confirm our hypothesis. We examined several additional mechanisms as possible causes of increased sensitivity to lovastatin, but found no correlation between expression of HMG CoA-reductase, Bcl-2, survivin or P-glycoprotein with the level of sensitivity. Similar sensitivity pattern of $CA3_{ST}$ and CK2 cells to lovastatin and statins with different lipophility and metabolic pathways suggests that altered intracellular accumulation of active drugs was not the cause of altered sensitivity.
Conclusions: Increased sensitivity of cDDP-resistant cells to lovastatin involves several geranylgeranylated proteins, among others Rac1 and Cdc42. Including lovastatin in the treatment of cancer patients could improve the success of chemotherapy for patients with cDDP-resistant tumors.

1027 POSTER

Sorafenib overcomes TRAIL resistance of hepatocellular carcinoma cells through the inhibition of signal transducers and activators of transcription 3

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Background: Hepatocellular carcinoma (HCC) is one of the most common and lethal human malignancies. Recombinant tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) is a promising anti-tumor agent. However, many HCC cells show resistance to TRAIL-induced apoptosis. Sorafenib, a tyrosine kinase inhibitor, was recently approved by FDA for HCC. In this study, we showed that sorafenib sensitizes resistant HCC cells to TRAIL-induced apoptosis.
Material and Methods: HCC cell lines (PLC5, Huh-7, Sk-Hep1, and Hep3B) were treated with sorafenib and/or TRAIL and analyzed in terms of apoptosis, signal transduction.
Results: HCC cells, including PLC5, Huh-7, Hep3B and Sk-Hep1, showed significant resistance to TRAIL-induced apoptosis (up to 1000 ng/ml). The combination of sorafenib (starting at 5 μM) and TRAIL restored the sensitivity of HCC cells to TRAIL-induced apoptosis. Thorough comparisons of the molecular change before and after treatment with these agents, we found signal transducers and activators of transcription 3 (Stat3) played a significant role in mediating TRAIL sensitization of sorafenib. Our data showed that sorafenib down-regulated phospho-Stat3 (Tyr 705) and subsequently reduced the expression levels of two Stat3-related proteins, Mcl-1, and survivin, in a dose- and time-dependent manner in TRAIL-treated HCC cells. Knocking down Stat3 by RNA-interference reversed overcame apoptotic resistance to TRAIL in HCC cells, and ectopic expression of Stat3 in HCC cells abolished the TRAIL sensitizing effect of sorafenib, indicating Stat3 inactivation plays a key role in mediating the combination effect.
Conclusions: Sorafenib sensitizes resistant HCC cells to TRAIL-induced apoptosis at clinical achievable concentrations, and this effect is mediated via the inhibition of Stat3.
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1028 POSTER

Aloe emodin, a natural anthraquinone targeting multiple facets (migration, invasion, angiogenesis) of tumour metastasis

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Background: The present study evaluated the apoptotic, antimetastatic and antiangiogenic property of a naturally occurring anthraquinone, aloe emodin (AE) in colon cancer cells. This compound is known to induce apoptosis in various other tumour cell types *in vitro*. Studies validating its role in influencing the regulatory molecules involved in metastasis and angiogenesis are rare. Colorectal tumors are one of the rapidly metastasizing tumours and a major cause for cancer deaths world wide.
Methods: Cell viability was assayed by MTT staining for the detection of the antiproliferative activity of drug. Ability of this drug to induce apoptosis was identified by annexin/propidium Iodide staining, loss of mitochondrial membrane potential and TUNEL assay. Inhibition of cancer cell migration was assessed by wound healing and transwell migration/transwell invasion assays. Cell growth inhibition and cell cycle distribution induced by AE was evaluated by FACS. Endothelial cell proliferation and migration assays as well as *in vitro* tube formation assays were used to evaluate the antiangiogenic activity of AE. Effect of AE on the expression of molecular players involved in apoptosis (caspases, PARP, MAPKs) migration (MMP2, MMP9, MAPKs) angiogenesis (VEGF) was assayed using substrate zymograpghy, PCR, western blot and fluorescent tagged peptide assay.
Results: Treatment with AE we observed positive annexin staining, loss of mitochondrial membrane potential and strong TUNEL positivity. The growth inhibitory capacity of AE was through induction of G2/M arrest. We have observed down regulation of phosphoERK1/2, activation of caspase and fragmentation of PARP on treatment with AE. Our results showed that a relatively non toxic level of AE suppressed the phorbol-12-myristyl-13-acetate (PMA) induced migration and expression/activity of MMP2/MMP9. We have also analyzed the involvement of signaling molecules (MAPKs) and specific transcription factors in AE mediated up regulation of MMP2/9. AE also inhibited Human Umbilical Vein Endothelial cells (HUVECs) proliferation migration/invasion and *in vitro* tube formation. We have also analyzed its effects on VEGF expression.
Conclusion: *In vitro* anticancer activity of AE on colon cancer cells depends on the ability to induce apoptosis, inhibit cell migration, *in vitro* tube formation and down regulation of key MMPs involved in metastasis. Thus AE can be projected as a prospective antitumor agent even though further research is warranted.

