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

H.P. Hsieh[1], M.S. Coumarrs[1], C.Y. Chu[1], Y.L. Ho[1], M.T. Tsai[1], W.H. Lin[1], C.H. Chen[1], J.S. Song[1], J.T.A. Hsu[1], Y.S. Chao[1]. *[1]National Health Research Institutes, Division of Biotechnology and Pharmaceutical Research, Zhunan Miaoli County, Taiwan*

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

E. Ulukaya[1], E.I. Taskin[2], K. Akgun Dar[3], A. Kapucu[3], E. Osanc[4], H. Dogruman[2], H. Eraltan[5]. *[1]Uludag University Medical School, Biochemistry, Bursa, Turkey; [2]Istanbul University Veterinary School, Morphology, Istanbul, Turkey; [3]Istanbul University Faculty of Science, Biology, Istanbul, Turkey; [4]Kocaeli University, Stem Cell Research Center, Izmit, Turkey; [5]Acupuncture and Health Clinic, Acupuncture, Istanbul, Turkey*

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

O. Basaraba[1], N. Kozlova[2], A. Pasichnyk[3], I. Dikic[4], L. Drobot[5]. *[1]Palladin Institute of Biochemistry National Academy of Sciences of Ukraine, Laboratory of Cell Signalling, Kiev, Ukraine; [2]National University of "Kyiv-Mohyla Academy", Department of Natural Sciences, Kiev, Ukraine; [3]Taras Schevchenko National University of Kyiv, Department of Biology, Kiev, Ukraine; [4]Institute of Biochemistry II Goethe University Medical School, Molecular Signaling Group, Frankfurt am Main, Germany; [5]Palladin Institute of Biochemistry National Academy of Sciences of Ukraine, Laboratory of Cell Signalling, Kyiv, Ukraine*

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

and proliferation in transformated epithelial tissue and significantly contribute to cancerogenesis and progression of cervical cancer.

1034 POSTER

Multidrug resistance (MDR) transporters and vault protein LRP as tamoxifen molecular targets

E.A. Dudko[1], T.A. Bogush[1]. [1]*Russian N.N. Blokhin Memorial Cancer Research Center Russian Academy of Medical Sciences, Laboratory of Medical Chemistry, Moscow, Russian Federation*

Background: Tamoxifen (TAM) is an effective antiestrogen in therapy of breast cancer. But there are other clinical important activities of TAM, one of them is overcoming the chemotherapy resistance under TAM action. Thinking over the reasons of that we have supposed TAM interaction with multidrug resistance (MDR) mechanism namely TAM binding to the MDR-transporters and vault protein LRP extruding anticancer drugs out of the cells.
Materials and Methods: TAM influence on specific monoclonal antibody (mAb) binding to Pgp, MRP1 and vault protein LRP was estimated by flow cytometry in human cell cultures Jurkat (T-lymphoblastic lymphoma) and HeLa (cervical cancer), overexpressing the MDR-markers. Mean fluorescence of mAb-labelled cells as well as the number of mAb-labelled cells were calculated over fluorescence area of isotypic controls.
Results: 1. Incubation of the cells with mAbs increased significantly their fluorescence intensity compared to the isotypic controls. 2. It was not any influence of TAM on isotypic Abs binding to the cells. 3. Incubation of the cells with 50×10^{-6} M TAM changed interaction of mAbs with the MDR-markers investigated. The mean cell fluorescence intensity in the area of specific fluorescence of mAbs and the number of mAb-labeled cells was changed but with different manner for different MDR-markers. Under TAM action the indexes for MRP1 and LRP mAbs decreased up to more than 2 times. TAM effect on mAb interaction to Pgp was different in living cells and in the cells after 0.5% Tween 20 permeabilization. For the first one, TAM increased the mean specific cell fluorescence intensity and the number of mAb-labeled cells up to more than 4 times. For the second one, the indexes decreased up to more than 2 times.
Conclusion: These data are direct evidence for the TAM interaction with the Pgp, MRP1 and LRP in tumor cells. It should decrease further binding of anticancer drugs with the MDR-markers and thereby inhibit MDR-mechanism through decrease of the MDR-drug transport out of the cells. This can be regarded as confirmation of our assumption that TAM interaction with Pgp, MRP1 and LRP may be one of the reasons for clinical overcoming chemotherapy resistance under TAM action. The conclusion is true for the MDR-drugs and the tumors expressing MDR-phenotype only and explains the TAM insufficient in increase chemotherapy efficacy in some patients.
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1035 POSTER

Autophagic cell death of the nutrient deprivation augmented by cytotoxic drugs in lung cancer cell

S. Yang[1], H. Jo[1], H. Jang[1], M. Lee[1], K. Lee[1], S. Moon[1], J. Park[1], E. Jeong[1]. [1]*Wonkwang University School of Medicine, Deparment of Internal Medicine, Iksan-city Jeonbuk, South Korea*

