A2058), glioblastoma (BBTRG-05MG), colorectal carcinoma (HT-29), gastric carcinoma (TSGH), and non-small-cell lung carcinoma (H460). Annexin-V-propidium iodide binding, caspase 3 activity, and PARP cleavage assay indicated that combined ATO with BCNU did not induce cellular apoptosis. Instead, special biological staining with acridine orange and microtubule-associated protein 1 light chain 3, revealed ATO plus BCNU resulted in an increased percentage of autophagic cell death in HONE-1 cells compared to ATO alone. Further analysed indicates that the synergistic augmentation of the cytotoxicity trough autophagic cell death by ATO with BCNU majority through the depletion of reduced glutathione followed augmentation of reactive oxygen species (ROS). Moreover, depletion of reduced glutathione is through the inhibition of catalytic activity of thioredoxin reductase and glutathione reductase.
Conclusion: Taken together, the synergistic interaction of ATO with BCNU is through ROS related-autophagic pathway. These findings will be useful in designing future clinical trial of combination chemotherapy with ATO and BCNU with a potential for a broad use against human cancers.

34LBA LATE BREAKING ABSTRACT

Activated cPLA2a correlates with HER2 over-expression and mediates estrogen-induced cell growth of breast cancer cells

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The cytosolic phospholipase A2 (cPLA2α) catalyzes the hydrolisis of membrane glycerophospholipids to release arachidonic acid, which is converted to biactive eicosanoid lipid mediators, including prostaglandins produced through cycloxigenases, promoting activation of downstream proliferative cell signaling pathways. The eicosanoid signalling pathway contributes to cell proliferation in breast cancer. Numerous studies demonstrated a crucial role of COX-2 and PGE2 in breast tumorigenesis and progression. The specific role of cPLA2α, however, is not established. Recent work from our group demonstrated that 17β-estradiol (E2) can rapidly activate cPLA2α in the breast cancer-derived MCF-7 cell line, leading to the hypothesis that the rapid release of bioactive lipids may play a role in the proliferative signalling responses stimulated by E2 in breast cancer cells. We have shown that the E2-induced rapid activation of cPLA2α was dependent on specific trans-activation of EGFR/HER2

heterodimers and downstream signalling through ERK1/2 MAPK to phosphorylate cPLA2α on Ser505. Over-expression or amplification of HER2 is found in approximately 30% of breast cancer patients and correlates with a poor clinical outcome and resistance to endocrine therapy. We have found an increased expression of cPLA2α at both mRNA and protein levels in SKBR3 breast cancer cells over-expressing EGFR and HER2, as compared with MCF-7 cells which have low expression of EGFR and HER2. The increased protein expression of cPLA2α in SKBR3 was accompanied with a two-fold increase in enzymatic cPLA2α activity. Inhibition of HER2 with either the monoclonal antibody Trastuzumab or short-interfering RNA caused a reduction in both total and phosphorylated levels of cPLA2α in SKBR3. Pharmacological blockade of cPLA2α with the specific inhibitor (525143) impacted on cell growth of SKBR3 cells, by reducing E2-induced proliferation and inducing both apoptotis and necrosis. Selective gene silencing of cPLA2α also reduced both E2-dependent and E2-independent cell growth. To investigate the clinical significance of our in vitro studies, we analysed cPLA2α expression by real time qRT-PCR in tumor samples from HER2-negative and HER2-positive breast cancer patients: our preliminary data show a significant increase in cPLA2α in tumor samples over-expressing HER2. This study highliths cPLA2α as a potential target for therapeutic intervention in HER2-positive breast cancer.

35LBA LATE BREAKING ABSTRACT

Zoledronic acid affects the ability of mesenchymal stem cells to sustain breast cancer progression

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Background: Zoledronic acid (ZA) very rapidly concentrates in the bone following intravenous administration. ZA has been recently shown to increase the progression free-survival of estrogen receptor (ER)-positive breast cancer patients by reducing both loco-regional and distant metastases. Recent reports have also shown that bone-marrow-derived mesenchymal stem cell (MSCs) are recruited to the stroma of developing tumors, where they increase the metastatic potential of breast cancer cells by secreting the chemokine RANTES (CCL5) that sustains breast cancer motility and invasion.
Materials and Methods: The antiproliferative effects of ZA on human primary MSCs were evaluated with an anchorage-dependent growth assay. The effects of ZA on the secretion of RANTES, IL-6 and angiogenic factors were assessed by using the Luminex-based Bio-Plex Suspension Array. The ability of MSCs and breast cancer cells to migrate through a fibronectin-coated membrane was evaluated by using a commercially available assay.
Results: We found that treatment with ZA produced marginal effects on the growth of human primary MSCs, with an approximately 25% growth inhibition following treatment with 20 μM ZA for 48 hours. In contrast, treatment with similar concentrations of ZA almost completely suppressed the ability of MSCs to secrete RANTES. The effect of ZA on RANTES was quite specific, since marginal inhibition of the secretion of different angiogenic growth factors, such as VEGF, IL-8 and bFGF, was observed. ZA also significantly reduced the secretion by MSCs of IL-6 that has been previously demonstrated to act as a potent paracrine growth factor for human breast cancer cells. Conditioned medium from ZA-treated MSCs showed a reduced ability to promote the migration of ER-positive MCF-7 breast cancer cells through a fibronectin-coated membrane as compared with conditioned medium from untreated cells. In co-culture assays, treatment with ZA reduced the ability of MSCs to sustain the growth of breast cancer cells. Finally, the migration of MSCs was significantly reduced by ZA.
Conclusions: Taken together, these data suggest that ZA might exert its antitumor activity in the bone marrow microenvironment by inhibiting the migration of MSCs and by blocking the ability of MSCs to secrete factors involved in breast cancer progression.