1029 POSTER

Improved distribution and efficacy of oncolytic virus in solid tumors by apoptosis-inducing pretreatments

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Background: For successful eradication of the tumor by oncolytic gene therapy, initial widespread distribution of the virus within the tumor is crucial. However, viral distribution is limited by the large size of viral vectors, which limits their penetration through the interstitial matrix and the narrow spaces separating tumor cells. This study tested if the void space resulting from tumor cell apoptosis improves the distribution and efficacy of oncolytic HSV.
Material and Methods: We used two different approaches to induce apoptosis, which are 1) tet-regulated expression of apoptotic gene and 2) cytotoxic agents. For tet-regulated apoptosis system, MDA-MB-435S cells were transfected with tet-inducible CD8/Caspase-8 plasmids. For cytotoxic agents, paclitaxel or recombinant TRAIL was used. *In vivo*, MDA-MB-435S cells were implanted into the SCID mice and apoptosis was induced by doxycycline-regulated expression of CD8/Caspase-8 or cytotoxic agents. To study the effect of pretreatments on the viral distribution, oncolytic HSV expressing GFP was injected intratumorally following to different pretreatments. Finally the effect on tumor growth was assessed in MDA-MB-435S tumors.
Results: *In vitro,* both caspase-8 activaion and cytotoxic treatments induced significant apoptosis on tumor cells. Paclitaxel followed by TRAIL induced significantly more apoptosis than single treatment. In mice with MDA-MB-435S tumors, both the activation of caspase-8 and pretreatment with cytotoxic agents induced 9.0% and 4.0% apoptosis, respectively. In contrast to the limited viral distribution of 13% of the tumor section in control tumors, viral distribution was significantly improved by both caspase-8 activation (42.4%) and paclitaxel-TRAIL (30.3%). In tumor areas with a high density of apoptotic cells, the cellular shrinkage produced interstitial void spaces and channels that facilitated HSV distribution. We also show that the intratumoral injection of oncolytic HSV after caspase-8 activation or the paclitaxel plus TRAIL pre-treatment produces a significantly longer tumor growth delay than the administration of HSV before the induction of cell death, demonstrating the importance of sequence of the treatments.
Conclusions: Cancer cell death improves the intratumoral spread and therapeutic efficacy of oncolytic HSV. Thus the administration of cytotoxic agents before the injection of virus could significantly enhance the efficacy of oncolytic viral therapy.

1030 POSTER

Complementary treatment with (−)-epicatechin enhances the anti-cancer effect of etoposide in the spleen of Brown Norway rats with acute myeloid leukemia

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Background: It has been proven that tea catechins possess antileukemic properties, which might be useful in complementary treatment. The aim of the study, was to examine whether the adjuvant therapy with (−)-epicatechin (EC) can affect the anti-cancer effect of etoposide (Eto) in Brown Norway rats with acute myeloid leukemia (BNML).