Autophagy is known for its role in cellular homeostasis, development, cell survival, aging, immunity, and cancer. Autophagy has emerged as another major 'programmed' mechanism to control life and death much like "programmed cell death" is for apoptosis in several types of cancer. To be elusive that autophgic cell death on nutrient starvation in combination with cytotoxic drugs, we investigated whether its increase synergistically in two mixed conditions. When cancer cells were subjected to extreme nutrient starvation by culturing in a medium without serum and amino acids or with 2-deoxyl-D-glucose, a chemical inhibitor of glucose metabolism, cells death occurred within early time. At nutrient deprived media with cisplatin or gemcitabine treatment, Cell survival revealed a markedly decrease in percentage of living cells undergoing nutrient starved medium with each of two cytotoxic drugs compared with those drugs respectively. The staining of cells in normal media with acridine orange displayed green fluorescence with cytoplasmic and nuclear components in normal media but showed considerable red fluorescence in combined medium or cytotoxic drugs in each treated cells, suggesting formation of numerous acidic autophagolysosomal vacuoles. LC3 modification, as autophagy marker, was analyzed by western blotting. LC3 proteins have two forms: type I is cytosolic and type II is membrane-bound. During autophagy is advanced, LC3 type II increased by conversion from LC3 type I. We figured out that the autophagosome-incorporated LC3 II protein expression more increased in cell contained nutrient-deprived medium with cytotoxic drugs compare with cisplatin or gemcitabine alone. These results demonstrated that the autophagic cell death potentially increased in nutrient-deprived conditions combined with cytotoxic drugs in human lung cancer cell lines.

1036 POSTER

Response of CD133+/− subpopulations of CRC cell lines to radio- and chemotherapy

C. Dittfeld[1], S. Peickert[1], A. Dietrich[1], M. Grade[2], T. Ried[3], L.A. Kunz-Schughart[1]. [1]*OncoRay – Center for Radiation Research in Oncology, Tumor Pathophysiology, Dresden, Germany;* [2]*Department of General and Visceral Surgery, University Medicine, Göttingen, Germany;* [3]*National Cancer Institute, National Institutes of Health, Bethesda, USA*

Background: More than 40% of colorectal cancers (CRC) are located in the rectum. Preoperative 5-FU based chemoradiotherapy is recommended for locally advanced rectal adenocarcinomas. However, therapy response of individual tumors is not uniform. Complete regressions but also resistant tumors are described which may be related to a discrepancy in the presence or survival of tumor-initiating/cancer stem (TIC/CSC) cell populations leading to an individual risk of recurrence. The hypothesis of such cell populations to relate to therapy resistance and recurrence of disease is challenging because the only tool to identify or isolate such cells are surface markers with limited causal evidence. CD133 has recently been described as a potential marker to enrich TIC/CSC from primary CRC material and from the cell line HT29. The aim of our study was to investigate tumorigenic potential, radioresponse and drug efficacy in another CRC cell line which contains two distinct populations defined by their CD133 expression.
Material and Methods: The cell line HCT-116 showed two clearly distinguishable $CD133^+$ ($74.3\pm6.2\%$) and $CD133^-$ subfractions. Subpopulations were isolated via FACS and analyzed in parallel to the original, mixed cells for colony formation and therapy response *in vitro*. $SF_{2\,Gy}$ and IC_{50} values after single dose irradiation or treatment with 5-FU or Oxaliplatin (Oxa) were calculated from dose response curves. Tumorigenicity was evaluated in a subcutaneous xenograft model.
Results and Conclusions: Colony forming capacity and radioresponse of $CD133^+$ and $CD133^-$ HCT-116 subpopulations did not differ. The $SF_{2\,Gy}$ was $32.0\pm4.2\%$ for $CD133^+$ and $34.1\pm3.8\%$ for $CD133^-$ HCT-116 cells. Also, the IC_{50} values after 5-FU and Oxa treatment were comparable for HCT-116 cells with discrepant CD133 expression. The mean IC_{50} for 5-FU was $5.8\pm1.0\,\mu M$ for $CD133^+$ and $6.3\pm1.6\,\mu M$ for $CD133^-$ cells and reflected the original HCT-116. Oxa efficacy was slightly lower but revealed the observation with respect to $CD133^{+/-}$ subpopulations. Since $CD133^+$ and $CD133^-$ HCT-116 cells showed a similar xenograft formation capacity, CD133 can neither be regarded as a TIC/CSC marker in HCT-116 cells nor does it define a subpopulation with higher resistance to radio-/chemotherapy *in vitro*. The underlying reasons for differences between HT29 (literature) and HCT-116 are unknown. Extended studies including HT29 cells are ongoing.
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1037 POSTER

Cytostatic agents, radiosesitizers and immunomodulators derived from tropolone alkaloids

Z.M. Yenikeeva[1], S.N. Navruzov[2]. [1]*National Research Center of Oncology, Science, Tashkent, Uzbekistan;* [2]*National Research Center of Oncology, Proctology, Tashkent, Uzbekistan*

Background: It has been implemented – synthesis of the new compounds obtained on the basis of tropolone alkaloids – colchicine and colcemid with a range of amino-substitutes in tropolone and heptadiene rings of the alkaloids, in the laboratory on development of anti-tumor agents of NSCO of MH of RUz. Biological properties of those are being studied and have shown to possess lowered toxicity (in 10–400 times) and high anti-tumor activity in vitro found in NCI USA.
Results: On the basis of study of toxicity and anti-tumor activity in vivo among the new derivatives 5 new agents has been selected: K-48, K-42, Decocine, Decovine, K-20, which have passed (Decocine) or are at stage of pre-clinic study. These compounds are in 13–360 times less toxic than colchicines, their anti-tumor efficiency exceeds activity of both colchicine and colcemid for 20−70%, and in a range of known cytostatics used as control. K-48 together with expressed anti-tumor activity doesn't lower immunity and hemopoiesis, that is reasoned by its ability towards CFUs increase. Cytogenetic studies has shown that K-48 in therapeutic dose doesn't cause chromosome aberrations, k-mitosis and polyploidy in bone marrow, that is peculiar to tubulin-interacting and alkylating drugs, and also lowers amount of chromosome aberrations since treatment. All it is characteristic in some degree for the ?-42 agent at per oral application.