Tamoxifen resistance and tamoxifen agonistic effect were induced mainly via ERK pathway activation, while the PI3K pathway is linked to cell proliferation.
Conclusions: Our data provided the first evidence for the causal role of IGF-1R signaling in acquired anti-estrogen resistance and agonistic action of tamoxifen in human breast cancer cells.

540 Post-translational mechanisms involved in downregulation of the gap junction protein Connexin43 in colorectal cancer

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Background: The purpose of this study was to elucidate the molecular mechanisms involved in down-regulation of the gap junction protein Connexin43 in colorectal carcinogenesis.
Materials and Methods: The expression level, localization and phosphorylation status of Connexin43 was analyzed in normal colon tissue and colorectal tumours, as well as in colorectal cancer cell lines. As a model system for studying the post-translational mechanisms involved in regulation of Connexin43, the IAR20 rat liver cell line was used. These cells express Cx43 endogenously and form functional gap junctions. The cells were exposed to the tumour-promoting phorbol ester 12-*O*-tetradecanoylphorbol 13-acetate (TPA), which induces endocytosis and degradation of Connexin43. Candidate proteins involved in Connexin43 endocytosis and degradation were depleted by small interfering RNA (siRNA). Connexin43 localization was analyzed by confocal microscopy. The Connexin43 protein level and ubiquitination status were analyzed by western blotting and immunoprecipitation.
Results: Connexin43 was expressed in the plasma membrane in normal colon tissue, while in colorectal tumours Connexin43 was found to localize in intracellular compartments. Among 19 colorectal cell lines examined, 7 were found to express Connexin43 protein. None of the cell lines were able to form functional gap junctions, and Connexin43 was localized intracellularly, indicative of enhanced gap junction endocytosis and degradation. To elucidate the molecular mechanism involved in aberrant trafficking of Connexin43, IAR20 cells were used as a model system. Connexin43 organized in gap junction plaques was found to undergo ubiquitination in response to TPA treatment. Depletion of the ubiquitin-binding proteins Hrs or Tsg101 by siRNA counteracted trafficking of Connexin43 from early endosomes to lysosomes. Under these conditions, Connexin43 was able to undergo dephosphorylation and deubiquitination, locate to the plasma membrane, and form functional gap junctions.
Conclusions: Colorectal cancer cells are unable to form functional gap junctions, and express Connexin43 in intracellular compartments, indicative of aberrant endocytic trafficking. Using the IAR20 cell line as a model system, the TPA-induced endocytosis and degradation of Connexin43 organized in gap junctions was found to involve ubiquitination and to be mediated by the ubiquitin-binding proteins Hrs and Tsg101.

541 Huntingtin interacting protein 1 (HIP1) induces Epithelial-to-Mesenchymal Transition (EMT) in prostate cancer cells

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Background: HIP1, an adaptor protein classically involved in clathrin mediated endocytosis affecting cell signalling, is overexpressed in prostate cancer and drives fibroblast and prostate epithelial cell transformation by perturbing growth factor receptor signalling. It has also been shown to translocate to the nucleus and have a role as an androgen receptor coactivator.
Methods: To explore its role in prostate cancer we used HIP1 overexpressing ($HIP1^+$) prostate cell lines in soft agar colony formation, invasion, migration, and adhesion assays, gene expression arrays, and real-time PCR.
Results: $HIP1^+$ epithelial PNT1a cell lines showed cell transformation. $HIP1^+$ PNT1a and $HIP1^+$ LNCaP showed significantly increased anchorage independent cell growth. $HIP1^+$ LNCaP also showed significantly increased cell adhesion to ECM protein fibronectin, implicated in cancer growth/survival and drug resistance. Epithelial-to-mesenchymal transition (EMT) is associated with increased propensity for cell migration, invasion, and metastasis. Here, we show a >2 fold upregulation of Wnt7b, Snail, and vimentin in $HIP1^+$ cell lines, which have been implicated in EMT. Gene expression arrays showed enrichment of pathways involved in cell-cell signalling, cell movement and metabolic pathways in these $HIP1^+$ cell lines. Furthermore, $HIP1^+$ PNT1a cell lines showed a resistance to paclitaxel treatment in soft agar colony formation assays.
Conclusion: HIP1 may contribute to prostate cancer progression by altering cell-cell interaction, migration, and invasion through the induction of an EMT-like phenotype.
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542 Tumour stem cells in oncogenic RAS-dependent rhabdomyosarcoma

