structures and to stabilize them. The very promising strategy of studies focuses on telomerase, which is responsible for cancer cells longevity. It was shown that telomerase is detected in over 90% of cancer cells while it is generally inactive in most normal cells.
The aim of the study was to estimate the influence of two ligands (1 and 2), papaverine oxidation products, on cells viability and DNA-quadruplexes stabilization and thus, inhibition of telomerase activity. The two ligands were shown to have high affinity to guanine quadruplexes (G-4 DNA) in vitro, which suggests that they could be able to block DNA-telomerase interactions.
The cytotoxicity of ligands was measured in Cell Proliferation MTT Kit and the influence of the compounds on Telomerase activity was assessed by Telo TAGGG Telomerase PCR ELISA Plus assay.
Cytotoxicity tests showed that both ligands inhibit cell viability with their IC50 values for ligand 1 and ligand 2 respectively, at 72 h incubation time in HL60 cells: 0.15 and 0.19 μM; in HL60AR cells: 46.21 and 16.48 μM; in MCF-7 cells: 1.16 and 0.42 μM; in MDA-MB-231 cells: 16.55 and 5.1 μM. Moreover, it was also reported that ligand 1, showing fluorescence at 365/397 (exc./emis.), binds the growing cells permanently, persisting even through a few cell passages what was observed in a fluorescence microscopy.
Telomerase activity assay showed that both ligands significantly inhibit telomerase activity at the concentration of 0.1 μM. However, the action of both ligands resulted also in Polymerase activity inhibition, which might suggest interactions specific not only to quadruplexes but also to DNA helix or maybe even enzyme structure.
It is suggested that both studied ligands could be strong and selective cancer cells growth inhibitors that results from their telomerase inhibition specific action. It is also possible that the studied compounds could be new promising fluorescent probes for DNA detection and labeling, however further studies concerning their specificity and sensitivity are required.

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Non-clinical pharmacokinetics, distribution and excretion of SNS-314, a novel, selective aurora kinase inhibitor

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Background: The Aurora kinase family is comprised of three proteins, Auroras A, B and C that function as key regulators of cell progression through mitosis and cytokinesis and may be important targets in anti-cancer therapy. SNS-314 is a novel small molecule that potently inhibits all three Aurora proteins in the low-nanomolar range. SNS-314 has robust anti-tumor activity in a wide range of human xenograft tumor models in mice using an intermittent dosing schedule. SNS-314 currently is being investigated in a Phase 1 trial to evaluate its safety and pharmacokinetic properties in humans.
Methods: Pharmacokinetic studies were conducted in mice, rats and dogs dosed with SNS-314 or [14C]SNS-314. Blood, tissue, bile, and urine were collected between 0−48 hours and analyzed via LC/MS/MS. Pharmacokinetic parameters were estimated using WinNonLin. Quantitative whole body autoradiography was used to measure tissue distribution in rats.
Results: Pharmacokinetic studies were conducted in mice, rats and dogs after single and repeated administration. In rising dose pharmacokinetic studies, SNS-314 displays non-linear systemic exposure; the area under the concentration curve increases more than dose linearly. This is most pronounced in rats and mice and occurs to a lesser extent in dogs. Sex-related differences in pharmacokinetic parameters are observed in rodents and to a much lesser extent in dogs. Female rats had 1.3 to 2 fold greater plasma AUC than male rats. SNS-314 is rapidly and extensively distributed in both mice and rats when dosed IV, IP, or PO. Administration at 170 mg/kg to tumor bearing mice shows drug levels persisting in the tumor for more than 96 hours post-dose (T1/2 = 7.5 hr), even though plasma levels were not measurable beyond 40 hours post-dose (T1/2 = 4.7 hr). Whole-body autoradiography indicates [14C]SNS-314 related radioactivity is widely distributed in tissues after an IV bolus dose with maximum concentrations observed 1 hour post dose. Approximately 70% of SNS-314 is eliminated through biliary excretion 48 hours post dose.
Conclusion: The favorable pharmacokinetic properties of SNS-314 including elevated tumor over plasma drug levels support clinical investigation of this oncology agent.

