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.

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

S. Fedida-Metula[1], N. Sion-Vardy[2], Z. Suzlovich[2], L. Osyntsov[2], S. Segal[1], D. Fishman[1]
[1]Ben-Gurion University of the Negev, Microbiology and Immunology, Beer-Sheva, Israel; [2] Soroka University Medical Center, Institute of Pathology, Beer-Sheva, Israel

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-responding 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-responding carcinoma cells indicate that IL-1beta may attenuate the initial steps of prostate cancer.

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miRNAs expression profiles during ErbB2 driven mammary carcinogenesis

E. Quaglino[1], R. Calogero[1], S. Saviozzi[1], G. Forni[1], F. Cavallo[1]
[1]University of Torino, Department of Clinical and Biological Sciences, Torino, Italy

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 authoctonous 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 disregulation in the mammary gland of BALB-neuT females is an early event which is then stably maintained during the tumor progression.

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

R. Cojoca[1], S. Rolla[1], S. Occhipinti[1], H. Pinto[1], G. Forni[1], F. Cavallo[1]
[1]University of Torino, Clinical and Biological Sciences, Torino, Italy

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 citotoxicity 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.

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

I. Horwacik[1], A. Kowalczyk[1], M. Bzowska[2], D. Czaplicki[1], H. Rokita[1]
[1]Faculty of Biochemistry Biophysics and Biotechnology, Laboratory of Molecular Genetics and Virology, Krakow, Poland; [2] Faculty of Biochemistry Biophysics and Biotechnology, Department of Immunology, Krakow, Poland

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

characterized the molecular mechanism of GD2 mimicry observed with our isolated peptides by application of alanine scanning experiments. This has allowed us to determine the involvement of consecutive aa residues of the peptides in the 14G2a mAb binding. Using competition assays we have identified the aa residues that are critical for the binding. Furthermore, in an attempt to optimize the GD2 mimotopes we have designed and characterized a peptide sub-library containing aa substitutions at the pivotal positions for the 14G2a mAb binding. Finally, we have screened the peptides for their ability to bind to mAb specific for other gangliosides.

The accumulated data allowed us to gain insight into the molecular mechanism of GD2 ganglioside mimicry by the mimotopes. This can lead to increase of therapeutic potential of our GD2 mimotopes. More research is planned to optimize the GD2-specific immune responses induced with the mimotopes, by testing their anti-tumor activity on a NB model based on the A/J mouse strain and syngenic NXS2 cells.

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An MVA based vaccine targeting the oncofoetal antigen 5T4 in patients undergoing surgical resection of colorectal cancer liver metastases

P.L. Stern[1], E. Elkord[1], A. Dangoor[1], N.L. Drury[2], R. Harrop[2], D.J. Burt[1], J.W. Drijfhout[3], S. Naylor[2], D. Sherlock[4], R.E. Hawkins[5]
[1]Paterson Institute for Cancer Research, Immunology, Manchester, United Kingdom; [2] Oxford BioMedica, Oxford Science Park, Oxford, United Kingdom; [3] Leiden University Medical Center, Immunohaematology and Bloodbank, Leiden, The Netherlands; [4] North Manchester Healthcare Trust, Surgery, Manchester, United Kingdom; [5] University of Manchester, Medical Oncology, Manchester, United Kingdom

Purpose: This study investigated the use of a therapeutic vaccine, TroVax? in patients undergoing surgical resection of colorectal cancer liver metastases. Systemic immunity generated by vaccination before and after resection of metastases was measured in addition to assessing safety and toxicity and analyzing the function and phenotype of tumour associated lymphocytes,.

Experimental Design: Twenty patients were scheduled to receive 2 TroVax vaccinations at 2 week intervals pre-operatively and 2 post-operatively; if immune responses were detected 2 further vaccinations were offered. Blood samples were taken at trial entry and 2 weeks after each vaccination; tumor biopsies were taken at surgery. 5T4-specific cellular responses were assessed by lymphocyte proliferation and ELISPOT, while antibody responses were measured by ELISA. Immunohistochemistry was used to characterize antigens expressed on the tumour and to analyze the phenotype of infiltrating lymphocytes.

Results: Twenty patients were recruited, one of whom was found to have hepatocellular carcinoma. Of the 19 colorectal cancer (CRC) patients, seventeen showed 5T4 expression in the tumour or surrounding stroma and 18 mounted a 5T4-specific cellular and/or humoral response. In patients where surgery was at least potentially curative (n=15), those with above median 5T4-specific proliferative responses or T cell infiltration into the resected tumour showed significantly longer survival compared to those with below median responses. A similar, but non-significant, trend was also associated with the 5T4 antibody response.

Conclusion: These data suggest that the magnitude of 5T4 (but not MVA) specific antibody and proliferative responses and the density of CD3 cells in colorectal cancer liver metastases are associated with clinical benefit. Such encouraging observations warrant more extensive studies to identify the precise underlying mechanisms.

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Tumor antigen NY-CO-58/KIF2C is strongly overexpressed in a variety of human cancers and evokes spontaneous T cell responses

D. Atanackovic[1], Y. Cao[1], Y. Hildebrandt[1], K. Bartels[1], C. Horn[1], T. Stahl[1], L.J. Old[2], S. Gnjatic[2], C. Bokemeyer[1]
[1]University Medical Center Hamburg-Eppendorf, Oncology/Hematology, Hamburg, Germany; [2] Ludwig Institute for Cancer Research, New York branch, New York, USA

INTRODUCTION: A recent study indicated that NY-CO-58/KIF2C might be overexpressed in colorectal cancer. However, NY-CO-58/KIF2C expression has not been examined in detail in this tumor type, little is known about the expression of NY-CO-58/KIF2C in other cancers, and it is unclear whether this tumor antigen is able to induce spontaneous T cell responses in cancer patients.