Material and Methods: 40 rats were injected (i.v.) with BNML cells and divided into 4 groups treated with: 1) EC (40 mg/kg b.w. by gavage) for 19 consecutive days, 2) Eto (14 mg/kg b.w., i.p.) for 3 consecutive days, from day 17 to 19 of the experiment, 3) EC + Eto, or 4) water as a solvent of EC (control group). The rats were killed 2 hours after the last dose of EC and/or 1 hour after Eto administration. The spleen and the bone marrow were isolated. The BNML cells were identified using an RM-124 antibody and analysed by flow cytometry. The mean concentrations of EC and *O*-methylated EC (Methyl-EC) in plasma were analyzed by HPLC-ECD.
Results: The mean concentration of EC in the plasma was 4.7 mM, and that of Methyl-EC was 5.5 mM. After simultaneous treatment (EC+Eto), the concentration of EC in the plasma decreased significantly to a level of 2.3 mM and that of Methyl-EC to 2.5 mM. The weight of the spleens isolated from the rats of each experimental group, was significantly diminished in comparison to the control group. EC significantly diminished the extent of BNML cells in the spleen but not in the bone marrow of rats in comparison to the control. The simultaneous treatment of rats (EC+Eto) led to a significant decrease in the number of BNML cells in the spleen of rats compared to Eto or EC applied alone. EC did not affect significantly, the number of leukemic cells diminished by Eto in the bone marrow of BNML rats.
Conclusions: Complementary treatment enhanced the anti-cancer effect of Eto in the spleen, although the bioavailability of EC decreased by about of 50% when it was administered simultaneous with Eto. It can be surmised that the increase in bioavailability of EC during Eto administration can enhance the anti-cancer effect of this polyphenol.

1031 POSTER

Fast-forwarding kinase drug discovery: aurora and EGFR kinase inhibitor lead development

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Background: Drug discovery is a complex, risky, costly and time consuming process, nevertheless essential. Means to improve the efficiency and shorten the time frame required for drug discovery are being sought after continuously by researchers. Here we present our synthetic, screening and design strategies to speed-up drug discovery lead identification process for kinase inhibitors, using Aurora A and EGFR kinase inhibitor development as a prototype.
Material and Methods: Compounds were synthesized using batch reactor and screened on 96-well plates. X-ray co-crystal studies were performed as reported by us previously (J Med Chem, 2009, 52 (4), 1050–1062).
Results: Furano-pyrimidine compound BPR1K224 was identified as an Aurora kinase A inhibitor with an IC_{50} of ~300 nM, by screening in house compound library. Over 350 analogs of the hit were rapidly synthesized in batch reactor and screened for Aurora inhibition without purification to identify 2nd generation lead compounds. Based on the X-ray co-crystal structure of the lead compounds in complex with Aurora A, a potent Aurora A inhibitor BPR1K432 (IC_{50} ~50 nM), which possessed anti-proliferative activity in HCT-116 cell line (IC_{50} ~400 nM) was synthesized. Counter-screening of 350 library compounds for EGFR kinase inhibition, led to the identification of an EGFR kinase inhibitor BPR1K0317 with an IC_{50} ~200 nM. Based on the molecular docking studies of BPR1K0317 in the active site of EGFR kinase protein, acrylamide michael acceptor group was introduced, which resulted in the identification of a potent EGFR kinase inhibitor BPR3K007, which inhibited both wild type (IC_{50} 7 nM) and DM (IC_{50} 22 nM) EGFR kinase and possessed anti-proliferative activity in HCC827 cell line (IC_{50} 8 nM).

Compound	Inhibition, IC_{50} (nM)				
	Aurora A based		EGFR based		
	Kinase	HCT-116	Kinase		HCC827
			WT	DM	
BPR1K224	309	>10000	>10000	–	–
BPR1K432	43	400	>10000	–	–
BPR1K317	>10000	–	223	>10000	518
BPR3K007	~10000	–	7	22	8

WT – Wild type EGFR kinase; DM – Double mutant (L858R/T790M) EGFR kinase.