36LBA LATE BREAKING ABSTRACT

Long-term safety and tolerability of fentanyl pectin nasal spray in opioid-tolerant patients in the treatment of breakthrough cancer pain

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Background: The authors are submitting this abstract on behalf of the Fentanyl Nasal Spray Study 045 Investigators Group. Placebo-controlled, randomized controlled trials have demonstrated efficacy with a rapid onset of effect for fentanyl pectin nasal spray (FPNS), a new nasal formulation of fentanyl. The aim of this study was to assess the long-term safety and tolerability of FPNS in treating patients with breakthrough cancer pain (BTCP).
Material and Methods: Patients (new and rolled over from previous controlled studies) with cancer experiencing 1−4 episodes/day of BTCP whilst taking ⩾60 mg/day of oral morphine (or equivalent) for cancer pain were eligible to enter an open-label safety study: 16-week initial phase and extension phase. FPNS was used to treat up to 4 BTCP episodes/day. Safety and tolerability were assessed by: adverse events (AEs), withdrawal due to AEs and nasal assessments. Objective nasal assessments examined treatment effect on the nasal mucosa. Subjective nasal assessment included: stuffy/blocked nose, runny nose, itching/sneezing, crusting/dryness of nose, burning/discomfort, nasal bleeding, cough, postnasal drip, sore throat and taste disturbance. Additional rescue medication use was also recorded.
Results: 403 patients (234 new, 47 exposed to FPNS during titration phase but did not enter the treatment phase, 122 rolled over) were included in the safety analysis (42,227 FPNS-treated episodes) with 110 patients completing the full 16 weeks. For the entire course of the study, mean duration of treatment was 60 days, with 138 patients treated for ⩾90 days. A total of 99 (24.6%) patients reported treatment-related, treatment-emergent AEs (TEAEs) that were generally mild or moderate in severity. TEAEs were not dose related and were typical of opioid therapy. Of the 80 deaths that occurred during the study, 1 death was possibly related to study drug (constipation, intestinal perforation, peritonitis). Nonfatal serious AEs were reported in 61 (15.1%) patients – 6 possibly and 1 probably related to study drug. Of the 20 patients who discontinued treatment due to an AE, 9 patients withdrew due to treatment-related AEs. Objective and subjective nasal assessments revealed no clinically significant effects. 94% of FPNS-treated episodes required no rescue medication and 90% of patients required no dose change.
Conclusions: FPNS was safe and well tolerated both systemically and nasally. Overall, FPNS delivered consistent and reliable clinical effects that were sustained through up to 4 months of BTCP treatment.

37LBA LATE BREAKING ABSTRACT

Impact of p53 protein overexpression on survival of stage II young breast cancer patients

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Background: p53 is a tumor suppressor gene and plays important role in the etiology of breast cancer and has been linked to breast cancer survival. The prognostic potential and impact on 5-year survival of p53 protein overexpression was investigated in 34 primary breast cancers from stage II young breast cancer patients (<50 years).
Material and Methods: using archived tumor tissue from 34 patients diagnosed with stage II breast cancer between 2001–2003, we determined p53 protein overexpression by immunohistochemistry. We examined the association of p53 overexpression and HER2 scores, ER/PR status and anthracyclines doses in adjuvant setting. Tumour sections were stained for p53 and HER2. p53 and HER2 scores were based on staining intensity, 2+ and 3+ being considered HER2+, nuclear staining score ⩾1% being considered p53+. The material from medical records was obtained and the adequacy of adjuvant chemotherapy was assessed. The dose of anthracyclines ⩾400 mg was considered adequate and <400 mg was considered inadequate.
Results: The prevalence of protein overexpression in the tumor was 20.6% and HER2 overexpression was 26.4%. Our results suggest that patients with tumors that were positive for p53 protein, negative estrogene receptors and treated with inadequate anthracyclines dose died within shorter period of time after diagnosis (*log rank* $p = 0.016$, *log rank* $p = 0.027$, *log rank* $p = 0.013$, respectively). There were no significant correlations with HER2 overexpression and 5-year survival in this population (*log rank* $p = 0.51$). In multivariate analysis, inadequate anthracyclines dose ($p = 0.028$) was independent factor of poor outcome (Table 1).
Conclusions: The results of this study demonstrate a consistent relationship between p53 protein overexpression, negative estrogene receptor status, inadequate anthracyclines dose and worse survival of young early stage breast cancer patients. Our data do not support a significant prognostic role for HER2 overexpression in predicting survival. The independent prognostic factor is inadequate anthracyclines dose in the adjuvant setting.