METHODS: We examined the expression of NY-CO-58/KIF2C in colon cancer cell lines, a broad series of healthy human tissues, and malignant as well as autologous healthy tissues from patients with colorectal cancer (N=22). In addition, normal and tumor-infiltrated samples from patients with pancreatic (N=17), gastric (N=10), head-and-neck (N=30), and breast cancer (N=44) were examined for NY-CO-58/KIF2C expression using conventional RT-PCR, real-time PCR, Western blot, immunofluorescence, and immunohistochemistry. Finally, we analyzed peripheral T cells of 43 patients with colorectal cancer and 35 healthy controls for responses against nine 30mer peptides of NY-CO-58/KIF2C following one cycle of antigen-specific stimulation.

RESULTS: Colon cancer cell lines strongly expressed NY-CO-58/KIF2C on the RNA and protein levels. Among 20 normal tissues, human testis expressed the highest levels of NY-CO-58/KIFC, thymic tissue showed an intermediate level, and the remaining healthy tissues only evidenced trace levels of NY-CO-58/KIFC. Examining samples of patients with colorectal cancer using real-time PCR, we found that NY-CO-58/KIF2C was strongly overexpressed in the malignant compared to autologous healthy colon tissue. Immunohistochemisty localized NY-CO-58/KIF2C expression to malignant epithelial tissue. Analyzing malignant and autologous healthy tissues from patients with pancreatic, gastric, breast, and head-and-neck cancer, we found that NY-CO-58/KIF2C was significantly overexpressed in all these tumor types. CD8+ T cell-mediated responses were only detected in less than 10% of patients or healthy controls and were generally weak. In contrast, we found CD4+ T cell responses against one or more NY-CO-58/KIF2C peptides in close to 50% (20/43) of patients with colorectal cancer. Surprisingly, we observed equally frequent NY-CO-58/KIF2C-specific CD4+ T cell responses in the healthy blood donors with the majority (21/35) of subjects evidencing a response against at least one NY-CO-58/KIF2C peptide. Importantly, NY-CO-58/KIF2C-specific CD4+ T cells were of high avidity, recognized the naturally processed antigen, and secreted Th1-type cytokines.

CONCLUSION: Based on its overexpression in a number of human cancers and its high immunogenicity we suggest that NY-CO-58/KIF2C represents an attractive target for active tumor immunotherapies.

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Tumor antigen-encoding mRNA for the analysis of spontaneousand vaccine-induced immune responses in cancer patients

N. Nuber[1], A.J. Knights[1], S. Pascolo[1], C.W. Thomson[1], A. Knuth[1], M. van den Broek[1], A. Zippelius[1]
[1]University Hospital Zurich, Department of Oncology, Zürich, Switzerland

Background: For the development of effective cancer vaccines there is a requirement for the assessment of vaccine induced immunity. Current immunomonitoring strategies do not allow for the optimal investigation of the full breadth of T cell responses, and is hampered by the limited number of known epitopes for most tumour antigens.

Methods: In this study transfection of antigen-presenting cells (APC) with modified mRNA constructs encoding for tumour antigens was optimized. mRNA encoding for full length NY-ESO-1 and CT-7/MAGEC1 has been applied to monitor T cell responses in cancer patients with naturally occurring immune responses to their tumour or following vaccination.

Results:CD8 T cells obtained from lung cancer patients with humoral immune responses directed towards NY-ESO-1 could be successfully amplified in vitro following only one stimulation round with mRNA-transfected APC. Specific killing of a panel of HLA-matched allogeneic NY-ESO-1 expressing tumour cell lines by the monoclonal CD8 T cells indicates an oligoclonal response including a novel HLA-B49 restricted epitope. Detection of NY-ESO-1 specific CD4 T cells in patients could be enhanced using a modified mRNA construct that targets the MHC class II pathway. The establishment of functional CD4 T cell clones specific for NY-ESO-1 has enabled the definition of the restriction element HLA-DQB10301 and HLA-DPB10402. Oligoclonal CD8 and CD4 T cell responses were detected in patients following an NY-ESO-1 vaccination. Using a modified CT-7 encoding mRNA, CT-7 specific CD4 T cells were detected in melanoma patients.

Conclusion: This methodology allows for a more precise monitoring of responses to tumour antigens in a setting that addresses the breadth and magnitude of antigen-specific T cell responses, and that is not limited to a particular combination of known epitopes and HLA-restrictions.

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Epigallocatechin-3-gallate inhibits monocyte adhesion and migration to sites of inflammation

E. Melgarejo[1], M.A. Medina[1], F. Sánchez-Jiménez[1], J.L. Urdiales[1]
[1]Universidad de Málaga and CIBERER, Biología Molecular y Bioquímica, Málaga, Spain

Monocytes/macrophages play an important role on initiation, development, and outcome of the immune response. Epigallocatechin-3-gallate (EGCG), a major component of green tea, has been reported to have antiallergic and anti-inflammatory activities. Our group demonstrated previously that EGCG