Materials and Methods: We used the new automated two-dimensional chromatography system *PF2D* to fractionate the proteome of tumour tissues and tested protein fractions for recognition by pre-existing tumour-specific CD4+ T-helper cells and cytotoxic T-cells.
Results: Applying this method to the Ovalbumin (OVA)-specific, TCRtg OT-I mouse model demonstrate efficient separation, processing and cross-presentation to CD8+ T-cells by dendritic cells of OVA expressed by the OVA-transfected mouse lymphoma RMA-OVA. Applying this method to human tumour tissues we identified in patients with head and neck cancer MUC-1 and EGFR as tumour-associated antigens selectively recognized by patients' T-cells. Finally, we detected on an exemplary patient with a malignant brain tumour CD4 and CD8 T-cell responses against two novel antigens, transthyretin and calgranulin B/S100A9, which were expressed on tumour and endothelial cells. Immunogenicity of these antigens could be confirmed in 4 out of 10 other brain tumour patients.
Conclusions: This fast and cheap method appears suitable to identify candidate T-cell antigens in various disease situations, such as autoimmune and malignant diseases without restriction to their expression by a certain cell type or HLA allele.

293 Cyclooxygenase 2-driven inflammation in pancreatic cancer

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Prostanoids perform important tasks in normal and diseased tissues by acting as local signals which coordinate the concerted activities of different cell types. In our work, we substantially focus on the role related to inflammation and cancer. Chronically inflamed pancreas, urinary bladder, and colon represent high-risk environments for tumourigenesis.
Epidemiological, pharmacologic, clinical, and genetic studies show the cause/effect relationship between over-expression of pro-inflammatory cyclooxygenase (COX)-2, COX-2-mediated prostaglandin (PG) signaling and the development of epithelial cancers. Depending on the cellular context, PG stimulate growth, angiogenesis, and modulate immune functions in inflammation-driven cancers.
Keratin 5 promoter-driven COX-2 (K5 COX-2) transgenic mouse lines established in our group develop pre-invasive neoplasms in various epithelial tissues including pancreas. Pancreata of the K5 COX-2 mice develop ductal neoplasms which resemble, on molecular and morphological levels, human precursor lesions of pancreatic ductal adenocarcinoma (PDAC), i.e. cystic intra-ductal papillary mucinous neoplasms (IPMN) and pancreatic intra-epithelial neoplasias (PanIN) (Gastroenterology 130, 2006).
These phenotypic changes are associated with pronounced inflammatory infiltrates in pancreas, thus representing a putative high-risk environment for tumourigenesis. Cytokine gene expression analysis depicts the presence of TH-1 (elevated IFN-gamma, TBX21), TH-17 (elevated Il-6, Il-17A), and T-regulatory (elevated FOXP-3) cells. At the cellular level, diffuse inflammatory infiltrates are observed, besides prominent inflammatory clusters, in the diseased pancreata of K5 COX-2 mice. These are comprised of B-cells, T-cells, follicular dendritic cells, macrophages, and high endothelial venules decorated with lymphocyte adhesion molecules. In addition, a panel of TLO-relevant chemokines is expressed. Altogether are known to be hallmarks of ectopic tertiary lymphoid organs (TLO) which arise in chronic inflammatory diseases but with a yet un-known function. Such an inflammatory phenotype is suppressed by inhibition of COX-2 activity whereby celebrex-fed transgenics exhibit fewer and smaller clusters, indicating the involvement of COX-2/PG signaling in the establishment of TLO; a putative novel COX-2-effect on local immunity. Follicular clusters rich in B-cells and T-cells, with vessels that express peripheral lymph node addressin (PNAd) are also observed in COX-2-positive human PDAC. This hypothesizes that the induction of TLO might be relevant in humans as well.

