inhibits monocyte chemotactic protein-1 (MCP-1) in the human mast cell line HMC-1, decreasing its abiliy of monocyte recruitment, but the effects of EGCG directly on monocytes has not yet been explored. This work shows that EGCG decreases monocyte migration ability in response to MCP-1 and inhibits MCP-1 secretion and CCR2 expression, the specific receptor for MCP-1, using the human monocyte cell line THP-1. Moreover, EGCG has been described to inhibit the expression of some integrins. Our work demonstrates that EGCG decreases the levels of integrin beta1 activated, one of the primary integrins that can assembly monocytes to extracellular matrix under normal conditions, and THP-1 adhesion to fibronectin. We conclude that this study support the effects of EGCG as an anti-inflammatory compound.

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High excretion of etheno adducts in liver fluke-infected patients: protection by praziquantel against DNA damage

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Background: Chronic infection by liver fluke (Opisthorchis viverrini, OV), is a strong risk factor for developing cholangiocarcinoma (CCA). To clarify the involvement of oxidative stress and lipid peroxidation (LPO)–derived DNA damage, the excretion of LPO-derived etheno DNA adducts was measured in urine samples collected from healthy volunteers and OV-infected Thai subjects.

Materials and Methods: The study was performed in healthy volunteers (n=20, 9 males and 11 females) and OV-infected subjects (n=50, 26 males and 24 females). Urinary 1,N^6-etheno-2'-deoxyadenosine (εdA)- and 3,N^4-etheno-2'-deoxycytidine (εdC)-levels were quantified by immunoprecipitation-HPLC-fluorescence detection and ^{32}P-postlabeling thin-layer chromatography. Urinary malondialdehyde (MDA) was measured by the thiobarbituric acid-based method. Urinary nitrate/nitrite was measured by a simple Griess-based method. Plasma alkaline phosphatase (ALP) activity, a marker of hepatobiliary tract damage, was analyzed by a standard automated spectrophotometer using a commercial kit.

Results: Excreted etheno adduct levels were related to indicators of inflammatory conditions, MDA-, nitrate/nitrite-levels in urine and plasma ALP activity. Mean εdA- and εdC-levels were 3-4 times higher in urine of OV-infected patients; MDA, nitrate/nitrite and ALP were also increased up to 2-fold. MDA and ALP were positively related to εdA excretion. Two months after a single dose of the anti-parasitic drug praziquantel, εdA and εdC concentrations in urine of OV-infected subjects were decreased; MDA, nitrate/nitrite and ALP were concomitantly lowered.

Conclusions: We conclude: chronic OV-infection through oxidative/nitrative stress leads to massive urinary excretion of the etheno-bridged deoxy-ribonucleosides, reflecting high LPO-derived DNA damage in vivo. These promutagenic DNA etheno-adducts in bile duct epithelial cells may increase the risk of OV-infected patients to later develop CCA. Urinary εdA and εdC levels should be explored (i) as non-invasive risk markers for developing opisthorchiasis-related CCA and (ii) as promising biomarkers to assess the efficacy of preventive and therapeutic interventions.

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Tumor associated antigens identify a high risk benign disease group