Conclusion: Rapid synthesis of 350 compounds and screening them without purification, led to the identification of potent Aurora A and EGFR kinase inhibitors with furano-pyrimidine scaffold. Similar synthesis and screening protocols could be applied to diverse scaffolds and could help in identifying inhibitors for other kinase in shorter periods, relative to traditional methods resulting in newer targeted anticancer therapy.

1032 POSTER

Apoptosis-inducing effects of morinda citrifolia and doxorubicin on the Ehrlich ascites tumour in balb-c mice

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Background: Morinda Citrifolia (noni) is a herbal remedy with promising anti-cancer properties. However, its effects on various cancers are to be investigated to make a firm conclusion before implementing it into the clinical practice. Therefore, we investigated the cytotoxic potential of noni on Ehrlich ascites tumor grown in female Balb/c mice and also combined it with a potent anti-cancer agent, doxorubicin.
Materials and Methods: One group received noni only (n = 8), another one doxorubicine (n = 8), and the other one noni+doxorubicine (n = 8) for 14 days after the inoculation of cells. The control group (n = 7) received 0.9% NaCl only.
Results: We found that short and long diameters of the tumor tissues were about 40−50% smaller, compared to those in control group. This anti-growth effect was resulted from the induction of apoptosis, which was proved by the positivity of TUNEL and active caspase-3 cells in tissues and confirmed by caspase-cleaved cytokeratin 18 elevation in serum of the treated groups. In addition, the proliferation was decreased, which is immunohistochemically shown by the PCNA staining.
Conclusion: We conclude that noni may be useful in the treatment of breast cancer either on its own or in combination with doxorubicine, which seems to warrant further clinical studies.

1033 POSTER

Study of adapter protein Ruk/CIN85 subcellular redistribution after stimulation of HeLa cells with activators of PI3K

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Background: Ruk/CIN85 belongs to a family of ubiquitously expressed adaptor molecules. Adaptor protein Ruk/CIN85 is important component of different cellular pathways and directly involved in regulation of multiple cellular functions, including proliferation, adhesion, invasion, and survival.
Material and Methods: Serum-starved human cervical cancer (HeLa) cells were treated with 5α-dihydrotestosterone (DHT), interferon-gamma (IFNγ) and isoproterenol (ISO). Expression of adapter protein Ruk/CIN85 full-length form was studied using Western-blot analysis as well as confocal microscopy.
Results: There are data that stimulation of HeLa cells with DHT lead to the activation of PI3K-dependent signaling. It was established that the content of full-length CIN85/Ruk form in Triton-X-100-soluble fraction of untreated human cervical adenocarcinoma HeLa cells in logarithmic growth phase was very low. Interestingly, stimulation of cells with DHT resulted in up-regulation of the full-length form content. The maximal effect of DHT on p85 content (10 min of stimulation) was observed at 0.01 nM of ligand concentration followed by the decrease of this effect at 30 min. It should be stressed, that 10 min stimulation of HeLa cells with high (100 nM) or intermediate (1 nM) concentration of DHT led to the least up-regulation of p85 content, while the maximal p85 level after 30 min stimulation with DHT was revealed at 0.1 nM ligand concentration. In transfected HeLa cells, a punctate Ruk/CIN85 localization pattern, common for proteins involved in membrane trafficking was revealed using confocal microscopy. In addition, the obtained data demonstrate partial colocalization between Ruk/CIN85 and actin in untreated HeLa cells. After DHT treatment partial redistribution of Ruk/CIN85 signal was observed. The main changes were connected with decrease of Ruk/CIN85-actin colocalization in near membrane area and corresponding accumulation of protein in perinuclear region. The same time- and concentration-dependent changes in Ruk/CIN85 content were characteristic to IFNγ and ISO action.
Conclusions: The obtained results suggest that changes in the expression level as well as subcellular redistribution of Ruk/CIN85 in human cervical adenocarcinoma can lead to loss of consistent control of both apoptosis