294 Successful engraftment of glioblastoma biopsy spheroids in immunocompetent rats

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Standardized implantation of biopsy spheroids generated from GBM patients into athymic nude rats has now been well established and characterized, with tumour takes close to 100%. The resulting lesions resemble human glioblastomas in their presentation, containing invasive single cells, vascular proliferations, thrombotic vessels, and necroses. Given that the brain is a site of immune privilege, and human glioblastomas evade immune surveillance, we evaluated whether human GBM spheroids would survive in fully immunocompetent rats and kill their hosts. We compared the engraftment rates of spheroids generated directly from patients or prepared after several generations of passaging in nude rats. Spheroids originally derived from four GBM patients were implanted, followed up by weekly MRI, and engraftment rates and survival data were collected.
Xenografts that were generated directly from patient biopsy tissue appeared on MRI scans only in two cases and were thereafter rejected. In contrast, xenograft tumours based on glioblastoma tissue that has been previously passaged in nude rats displayed engraftment rates of over 50% in immunocompetent rats, and once appearing on MRI scans, the lesions invariably killed their hosts. The survival time lengths for immunocompetent animals were similar to those for nude rats when implanted with the same biopsy spheroids. We sought to further characterize the mechanisms that permitted the development of human tumours in immunocompetent rats, such as the nature of immune-inflammatory host cells present and the production of immunomodulatory cytokines by the tumour and the host. The current data suggests that the elevated numbers of both CD4+ and CD8+ lymphocytes together with higher serum levels of rodent IL-1a, IL-2 and IL18 were significantly correlated with tumour xenograft rejection in immunocompetent rats. Furthermore, survival of the xenografts was associated with the inability of activated lymphocytes to penetrate the tumour bed.
In conclusion, we established that passaging of human GBM biopsy spheroids in nude rats facilitates more efficient engraftment in immunocompetent rats.

295 Cyclooxygenase-2 (COX2) gene silencing with siRNA could enhance DNA vaccination to inhibit established ErbB-2 carcinomas

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Background: Cyclooxygenase-2 (COX2) is the key enzyme in the synthesis of prostaglandin E2 from its precursor, arachidonic acid. The level of COX2 is normally very low in cells but increased amounts of COX2 are commonly detected in both premalignant and malignant tissues. In order to investigate COX2 as a potential target for the prevention and treatment of cancer, we propose a novel immunotherapeutic approach for the prevention of ErbB-2 positive breast carcinomas, based on DNA vaccination against a specific antigen (ErbB-2) in combination with the silencing of COX2.
Material and Methods: The short hairpin RNA interference cassette targeting COX2 mRNA, containing the mouse RNA promoter U6, was amplified and cloned into the Eco72I site of pVAX-ratECTM (RRT), a vector carrying the extracellular and transmembrane domains of rat ErbB-2, and into pVAXI, to obtain respectively RRT-COX2 and pVAX-COX2. The gene silencing efficacy of both plasmids was checked by transfecting COX2 over-expressing A17 cells. The plasmids were used for electroporation-based vaccination of female BALB-neuT mice of different ages, corresponding to various stages of cancer progression, from atypical hyperplasia to invasive lobular carcinoma. We are evaluating mammary cancer progression in vivo, the titer of anti-ErbB-2 antibodies in the immune sera, and the ability of spleen cells to release IFNg in response to the $H2^d$ immune dominant peptide of ErbB-2.
Results: Vaccination of 10 week-old female BALB-neuT mice with RRT plasmid significantly triggers a protective immune response toward the development of autochthonous mammary cancer in BALB-neuT mice (Quaglino et al. 2004, Cans Res). 30% of treated mice were still fully free from palpable tumours one year after vaccination, when all control animals had already died because of mammary cancer. Till now (week 46 of age) we see a similar protection using RRT-COX2. The level of anti-ErbB-2 antibodies in the sera from RRT-COX2 vaccinated mice is slightly higher than that of RRT vaccinated mice, but not significantly different. We are now collecting spleen cells from vaccinated mice to evaluate the specific CD8 response against ErbB-2. Experiments with mice vaccinated when they already have lobular carcinomas are ongoing.
Conclusions: COX2 suppression induced by shRNA might help to overcome tumour-mediated immunosuppression and generate an effective anti-tumour immunity not only in prophylactic but also in therapeutic vaccination.

296 Macrophage migration in tolerance

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Tumour Associated Macrophages (TAM) and lipopolysaccharide- (LPS)-tolerant macrophages share several characteristics, such as a massive accumulation of the p50NF-kB homodimer in the nucleus and the incapacity to express strong inflammatory programs (eg. impaired TNFa production) in response to inflammatory signals such as LPS (tolerance). Our recent study has described that both TAM and LPS-tolerant macrophages express