up-regulation of miR-31 (p = 0.024) expression. Higher serum levels of CEA were associated with down-regulation of miR-145 (p = 0.05). Tumors with high expression of p53 protein had significantly lower expression of miR-143 (p = 0.02). We have not associated any of studied miRNAs to tumor grade and tumor size. Tumors with down-regulated miR-143 and miR-145 were bigger and more frequent (not significantly) in proximal colon.
Conclusions: Our results suggest possible roles of miR-21, miR-31, miR-143 and miR-145 in colorectal cancer pathogenesis and different histopathologic phenotypes. Supported by IGA MZ CR NR/9076−4

381 POSTER

Rapamycin potentiates the apoptotic effect of TGFb in lymphoma cells

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Lymphoid tumor cells often lose their sensitivity to signals provided by proapoptotic regulators – such as TGFb1 –, which could be reversed by lowering the survival threshold. The mammalian target of rapamycin (mTOR) signaling kinase integrates growth factor stimulation, energy and nutrient availability to regulate protein translation responsible for cell growth and proliferation. Promising results have been obtained with mTOR inhibitors in some clinical studies, therefore we set out to investigate the apoptotic effect of exogenous TGFb1 and rapamycin in lymphoma cells, focusing on the activity and the role of Smad and alternative signaling mechanism of induced apoptosis.
B-cell non-Hodgkin lymphoma cells (HT58, HT58r, BL41, BL41/95 and U266) were treated with recombinant TGFb1 and rapamycin in vitro. Apoptosis was detected by flow cytometry. The abundance, activity and localization of signaling elements (Smad2, Smad4, Erk1/2, JNK, mTOR, p-mTOR, p-4EBP1, p-p70S6K, p70S6K, p-S6) were determined by Western-blotting.
PP2A phosphatase, MEK1 kinase activity was estimated with the help of specific inhibitors and the role of Smad signaling was studied by transfection of Smad4 siRNA transfection.
Rapamycin treatment (5 ng/ml-10 microg/ml) alone showed no effect in the examined lymphoma cells. However, rapamycin (50 ng/ml or higher doses) combined with TGFb1 (1 ng/ml) treatment restored TGFb1 sensitvity in certain TGFb1 and rapamycin resistant lymphoma cell lines. The combination of rapamycin and TGFb completely eliminated the activity of p70S6K and the ribosomal S6 protein. Smad4 siRNA treatment abolished TGFb induced early gene upregulation, indicating the absence of the rapid activation of Smad signaling. Our results showed that Smad4 siRNA treatment had no influence on the apoptotic effect of TGFb and TGFb+rapamycin, however, PP2A inhibition reduced the apoptotic capacity of TGFb.
These data suggest that exogenous TGFb and TGFb+rapamycin use Smad4 independent, alternative PP2A phosphatase dependent signaling pathways in the TGFb induced apoptosis of lymphoma cells. The results support that lowering mTOR kinase activity and inhibiting protein synthesis dependent survival signals could provide a tool in lymphoma therapy, however, the exact mechanism of Smad4 independent signaling requires further studies.
Supports: OTKA (F048380, TS049887), ETT, NKFP (1A/002/2004).

382 POSTER

Interacting effect of TGFb and Notch signaling in B-cell lymphomas

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Many aspects of normal and malignant cellular processes are regulated by a few major signaling pathways, such as TGFb and Notch. These pathways play an important role in fine-tuning developmental and survival programs in lymphoid cells, and their deregulation may contribute to tumorigenesis. Cross-talk between TGFb and Notch signaling has been reported in epithelial and myogenic cells. We set out to investigate whether TGFb and Notch interact in B-cell lymphomas, and whether their interaction affects cell death.
B-cell non-Hodgkin lymphoma cell lines (Ramos, BL41, BJAB, MED-B1 and U266) were treated with TGFb1 (1 ng/ml), DAPT (Notch-inhibitor; 1 μM) or immobilized rhDLL4 (Notch-ligand; 1 microg/ml). Gene expression of Hairy/Enhancer of Split-1 (HES-1; a Notch-target) and TGFb-induced early gene (TIEG; a TGFb-target) was determined by real-time PCR following RNA isolation and reverse transcription. Apoptosis was assessed with flow cytometry following ethidium-bromide staining, and on hematoxylin-eosin stained cytospin preparations.
TGFb induced apoptosis in Ramos and BL41 cells and rapidly upregulated HES-1. No such changes were detected in the other cell lines. TIEG expression was moderately elevated only in Ramos cells 1h after TGFb treatment. Experiments with DLL4 and DAPT were performed on Ramos and BJAB cells. DLL4 treatment resulted in HES-1 induction in both cell lines, which was inhibited by DAPT. Basal HES-1 expression was inhibited by DAPT in BJAB cells, but not in Ramos cells. DLL4 induced apoptosis in Ramos cells, which was not inhibited by DAPT. Apoptosis induction by combined TGFb+DAPT and TGFb+DLL4 treatment was greater than by TGFb alone in Ramos cells. None of these treatments increased apoptosis in BJAB cells.
Our results suggest that HES-1 may be a transcriptional target of TGFb in certain B-cell lymphomas, at least in part in a Notch-independent manner. Notch-activation induces apoptosis in some B-cell lymphomas, and both Notch-activation and -inhibition may augment TGFb-induced apoptosis. The relationship between HES-1 upregulation, apoptosis and the fine-tuning of these processes by other factors is under investigation.
Supports: OTKA (TS049887, F048380), NKFP (1A/002−04).