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Tumour heterogeneity reflects the hierarchical organization of normal tissues. Many tumours have now been shown to retain cells with stem cell activity, also referred to as tumour stem cells (TSC). Our aim was to indentify TSC in rhabdomyosarcoma (RMS), childhood tumours displaying skeletal muscle differentiation. Recently, we found activating RAS mutations in nearly half of embryonal RMS cases, indicating that RAS mutations appear early and are relevant for RMS development. We showed that the putative tumour suppressor sprouty1 becomes essential for the maintenance and survival of RMS harboring oncogenic RAS. Following shRNA-mediated inactivation of sprouty1 RMS cells became apoptotic *in vitro*, while fully established RMS-grafts completely regressed upon sprouty1 silencing. Although these data show novel therapeutic promise for RMS, the clinical value of this finding depends largely on the efficacy against different tumour subpopulations.
RMS has been suggested to originate from muscle satellite cells. Therefore, we decided to express oncogenic RAS in normal muscle progenitors and determine if cells with stem cell activity were maintained after transformation. This approach resulted in the generation of transformed myoblasts that upon orthotopic transplantation generate tumours resembling embryonal RMS. Normal and transformed mouse myoblasts were first screened for the expression of (tumour) stem cell markers (including c-kit, CD24, CD44 and CD133) as well as for satellite cell markers (b-integrin and CXCR4). By using a combination of these markers, designated as SIG, we could discriminate between stem cell populations and their differentiated progeny in both normal and malignant muscle.
In normal myoblast cultures the cells with myogenic activity were contained in the SIG-positive population. SIG-positive-sorted myoblasts restored the original culture after replating. And again cells with myogenic activity were predominantly found within the SIG-positive population of these 'restored' cultures, suggesting that these cells selfrenew. In RAS-transformed myoblasts only the SIG-positive population generated colonies in soft agar, generated heterogeneous tumours and self-renewed *in vivo* as demonstrated by serial passaging in NOD/SCID mice. We conclude that RAS-driven RMS contains a population of highly malignant selfrenewing cells with an immunophenotype resembling stem cells in normal muscle. The identification of TSC is essential for development of stem cell-targeted therapies for the treatment of RMS.

543 RNA Polymerase III transcription deregulation in cancer: study of Brf1 expression in prostate cancer

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Background: Aberrant RNA Polymerase III (Pol III) transcription has been linked to increased cellular proliferation and progression to cancer. The importance of elevated Pol III transcripts and its associated transcription factor Brf1 in cancer has been demonstrated in cell culture and in mice. However, the significance of this deregulation has yet to be determined in patients. This study examines Brf1 expression in prostate cancer (PCa) tissues in comparison to benign prostate hyperplasia (BPH) to determine if elevated levels of Brf1 protein are detected in tumour samples and if there is any correlation with severity of disease.
Materials and Methods: Immunohistochemical staining was performed on Tissue Micro Arrays (TMAs) containing 149 cases of PCa and 21 BPH samples and the Brf1 expression was evaluated using the weighted Histoscore method. The proliferation status of these samples was assessed using Ki-67 staining.
Results: Brf1 expression was detected as heterogenous staining and localised predominantly in the nucleus. Brf1 expression is elevated in PCa compared to BPH ($p < 0.001$), however there is no association between Brf1 expression with increasing Gleason Grade ($p = 0.545$) and no significant correlation with Ki-67 expression was observed.
Conclusions: Assessment of Brf1 levels in prostate cancer tissues revealed higher expression compared to benign hyperplasia and suggests that it may be a potential biomarker for cancer therapy. However a larger patient cohort that includes both hormone sensitive and hormone resistant prostate cancer patients will be of further value to determine Brf1 significance in the clinical settings.