

polyclonal activation and transduction with retroviral vectors. In vivo experiments are in progress to determine whether in vivo survival of transduced T cells can be influenced by the in vitro transduction method.

633 **Expression pattern of IL-1 beta in malignant and benign prostate tissues coincides with its inhibitory effects on carcinoma cells** Poster

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Although overproduction of IL-1beta is often associated with aggressive growth of many tumors, data on expression of this cytokine in neoplastic and benign prostate tissue are limited. In our study performed on archived samples of prostate carcinoma (N=28, Gleason scores 6-9) and age-matched control benign hyperplastic tissue (BPH, N=30), an overall IL-1beta expression was lower in cancer relative to BPH (p=0.037). In BPH, IL-1beta was detected only in stroma, where its levels positively correlated with intensity of lymphocyte infiltrate (p=0.043). In low-grade cancer, IL-1beta was apparent in stroma and malignant glands, while high-grade tumors were negative. In cancer foci, stromal IL-1beta levels positively correlated with its glandular levels (p=0.017); both glandular and stromal levels negatively correlated with tumor grade (p=0.003) implying that IL-1beta exerts inhibitory effects on cancer cells. Indeed, IL-1beta-treated androgen-responsive LNCaP carcinoma cells exhibited time- and dose-dependent growth delay accompanied by redistribution of cells to G1 phase of the cell cycle. Although cells displayed an increase of cytosolic cytochrome C and mitochondrial Bak, no cytotoxicity was detected. However, pretreatment of LNCaP cells by IL-1beta markedly increased their susceptibility to etoposide-induced apoptosis with both caspase 2 and caspase 8 involved. IL-1beta also stimulated its own secretion in LNCaP cells. The experiments employing co-cultures composed of cytokine-pretreated and -not-treated cells or utilizing IL-1 receptor antagonist and media conditioned by IL-1-pretreated cells demonstrated that this amplification loop created by IL-1beta was essential for rendering these cells more susceptible to apoptosis. Amplification loop was also detected in androgen-insensitive PC3 and DU-145 carcinoma cells; however, proliferation and apoptotic resistance of these cells were not affected. In the contrast, non-neoplastic PWR-1E prostate cells failed to secrete IL-1beta even when pretreated by the cytokine. These cells were highly susceptible to etoposide-induced apoptosis; no further increase in apoptosis was observed in IL-1beta-pretreated cultures relative to controls treated by etoposide only. Identified tissue expression patterns considered together with growth-retarding and pro-apoptotic activities exerted by IL-1beta on androgen-responsive carcinoma cells indicate that IL-1beta may attenuate the initial steps of prostate cancer.

634 **miRNAs expression profiles during ErbB2 driven mammary carcinogenesis** Poster

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Since the expression of miRNAs is modulated during cancer development, miRNA profiles provide new classifications of human cancer. The epigenetic silencing of genes plays important roles in tumor evasion from the immune control: miRNAs are involved in regulation of the immune system and in the generation of T regulatory cells. In view of the roles played by miRNAs in cancer progression, we performed a miRNA microarray analysis aimed at identifying the modulation of miRNA expression profiles during the progression of autochthonous mammary carcinomas arising in mice transgenic for the activated transforming rat ErbB2 oncogene (BALB-neuT mice). BALB-neuT mice constitute a suitable cancer-prone model, since inexorably the females develop an invasive and metastatic mammary cancer in all of their ten mammary glands with a step wide pattern and a systemic metastatic spread similar to that observed in human mammary cancer. We analyzed three prototypic situations: i) normal hyperplasia (2 weeks pregnant BALB-c mice), ii) atypical hyperplasia (10 weeks old BALB-neuT mice) and iii) neoplastic lesion (19 weeks old BALB-neuT mice). Total RNA was extracted from the all mammary glands from 4 animals for each group of mice and miRNAs changes within these three prototypic situations were evaluated using LNA microarrays (EXIQON A/S, Denmark). Linear model analysis was performed in order to find out subsets of probes differentially expressed between the mammary glands of 10 and 19 weeks old BALB-neuT mice with respect to pregnant BALB/c mice. The principal component analysis (PCA) done on 169 miRNA, indicated a significant difference between

miRNAs expressed in the mammary tumors of BALB-neuT females and those expressed in the mammary glands of pregnant BALB/c females. This result suggest that the expression of miRNAs during physiological mammary hyperplasia and neoplastic hyperplasia is different. By contrast, when the analysis was performed to evaluate the differences between mammary atypical hyperplasia and neoplastic lesions, PCA showed only subtle differences suggesting that miRNA dysregulation in the mammary gland of BALB-neuT females is an early event which is then stably maintained during the tumor progression.

