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

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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.

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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