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Effect of Flt3-ligand gene transfer in experimental pancreatic cancer

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Pancreatic cancer is a malignant disease with poor prognosis. Recent observations suggest that immunotherapy may have relevance in its therapy. Fms-like tyrosine kinase 3 receptor (Flt3) is a growth factor receptor highly expressed on common lymphoid and myeloid progenitors, monocytes and steady-state dendritic cells (DCs). Administration of its ligand (FL) results in dramatic expansion of dendritic cells (DCs) *in vivo*, which is not comparable to the effect of any other known cytokines. Dendritic cells are central regulators of the cellular immune system; they are the most potent antigen presenting cells (APCs), and the only ones capable of priming naïve T lymphocytes. *In vivo* expansion of dendritic cells may offer an alternative to their *ex vivo* manipulation, which process is time-consuming, potentially increases the risk of infection and doesn't provide natural way of maturation. This method is currently being evaluated in experiments against tumors. Little is known about the local applicability of FL or genetic material coding for it, a method with various potential benefits. There is no data available concerning FL-based immunotherapies of pancreatic cancer at all.
We aimed in this study to (1) describe and clone the rat FL cDNA for further experiments with species specific sequence in rats, (2) to test the applicability of intratumoral gene delivery in rat pancreatic cancer using cationic liposomes and (3) to use intratumoral FL-gene delivery in the DSL6A rat pancreatic cancer model, follow the tumor growth and monitor the immune changes during and after therapy.
The rat FL was sequenced and cloned from total mRNA extract of the spleen. Transfection efficiency of subcutaneously growing rat duct-like pancreatic cancer (DSL6A) with DOTAP/cholesterol- based liposomes was tested using a pcDNA3.1-lacZ construct. FL production of *in vitro* transfected tumor cells and *in vivo* transfected tumors was measured by ELISA. Tumor induction was achieved in 20 Lewis rats by s.c. inoculation of DSL6A cells. Animals were allocated into 3 groups: control, mock treatment, treatment with FL-plasmid. Therapy was started after tumors reached a diameter of 5 mm. The plasmid (10µg/20µl) was injected intratumorally 6 times in 2 weeks and tumor growth was measured through 6 weeks. Tumor volume, lymphocyte infiltration, tumor proliferation and tumor vessel density were measured. Leukocytes from blood during therapy and splenocytes after the end of therapy were characterized by various surface markers (NKR-P1A, CD4-CD25, CD8-CD28, CD18-CD62L, CD11b/c-CD40, CD80, CD86) with flow cytometry. Plasma TNF-α and INF-γ levels during therapy were measured by ELISA.
The rat FL peptide sequence showed 72,92% and 89.65% identity compared to human and mouse peptids, respectively. Transfection rates at 10% could be achieved with the used cationic liposomes *in vivo*. DSL6A cells and tumors expressed FL after transfection. Reduction in tumor volume was found only in the FL-transfected group. The tumor volume remained significantly lower compared to controls during the first three weeks only. Total tumor regression was observed in one case. There were no differences in plasma TNF-α and INF-γ levels and leukocyte markers during therapy. Significantly elevated level of CD80 expression of splenic dendritic cells but not the elevation of total dendritic cell number was

found in FL-treated animals. Splenic NK-cell number was significantly elevated in therapy responders. No difference was found in other markers from spleen. Level of tumor infiltrating lymphocytes was extremely low in every case, proliferation and tumor blood vessel density also remained unaffected.

The antitumoral effect of FL production was abolished in most of the cases soon after therapy, a phenomenon frequently seen after systemic FL administration. It seems that tumors rapidly overcame the immune attack after a short period of time. Our data reinforces the role of NK cells in the antitumoral mechanism of FL. The therapy has led to partial activation of DCs from spleen. Studying the possible underlying mechanism of the therapeutic failure would need further experiments. DC recruitment was possibly not satisfactory to the tumor site, or they may have remained locally immature and induced tolerance. Antigen presenting but immature - tolerogenic DCs, or even tumors themselves may cause T cell deletion, anergy or development of T cells with regulatory/suppressive function. Although we did not find systemic or splenic elevation of regulatory T cell numbers, the role of antigen specific clones can not be excluded. Reaching higher level of intratumoral FL expression or exposing DCs to signals improving maturation may be beneficial.

The sequence data of the rat FL published here may broaden the field of research with FL-based immunotherapeutic models by allowing the use of the species specific sequence. Our results suggest that cationic liposomes may be used very efficiently in the DSL6A pancreatic cancer *in vivo*. The fact that liposome-based rFL gene delivery may lead to total tumor regression and some long-term immunological changes is promising, but since growth inhibitory effect was rare, usually week and not predictable, this treatment modality needs further refinement.