agents interfering with thymosin β4 (either its up-regulation or actin sequestering function) for the treatment of thymosin β4-overexpressing tumors with high invasive and metastatic potential.

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Erythropoietin and steroid membrane initiated actions interact in breast cancer cells leading to enhanced cell survival

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Background: Erythropoietin (EPO) is a hormone primarily involved in erythropoiesis but dotted equally with an array of autocrine/paracrine effects. EPO can regulate major cell functions of normal and cancer cell types including breast cancer, a steroid hormone dependent neoplasm. Steroid effects can be nuclear- and/or membrane-initiated, with the latter depending, among others, to a cross-linking with various growth factor receptors. Previously, in breast cancer specimens we have reported a correlation of erythropoietin, its receptor (EPOR) and membrane androgen sites. In the present work we further explore this interaction and the possible mechanism involved. Material and Methods: We assayed the effect of serum deprivation- and testosterone-BSA-induced apoptosis and cell migration in the presence of erythropoietin and explored the signaling pathways involved. Results: Testosterone-BSA-induced apoptosis and decreased cell migration was reversed by erythropoietin in a dose- and time- related manner. Moreover, the anti-apoptotic effect of EPO was potentiated by the addition of testosterone-BSA indicating an interaction between the two systems. This interaction is not at the membrane-receptor level but is the result of the modulation of specific signaling pathways (switching of p38 and Jnk from pro- to anti-apoptosis and from STAT to Akt and β-catenin signaling), and the enhanced transcription of EPOR by testosterone-BSA. Conclusions: Erythropoietin could be integrated to the ensemble of growth factors that cross-link with membrane steroid receptors, amending tumor cell survival. Their importance to patients' prognosis and selection of the appropriate therapeutic regimen should be considered.

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Src activity is increased in liposarcomas and in gastrointestinal stromal tumors—analysis of associations with clinical and molecular tumor characteristics

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Increased activity of the non-receptor protein tyrosine kinase Src can be found in a variety of human cancers in vitro and in vivo. Several studies have shown that elevated Src activity is associated with an increase in tumor malignancy, as well as poor clinical prognosis. The present study was done to determine whether Src activity is also increased in soft tissue sarcomas such as gastrointestinal stromal tumors (GISTs), and liposarcomas, and if Src activity in these tumors correlates with established tumor characteristics, other molecular determinants, or clinical prognosis. Tumors and normal tissues from 29 patients with GIST and from 17 patients with liposarcoma were analyzed for Src activity by immune complex kinase assays. There was a positive correlation for Src autophosphorylation and phosphorylation of MBP, reflecting the ability of Src to activate an external substrate in GISTs (r=0,751; p<0,001) and liposarcomas (r=0,912; p<0,001). Src activity was significantly higher in tumors than in normal tissues within the 16 GIST patients excluding imatinib responders (p=0,017), and in liposarcoma patients (p=0,033). There was a trend for increased Src activity in GISTs to correlate with positive PDGF-R (p=0,066). Elevated specific Src activity was observed as a trend in tumors with high risk of malignant behaviour according to Fletcher (p=0,07), and in those with positive CD117 (p=0,099). Five GIST patients with recurrence and recent surgery were also analyzed for Src activity and, as a statistical trend, Src activity was now lower than in the primary (p=0,08). Furthermore, specific Src activity was significantly lower in GISTs containing spindle cells (p=0,01) than in epitheloid tumors, or tumors containing both cell types. No significant association with clinical prognosis was observed in this series so far, however, this may be due to the duration of follow-up and will be re-analyzed in the future. This study demonstrates that Src activity is significantly increased in GISTs and liposarcomas as compared to normal tissues, and in trend is associated with CD117, PDGF-R, and the score for malignant risk of GIST.

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Integrin-Rab21-Rasa1 complex regulates integrin traffic in migrating cells

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During malignancy progression, tumour cells acquire the ability to break the basement membrane and invade underlying tissue, a process called metastasis. The seeding of tumour colonies to different sites in the body requires the activity of integrin cell surface receptors that anchor cells to the surrounding extracellular matrix (ECM). The regulation of cellular migration and adhesion is thereby dependent on the continuous turn-over of integrins that need to be internalized at retracting edges and transported to new adhesion sites of the cell.

We discovered that the small GTPase Rab21 critically regulates the endocytic traffic of integrins (Pellinen et al., J. Cell Biol., 2006). Our aim is now to further elucidate the GAPs (GTPase-activating proteins) and GEFs (GDP/GTP-exchange factors) that are decisive for the control of Rab21-activity. Our recent findings that Rasa1 (GAP) regulates integrin-internalisation and migration of breast cancer cells suggests that Rasa1 is a crucial regulator for Rab21-controlled integrin traffic and has therewith an impact on the motility of transformed breast epithelial cells during metastasis.

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Knockdown of oncogenic microRNA-21 displays cytotoxicity in p53 wild-type colon cancer cells

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Although the number of verified human microRNAs (miRNAs) 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 data suggest altered regulation of microRNA-21 (miR-21) expression in CRC. In our study, we examined by Real-Time PCR expression levels of microRNA-21 (miR-21) in 60 colorectal tumors and 40 paired adjacent non-tumor tissues and correlated them to selected clinicopathologic features and survival parameters. We used expression of U6 small nuclear RNA (RNU6B) for data normalization and standard ddCt method for relative quantification of miRNA expression. Levels of miR-21 were significantly higher in tumors comparing to normal mucosa ($p < 0,0001$, Wilcoxon matched-pairs test). High expression levels of miR-21 in tumors (based on high tertile) were associated also with a poor survival (long-rank p=0,043). Up-regulation of miR-21 was previously associated with high potential of invasion, intravasation and metastasis in pre-clinical colorectal cancer models. Till now no data exist focused on miR-21 effects on CRC cells proliferation. To elucidate potential involvement of miR-21 in regulation of colon cancer cells (DLD1, SW837, HCT116 wt-p53, HCT116 null-p53) proliferation we tested effects of synthetic 2´OMe-antisense-miR-21 (anti-miR-21) transfection (2´OMe-EGFP as control) on their growth by use of MTT test. Proliferation was not affected in a null-p53 cell line or cell lines expressing mutated p53 (DLD1, SW837). In a wild-type p53-expressing cell line we observed more than 20% decrease of cells proliferation by MTT test after transfection of anti-miR-21. Now we are testing attenuating effect of anti-miR-21 on CRC cells survival under conditions of p53-directed apoptosis induced by doxorubicin treatment. Simultaneously, we are evaluating also changes in invasive properties of anti-miR-21 transfected cancer cells by matrigel invasion assay. Our results suggest possible role of miR-21 in colorectal cancer pathogenesis. Supported by IGA MZ CR NR/9076 – 4 and project MZ0MOU2005

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Differential expression of annexin A1 modulates invasion of melanoma B16 cells

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Identification of proteins involved in melanoma dissemination should complete the knowledge of physiopathology and potentially the prognosis for patients with a primary tumour. We used the B16 mouse melanoma