635 **Silencing IL-10 gene with intra-mammary siRNA enables DNA vaccination to inhibit established ErbB2 carcinomas** Poster

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In this study we intended to locally perturb tumor microenvironment in order to circumvent peripheral tolerance and induce an effective and persistent immune response.

We have shown that a DNA vaccine coding for the extracellular and transmembrane (EC-TM plasmid) domain of rat-ErbB2 (neu) halts the early stages of carcinogenesis in neu transgenic BALB/c mice (BALB-neuT). These mice develop mammary carcinomas that closely resemble human carcinomas in both tumor progression and immune tolerance to ErbB2, and in the expansion of suppressor cells. Marked expansion of CD11b+GR1+ immature myeloid cells and CD4+ CD25+ FOXP3+ GITR+ Treg cells during tumor progression in BALB-neuT mice makes immunotherapy against advanced lesions ineffective.

IL-10 is a key figure among the various tumor-derived and tumor induced factors that contributes to damp down the immune response. It is produced by both tumor cells and by cells with immunosuppressive activity such as T reg cells, tumor associated macrophages, tolerogenic DC and immature myeloid cells.

By using intra-nipple delivery it is possible to get a direct access to breast lesions and target immune suppressor cells that localize in the mammary tumor microenvironment. We have employed this technique to silence IL-10 gene in mammary glands of 18-week-old BALB-neuT mice with established lobular carcinomas. Intra-nipple injection of 50ug of IL-10 siRNA plasmid was followed 2 days later by intramuscular electroporation with 50ug of EC-TM plasmid. While EC-TM vaccination alone was only able to slightly delay the appearance of the first palpable tumor, prior silencing of IL-10 gene in the tumor microenvironment enabled the vaccine to elicit a protective anti-tumor immune response founded on a high titer of anti-neu antibodies and a significant cytotoxicity activity against ErbB2 peptides. All the mice that received both treatments were tumor free at 33 week of age, when all untreated mice, or mice treated with control IL10 siRNA or EC-TM vaccine alone were already dead.

In conclusion, this experiment planned to selectively perturb tumor microenvironment through intra-nipple injection of IL-10 siRNA may prove a new promising way of circumventing tolerance and eliciting protective immunity against established lesions.

636 **Probing the molecular mechanism of GD2 ganglioside mimicry with 12-amino acid constrained peptides** Poster

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Aberrant glycosylation is an universal feature of cancer cells. Over-expression of GD2 ganglioside (GD2) on neuroblastoma (NB) cells opens the possibilities to use the tumor associated carbohydrate antigen in immunotherapy. Moreover, the fact that about 60% of children with neuroblastoma have high risk tumors, which can rarely be cured by conventional therapy, stresses the need for new treatment protocols to control minimal residual disease.

Our goal is to design GD2-targeting active immunotherapy of NB by replacing the weakly immunogenic GD2 with its peptide mimotopes. We have identified 12-amino acid (aa) peptides that can mimic GD2 by screening the LX-8 phage-displayed peptide library with the GD2 specific mouse monoclonal antibody (mAb) 14G2a. In the current study, we have explored the observed GD2 mimicry phenomenon by vaccination studies in mice. Additionally, we have designed peptides with aa substitutions. In the next step, we have analyzed their 14G2a mAb binding in competition tests against GD2-expressing human NB cells, and microplate-bound GD2 using flow cytometry and ELISA, respectively.

We show that vaccines containing our peptides conjugated to KLH can induce GD2-targeting antibodies in A/J mice. Additionally, we have further