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Relationship between expression of CXCR4 and histological type in adenoid cystic carcinoma of the head and neck

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Background: Adenoid cystic carcinoma (ACC) is one of the most common malignant tumors of the salivary glands characterized by multiple recurrences and distant metastasis resulting in significantly worsening prognosis. CXCR4/CXCL12, a representative chemokine receptor and its ligand, has been reported to be involved in cancer metastasis, especially in breast cancer metastasis. In order to investigate the high invasive and metastatic potentials of ACC, CXCR4 expression in ACC was examined, and analyzed the relation to clinicopathological features and histological type.
Methods: We analyzed immunohistochemical expression of CXCR4 surgical specimen of ACC. We also used two established human tumor lines, ACCY and ACCI, in nude mice derived from ACC of the oral floor. The expression levels of protein and mRNA of CXCR4 in these tumor lines were examined by western blot and RT-PCR.
Results: Patients expressed CXCR4 at high levels showed lung metastasis, regional lymph nodes metastases, and poor prognosis. The solid type and cribriform type with distant metastases showed intense CXCR4 staining, while tubular type and cribriform type with no metastasis were weakly positive. In vivo model, ACCY tumor showed an increased growth rate as the passage levels proceeded, and the histological feature has been changed from a cribriform pattern to a solid one. CXCR4 was highly expressed in 15th passage level than in initial level of ACCY. ACCI tumor in nude mice developed spontaneous metastasis to the neck, and the histological feature changed from a cribriform pattern of ACC to undifferentiated carcinoma. This metastatic tumor (ACCIM) caused spontaneous metastasis to the lung at high incidence when transplanted subcutaneously in nude mice. Expressions of CXCR4 in ACCIM were higher than ACCI, and lung metastatic area was strongly positive immunohistochemically. Both ACCI and ACCIM had high levels of mRNA for human CXCR4 by RT-PCR.
Conclusions: Our results indicate that there is a close relationship between CXCR4 and histological type of ACC, and CXCR4 may play important roles in the process of metastasis and biological behavior of ACC.

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Association of miR-21, miR-31, miR-143, miR-145 and let-7a-1 levels with histopathologic features of colorectal cancer

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Background: MicroRNAs (miRNAs) are endogenously expressed short non-coding RNAs, that repress protein translation through binding to target mRNAs. Although the number of verified human miRNA is still expanding, only few have been functionally described. However, emerging evidences suggest the involvement of altered regulation of miRNA in pathogenesis of cancers and these genes are thought to function as both tumours suppressor and oncogenes. Previous studies, mainly based on microarrays technology applied on colorectal cancer cell lines, showed altered expression levels of several miRNAs in colorectal cancer (CRC).
Materials and Methods: In our study, we examined by Real-Time PCR expression levels of miR-21, miR-31, miR-143, miR-145 and let-7a-1 in bioptic samples of 29 colorectal cancer patients including 3 cases of IUCC Stage I, 11 of Stage II, 6 of Stage III, 9 of Stage IV. For 6 cases of CRC samples also adjacent non-tumor tissue was analyzed. MiRNAs expression levels were correlated with tumor stage, grade, size, anatomical localization, serum CEA levels and p53 protein expression in tumors. For data normalization we tried different approaches (18S rRNA, GAPDH, let-7a-1). Finally, variability of let-7a-1 expression was shown to be the lowest. P values were calculated using Mann-Whitney U test.
Results: Expression levels of all analyzed miRNAs significantly differ in tumor and normal mucosa, miR-21 ($p = 0.0001$) and miR-31 ($p = 0.0006$) were up-regulated and miR-143 ($p = 0.013$) and miR-145 ($p = 0.018$) were down-regulated in tumors. MiR-21 was also correlated with CRC stage. Although the highest levels of miR-143 and miR-145 were in normal mucosa, we identified positive correlation of tumor stage and their expression suggesting altered tumor suppressor function of these miRNAs in early events of colorectal carcinogenesis. Distal CRC showed significant

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).

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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).

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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