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Benign breast diseases appear in more than half of all women after 20. Although a history of benign breast disease (BBD) indicates some increase in breast cancer, only a fraction develops malignant disease. The relationship between benign breast diseases and cancer development remains a subject of controversy. The aim of the present report is to detect associated tumor antigens in 80 tissue samples belonging to BBD. Samples were classified in three risk groups depending on proliferation: without or minimal proliferation: no risk benign disease (NRBD); increased proliferation: low risk (LRBD) and atypical epithelial hyperplasia: high risk (HRBD). An immunohistochemical study was performed employing the following antibodies: anti-MUC1 protein core (C595, HMFG2 and SM3 monoclonal antibodies, MAbs), anti-MUC1-cytoplasmic tail (MUC1-CT) polyclonal antibody (Ab) (CT33), anti-MUC4 Ab, anti-MUC2 MAb (PMH1) and anti-carbohydrate associated antigens MAbs: sialyl Lewis x (KM93), Lewis x (KM380), Lewis y (Lewis y) and Tn antigen. Statistical analysis: Frequencies Analysis and Multiple correlation including principal components analysis (PCA) were performed. Results: In NRBD: MUC1 was detected in 62,9% with C595, 27,4% with HMFG2 and 17,2% with SM3. In LRBD, the percentages were: 53,3%, 50% and 31,3%, respectively while in HRBD: 50%, 50% and 16,7%, respectively. MUC1-CT percentages were: 80%, 93,8% and 50%, respectively. Lewis x was the carbohydrate antigen more frequently found in the three groups while sialyl Lewis x were less found (0% in HRBD); on the contrary, Lewis y was more expressed in HRBD than in the other risk groups. MUC2 was also mainly detected in HRBD while MUC4 in LRBD. A statistical significant ($p<0.05$) correlation between anti-MUC1 protein core MAbs and anti MUC1-CT Ab was found ($r=0,7373$). PCA explained 86% of data variability (risk groups and tumor antigenic expression); PCA first two components grouped all HRBD patients while LRBD and NRBD remained spread. Conclusions: 1- HRBD express a determine pattern of tumor associated antigens employed in this study and 2- In BBD, anti-MUC1 CT was the more useful MAb to detect MUC1 showing a high correlation with anti-MUC1 protein core MAbs.

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The immunoproteome of pancreatic cancer

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The purpose of our study is to identify novel tumour antigens by characterising a panel of proteins related to pancreatic cancer. These antigens may be used as tools for early diagnosis, prognosis and some may be good targets for cancer vaccine development.

In developed countries, pancreatic cancer is the fifth leading cause of cancer death. This cancer form is difficult to diagnose even at more advanced stages of the disease with prognosis very poor due to limited treatments offered. Thus, tools for early diagnosis and prognosis as well as new therapeutic agents are essential. Patients diagnosed with pancreatic cancer develop antibody responses against pancreatic tumour related proteins, also called tumour antigens, during the course of the disease. We utilised this and conducted an autologous SEREX analysis involving phage display and automated high-throughput screening of a cDNA tumour library made from a pancreatic cancer patient.

We identified cDNAs encoding 11 different identities. The dominating cDNAs encoded insulin, a hypothetical protein and NADH dehydrogenase (ubiquinone) flavoprotein 1. Other identities were the chromosome 19 open reading frame 60, keratin 19, calcineurin binding protein 1, coiled-coil domain containing 85B, heterogeneous nuclear ribonucleoprotein, TIMP metallopeptidase inhibitor 1, interferon alpha-inducible protein 27 and ADP-ribosylation-like factor 6 interacting protein 4. Furthermore, we found that of 37 pancreatic cancer patients examined 33% had autoantibodies against insulin whereas the percentage was 16% for healthy donors.

The antigens identified from the pancreatic cancer patient reflect a well known relationship between pancreatic cancer and diabetes since some of the antigens are related to the cancer development such as interferon alpha-inducible protein 27 and TIMP metallopeptidase inhibitor 1 and some e.g. insulin are related to diabetes. Due to the broad expression of the majority of the identified antigens they may not be targets for immunotherapy but further evaluation of the antigens will determine their diagnostic value.

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Development of monoclonal antibodies for the identification of novel invasion associated targets in human cancer

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Monoclonal antibodies (MAb's) have emerged as an ever increasingly important tool in cancer therapy. They have yielded promising results when used alone or in combination with current therapies. The development of MAb's also allows for the discovery of novel cancer associated antigens. In this study, monoclonal antibodies were generated by immunising Balb/c mice with the invasive melanoma cell line MDA-MB-435S/F and an invasive variant of the MiaPaCa pancreatic cell line. Following fusion, all resultant hybridomas were screened against their respective target immunogens using newly developed screening systems based on live cell immunofluorescence and a 96 well based invasion assay (Boyden chamber) system. MAb 7B7 has been shown to inhibit invasion (up to 50% of control level), and motility (up to 70% of control level). MAb 9E1 inhibits invasion (up to 70% of control level), but not motility. A dose response inhibitory effect on invasion has also been observed with MAb 7B7.