383 POSTER

Modulation of doxorubicin-induced endothelin-1 expression by phosphodiesterase-5-inhibitors

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Background: Doxorubicin (DOX) is a powerful and widely used oncotherapeutic. Its use is limited by cardiotoxic side effects whose underlying mechanisms are not fully understood. Interestingly, overexpression of Endothelin-1 (ET-1) leads to severe cardiomyopathy, and ET-1 is elevated in cases of DOX-induced cardiotoxicity. The latter can be prevented by sildenafil in a mouse model. GATA-4, playing a pivotal role in cardiac gene expression, has been shown to be down-regulated after DOX-treatment. Therefore, we investigated the effect of DOX and phosphodiesterase-5-inhibitors sildenafil and vardenafil on expression of ET-1 and GATA-4.
Material and Methods: Studies were conducted on HL-1 cells (murine cardiomyocytes) and isolated primary rat cardiomyocytes. Cells were pre-treated with 0.1 μM sildenafil or vardenafil for 1 h, followed by 48-h DOX-incubation (1 μM). mRNA expression of ET-1 and GATA-4 was measured by Realtime PCR. For determination of corresponding protein levels we carried out ET-1 ELISA.
Results: In HL-1 cells treated with DOX we found a 3.7-fold increase of ET-1 mRNA. Pre-treatment with sildenafil reduced ET-1 mRNA induction to 1.4-fold, whereas upon vardenafil it reached control level (1.2-fold). In rat cardiomyocytes, ET-1 mRNA was increased 16-fold upon DOX. Pre-treatment with sildenafil inhibited this induction completely, whereas vardenafil only slightly diminished ET-1 mRNA increase (13.5-fold). ET-1 peptide was increased 1.8-fold in HL-1 cells after DOX but was not influenced by sildenafil and reduced by vardenafil to 1.2-fold. In rat cardiomyocytes, DOX treatment resulted in a 1.3-fold raise in ET-1, which was abolished by pre-treatment with sildenafil but not with vardenafil (1.3-fold). GATA-4 expression was reduced in rat cardiomyocytes to about 50% but not in HL-1 upon DOX. In HL-1 cells sildenafil increased GATA-4 mRNA to 5.7-fold, whereas vardenafil reduced it to 0.8-fold. In rat cardiomyocytes sildenafil and vardenafil showed no influence on GATA-4 expression.
Conclusions: Sildenafil and vardenafil modulate DOX-mediated regulation of ET-1 and GATA-4 in a varying extent depending on the cell type. Altered expression of cardiotoxic ET-1 and cardioprotective GATA-4 by these drugs could influence the cardiomyocyte function and might be a tool to prevent DOX-induced cardiotoxicity.

384 POSTER

Targeting integrin alpha5beta1 on multiple tumour-associated cell types inhibits tumour growth in xenograft models

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Tumor angiogenesis is a complex process involving multiple growth factors, which stimulate vascular endothelial cell (EC) proliferation. Pro-inflammatory tumor associated macrophages (TAMs) contribute to this process by secreting additional growth factors and cytokines that further encourage angiogenesis and promote tumor progression. TAMs, ECs and many tumor cells express integrin alpha5beta1. An inhibitor of this integrin, volociximab (M200), inhibits EC growth and migration in vitro, independent of the growth factor milieu, and directly inhibits cancer cell migration and survival in vitro and in vivo. In addition, volociximab inhibits the secretion of pro-angiogenic cytokines from TAMs, without affecting the viability of these cells. Volociximab does not cross-react with mouse alpha5beta1, restricting its use in standard mouse xenograft models to targeting the tumor and not the invading ECs or TAMs. We therefore generated an anti-mouse antibody, 339.1, similar to volociximab in potency and selectivity relative to the mouse integrin, to target integrin on host cells in these models. In an A673 xenograft model, 339.1, which does not cross react with

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.

385 POSTER

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.

386 POSTER

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.

387 POSTER

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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388 POSTER

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