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