Identification of the antigen(s) being recognised (and any possible interacting proteins), by both antibodies, is being obtained through immunoprecipitation. Reactive bands will be identified using LCMS/LTQ. siRNA targeting, followed by proliferation and invasion assays, will be carried out in order to observe if any knockdown occurs.

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Tumour-derived high molecular weight M-CSF induces monocyte differentiation into M2- polarized macrophages

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Experimental and clinical evidence has highlighted that tumor-associated macrophages (TAM) represent the principal component of the leukocyte infiltrate and are usually associated with tumour growth, progression and metastasis. Macrophage population is generally divided into two distinct subsets: M1 and M2. M1 macrophages act as a first line of defence against pathogens whereas M2 cells participate in wound repair and maintenance of tissue integrity. In the tumour micro-environment TAM interactions with the extracellular matrix, neighboring cells, and soluble stimuli largely influence their gene expression and behavior.

To investigate the role of the tumor micro-environment on macrophage differentiation, we cultured freshly isolated human monocytes with pancreatic cancer cell line supernatants, in the absence of exogenous cytokine addition. In selected cultures, about 50% of the monocytes differentiated after 5 days into macrophages. The phenotype analysis of tumor-conditioned macrophages (TC-macro) demonstrated high expression of the mannose receptor, CD16, CD68 and low levels of MHC class II. TC-macro produced IL-10, IL-6, TNF but not IL-12, even after LPS stimulation. Moreover, TC-macro produced a panel of chemokines including CCL2, CXCL8, CCL17 and CXCL10. The transcriptional profile of TC-macro revealed that several genes in line with an M2 polarization are highly expressed. The nature of the tumor-derived factors inducing macrophage differentiation is currently under investigation; biochemical analysis indicated that the biological activity is excluded from exosomes and have a high molecular weight (>100.000 KDa). IL-3 and IL-6 were not detectable in tumor supernatants whereas M-CSF was present at low levels. By mass spectrometric techniques, we surprisingly found that the tumor-derived M-CSF had peculiar migration patterns which were different from those expected for the common human homodimeric glycosilated protein, suggesting an interesting structural differences for the tumor-secreted isoforms of this primary regulator of mononuclear phagocyte. The characterization of tumor-derived factors inducing macrophage differentiation could better clarify the intricate cross-talk between tumor cells and macrophages and thus might aid in the process of devising novel anti-tumor treatments.

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Neutralization of TGF-beta led to spontaneous elicitation of antitumor immune responses and elimination of tumors in mice administered of DNA encoding soluble TGF-beta receptor

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Tumor cells produce some cytokines to suppress the host immunity for the purpose of escaping from immunological attack by hosts. Among the immunosuppressive cytokines, TGF-beta is known as a key factor which weakens the host antitumor immunity by blocking activation and differentiation of immune cells or by accumulation of regulatory T cells. Although different kinds of cancer immunotherapy have been done, none of the treatments have reported successful clinical outcomes because of the difficulties in eliciting potent antitumor immune responses in cancer-bearing hosts with suppressed immunity. In this study, we tried to neutralize TGF-beta in tumor-challenged mice by administration of DNA encoding soluble TGF-beta type II receptor. B6 mice that were inoculated subcutaneously with EG.7 tumor cells were injected with plasmid DNA 10 to 12 days after tumor challenge. We monitored the tumor growth and examined for anti-tumor immune responses elicited after DNA administration in the mice. The treated mice acquired both humoral and cellular immune responses against the tumor. The frequency of tumor-specific cytotoxic T lymphocytes was significantly increased after treatment. Challenged tumors were eradicated in about 70% of the treated mice. In conclusion, potent antitumor immune responses can be elicited spontaneously by inhibiting TGF-beta function in cancer-bearing hosts. This strategy is applicable to clinical therapeutics against cancer.

