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The anaplastic lymphoma kinase receptor inhibits the apoptotic effect of the dependence receptor unc5

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Background: The Anaplastic Lymphoma Kinase (ALK) receptor is highly over-expressed and activated in several tumors protecting the cells from apoptotic cell death and promoting cell proliferation. The UNC5 receptors promote cell migration and axonal outgrowth when bound to their ligand Netrin-1 but induce apoptosis without ligand, therefore acting as dependence receptors. Recently it was shown that they also play an important role in tumorigenesis. Our study is aimed at determining the possible interaction of ALK, UNC5 and Netrin-1 and its effect on apoptosis and downstream signaling.
Methods: 293T cells over-expressing ALK/UNC5/Netrin-1 were analyzed for protein interaction and downstream signaling by Immunoprecipitation, Western Blotting or Dual Luciferase Assay, and for apoptosis by Annexin V staining.
Results: Interaction of the transmembrane receptors ALK and UNC5 depends on their extracellular domains and is blocked by a single chain anti-ALK antibody but not by Netrin-1. Apoptosis caused by UNC5 can be inhibited by ALK, similar to the protective effect of Netrin-1 on UNC5. Interaction of ALK and UNC5 led to decreased phosphorylation of Mitogen Activated Protein Kinase (MAPK) compared to ALK alone. Stimulation by additional Netrin-1 further decreased MAPK phosphorylation.
Conclusion: Our data provide evidence for an anti-apoptotic effect of the interaction of ALK and UNC5 accompanied by a decrease in MAPK phosphorylation. This makes the ALK receptor an ideal target for cancer treatment, by inhibiting its anti-apoptotic effect and simultaneously utilizing the dependence receptor UNC5 to induce apoptosis in tumor cells.

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The neuropilins: critical survival factors for non-small cell lung cancer cells

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Background: In this study, we examined the role of VEGF in non-small cell lung cancer (NSCLC) cells and the epigenetic mechanisms regulating expression of its receptors, in particular the Neuropilin receptors.
Methods: A549 and SKMES1 NSCLC cells were screened for VEGF receptors. The effect of VEGF on cell survival was examined using the BrdU assay. Cell cycle analysis was carried out following neutralisation of VEGF. Phosphorylation of Akt/PKB and Erk1/2 by VEGF was examined by confocal microscopy. VEGF receptor expression in response to the histone deacetylase inhibitor, TSA (Trichostatin A), was assessed, in addition to its effect on proliferation and apoptosis. VEGF levels were measured by ELISA in conditioned media of cells treated with TSA. The association between histone proteins and DNA was studied by ChIP analysis. The effect of TSA on acetylation of H3 and H4 histones was examined by Western blotting while the cell survival effects of VEGF following NP1 and NP2 gene silencing (siRNA) were determined using the MTT assay.
Results: VEGF increased proliferation of NP1- and NP2-expressing NSCLC cells. Neutralisation of VEGF induced growth arrest in the G0/G1 phase of the cell cycle. VEGF induced phosphorylation of Akt and Erk1/2. TSA upregulated the expression of VEGFR1 and VEGFR2 and downregulated NP1 and NP2. VEGF was unable to rescue cells from TSA-induced cell death. VEGF secretion by NSCLC cells was decreased in response to TSA. *De novo* protein synthesis was required for downregulation of the Neuropilin receptors by TSA but not for VEGFR1 and VEGFR2. TSA increased acetylation of H3 and H4 histones in both cell lines. Silencing of NP1 and NP2 receptors reduced survival of A549 and SKMES1 cells. VEGF was unable to rescue siNP1- and siNP2-treated NSCLC cells.
Conclusions: This study confirms that VEGF is a growth factor for NSCLC acting, at least in part, through NP1 and NP2, and implicates the Neuropilin receptors as critical survival factors for NSCLC cells.

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Analysis of Nanog gene in human gastrointestinal cancer

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Homeobox protein Nanog plays key roles in self-renewal and pluripotency in Embryonic stem (ES) cells. While Nanog expression was thought to be absent in somatic cells, recent reports suggested Nanog overexpression in several human tumor cells. We also found overexpression of Nanog gene in side population in human gastrointestinal (GI) tumor cell lines. However, expression of Nanog in human GI tumor tissue and functional role of Nanog in these cells still remains unknown. In order to clarify functional expression of Nanog, we investigated human GI cancer tissues and detected significant expression on Nanog by RT-PCR, western blot analysis and immunohistochemical staining. Nucleotide sequencing revealed that GI cancer cells we examined expressed Nanog pseudogene 8 (Nanog P8 gene). Overexpression and knockdown analysis of Nanog P8 in gastric cancer cell line, AZ521, and colon cancer cell line, SW480, exhibited cell proliferative activity of Nanog P8 in vitro. These data suggested that Nanog P8 might have a functional role in proliferation of human GI cancer cells. We are currently investigating tumorigenesis of Nanog P8-overexpressed cells in mice tumor xenograft model.

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Functional role of S100A4 in tumour stroma interaction

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Communication between tumor cells and host components, such as immune cells, fibroblasts and endothelial cells, contribute to the progression of cancer from its initial growth at a primary site in the body to its metastasis to distant organs. S100A4, one of many genes involved in stimulation of metastatic spread of tumor cells exerts its function as a stroma-cell derived factor. The exact mechanism of its metastasis-stimulating function remains poorly understood.
In the present study we investigated the effect of S100A4 genetic depletion in Polyoma Middle T oncoprotein (PyMT)- induced metastatic mammary tumors. Massive leukocyte infiltration is closely associated with the malignant transition in the PyMT tumors (adenoma/MIN). This is associated with increase in the concentration of extracellular S100A4 detected in the tumor microenvironment. In contrast, in PyMT S100A4(−/−) tumors, we observed substantial suppression in leukocyte infiltration in the transition period. S100A4 deficiency lead to significant decrease in particular in numbers of T-lymphocytes that invade developing tumor. A chemotaxis assay revealed that purified T lymphocytes migrate in response to S100A4. Invasion of T lymphocytes into the S100A4 positive fibroblast monolayer is greatly enhanced compared to the S100A4(−/−) ones. Both processes are blocked by anti-S100A4 antibodies. Moreover, co-injection of tumor cells with S100A4(+/+) but not the S100A4(−/−) fibroblasts in S100A4(−/−) mice, attracts significantly more T-lymphocytes to the site of growing tumor. This is also accompanied by increase in the amount of S100A4 released into the tumor microenvironment.
Engagement of high concentration of S100A4 in tumor microenvironment may cause T-cells to migrate to tumor sites and probably take part in stimulation of tumor vascularization and metastasis.

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Invasive breast tumour cells induce up regulation of tumour endothelial marker 8 (TEM8) in monocytes

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Background: Tumor cell migration and metastasis share many similarity with leukocyte trafficking which is critically regulated by chemokines/chemokine receptors interactions in a co-ordinate fashion with cell-surface adhesion molecules. We previously reported that Tumour Endothelial Marker-8 (TEM-8), a putative adhesion molecule though to be involved in extra cellular matrix-remodeling and migration processes, is selectively over -expressed in highly invasive vs. non-invasive breast cancer cell lines (e.g. MDA-MB231 vs. ZR-75 respectively). Here we investigated the effect of soluble factors released fom cycling cancer cells on TEM8 expression in immature myeloid monocytes.