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Implication of novel chemokine receptor CXCR7 in hepatocellular carcinoma

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The orphan chemokine receptor RDC1 was de-orphanized and re-baptized CXCR7 since the recent discovery in 2006 of its two ligands, CXCL11 and CXCL12. Membrane associated CXCR7 is expressed on many tumor cells types, promotes breast and lung tumor in-vivo, and increases invasiveness of prostate cancer cell lines. Hence, we investigated if and how CXCR7 is implicated in human hepatocellular carcinoma. To answer these questions we first studied the transcript expression of CXCR7, and of its two ligands in a cohort of 28 cases of human hepatocellular carcinoma. A significant 5 fold increase of CXCR7 was observed in HCC samples relative to normal liver (n=10), and of its two ligands only CXCL11 was over expressed in HCC. Thereafter, immunohistochemical staining performed for both CXCL11 and CXCR7 on HCC paraffin sections revealed that multiple cell types were positive for CXCR7 and CXCL11. Indeed, HCC cells, but as well hepatocytes in regeneration nodules, and proliferating biliary cells, were positive for CXCR7. CXCL11 showed a much broader tissue expression. Furthermore we investigated if in primary hepatic cells, notably hepatocytes and hepatic stellate cells, either CXCR7 or its ligands could respond to cytokines classically involved in the development of HCC. Our results showed that in isolated primary hepatocytes and hepatic stellate cells stimulated by IFN-g, TGF-b, IL-10 and IL-4, CXCL11 responds to IFN-g but no response was observed for either CXCL12 or CXCR7. Interestingly, quiescent human primary hepatocytes do not express membrane CXCR7. However HepaRG cell line, a human HCC cell line which can either differentiate into hepatocyte-like cells or remain in a proliferating phase, showed a strong up regulation of CXCR7 only during proliferation. When HepaRG cell line is cultivated in 0.1% serum conditions, CXCL12 induces proliferation. All together, our data shows that CXCR7 is over expressed in HCC and that activation of CXCR7 might induce pro-survival signals in malignant hepatic cells.

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Targeting CD4+ CD25+ FOXP3+ Treg cells abrogates established mechanisms of immune tolerance, reshuffles the T cell repertoire and results in effective anti-tumor immunity

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The presence of regulatory mechanisms that down-regulate the immune response to ErbB2 oncogene in the periphery has been recognized in human patients and transgenic mice. BALB-neuT mice genetically predestined to develop multiple, fast-growing, invasive, and metastasizing carcinomas are one of the most aggressive models of autochthonous mammary carcinogenesis. These mice are transgenic for the transforming rat-ErbB2 oncogene under the transcriptional control of the mouse mammary tumor virus. Due to Erbb2 transgene expression in the thymus and its over-expression in the mammary gland, CD8+ T cell clones reacting at high affinity with dominant Erbb2 epitopes are deleted. Despite the lack of such a crucial component of immune reactivity, DNA electroporation of a plasmid coding the extracellular and transmembrane (EC-TM) domains of Erbb2 markedly delays the onset of mammary carcinomas when microscopically detectable diffuse in situ carcinomas are present ("early vaccination") but fails to block the progression of invasive carcinomas ("late vaccination"). The protection afforded rests on the activation of CD4+ T cells releasing IFN-gamma and the induction of anti-neu antibodies. Nevertheless, when "early vaccination" is coupled with temporary Treg depletion trough the administration of anti-CD25 mAb, long lasting tumor immunity is induced and the antibody response is enhanced. BALB-neuT mice treated with anti-CD25 mAb and elelctroporated with EC-TM plasmids display a CTL response against the neu immunodominant peptide due to reshuffling of their CD8 T cell repertoire. This new CD8 T cell repertoire is different from that of vaccinated wild type BALB/c mice. Temporary interference with Treg is also instrumental for the induction of an effective immune response in BALB-neuT mice already bearing invasive carcinomas