comparison of the matching samples of cancer versus benign and cancer versus PIN showed that in 68% and 70% cases respectively, increased expression of PDEF was seen in cancer in comparison to the matching benign or PIN tissue.
Conclusions: This is the first report of the characteristics of PDEF protein expression in various stages of breast and prostate neoplasias. The data show frequent increase in the number and/or intensity of PDEF expressing epithelial cells in progression from benign breast or prostate tissue to carcinoma. These results together with: i) limited expression of PDEF in normal tissues; ii) its promotion of epithelial cell motility and invasiveness *in vitro* and tumorigenicity *in vivo*; and iii) immunogenicity of its mouse homologue support PDEF as a novel target in breast and prostate cancers.

120 POSTER

Transduction of tumor cell lines with adenoviral vectors expressing TLR ligands and scFv anti-IL-10 activates dendritic cell maturation and IL-12 secretion in the presence of IL-10

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In cancer patients and in mouse models, tumor progression has been associated with the accumulation of immature-type dendritic cells (DCs), known to be poor stimulators of Th1 cell responses and to participate in cancer-induced immunosuppression. The presence of "immunosuppressive" cytokines such as IL-10, secreted or induced by many types of tumors (melanomas, lung cancer, breast or cervical cancer, hepatocellular or renal carcinomas, lymphomas) contributes to maintain DCs in an immature state and profoundly affects their ability to produce IL-12 or to stimulate T cell-responses. In order to provide "danger" signals to DCs within tumors, we generated adenoviral (Ad) vectors driving the expression of TLR ligands in transduced cells. Thus, the outer membrane protein from Klebsiella pneumoniae (P40/OmpA) or Flagellin from Listeria monocytogenes, known to bind respectively TLR2 and TLR5, were expressed using Ad vectors in human tumor cell lines. Coculture with Ad-PAMP-transduced tumor cells was indeed able to induce the phenotypic maturation of human monocyte-derived DCs. With the goal of blocking the effect of IL-10 in the tumor milieu, we have constructed an Ad vector encoding a single-chain variable region (scFv) isolated from an anti-IL-10 blocking antibody. We show that in the presence of tumor cells transduced with the Ad-scFv vector, IL-10 is efficiently neutralized and LPS-stimulated human monocyte-derived DCs secrete increased levels of IL-12p70. Combined with Ad-PAMPs, Ad-scFv anti-IL-10 enhances the phenotypic maturation of DCs in the presence of IL-10. The combination of Ad-PAMP + Ad-scFv anti-IL-10 also restores IL-12p70 production by DCs in the presence of LPS and IL-10. The combination of an Ad-PAMP with Ad-scFv anti-IL-10 administered intratumorally may thus represent a promising strategy to reverse cancer-induced suppression of DC functions.

121 POSTER

Isolation and characterisation of anti-idiotypic scFv antibody fragments and llama VHH domains used as a surrogate tumour antigen to elicit an anti-HER-2 humoral response in mice

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HER-2 is a tumour antigen that is over-expressed in human breast tumours. Among the vaccine strategies developed to overcome immune tolerance to self-proteins, vaccination with anti-idiotypic (anti-Id) antibodies has been described as a promising approach for treatment of several malignant diseases. To develop an active immunotherapy for cancer patients positive for HER-2, we produced small molecular recognition units of Ab2beta type (scFv 40 and 69) and a single domain VHH antibody (VHH 1HE), specific for trastuzumab F(ab')$_2$ fragments (Ab1), a humanised anti-HER-2 monoclonal antibody. Using competitive ELISA and Biacore biosensor analysis, we showed that anti-Id scFv 40, scFv 69, and VHH 1HE could inhibit HER-2 binding to trastuzumab. Following vaccination of BALB/c mice with these molecules, Ab3 polyclonal antibodies, and among them Ab1' antibodies able to bind HER-2, were detected in the sera of the immunised mice. These results demonstrate that these anti-Id antibodies could act as a surrogate antigen for HER-2. The present study strongly suggests that the novel 30 kDa human mini-antibody and the 15kD llama VHH single domain could be used as an anti-idiotype-based vaccine formulation to induce an effective humoral response in patients bearing HER-2 positive tumours.

Chemoprevention

122 POSTER

An inhibitor of acetyl polyamine oxidase specifically blocks androgen induced oxidative stress and prevents occurrence of prostate cancer in TRansgenic Adenocarcinoma of Mouse Prostate (TRAMP)

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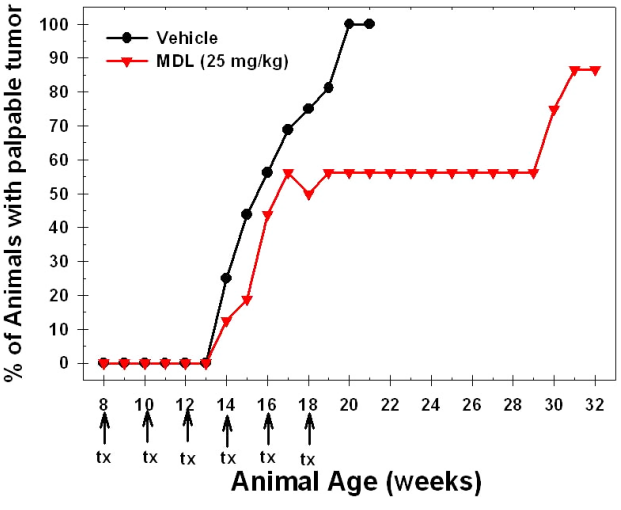
Background: Direct evidence linking oxidative stress with an increase in prostate tumor development both in human and in TRAMP animals has been reported. Reducing the oxidative stress in the prostate can be an effective strategy in preventing occurrence, recurrence and progression of prostate cancer. We have previously demonstrated that androgen treatment increases reactive oxygen species (ROS) levels in androgen dependent prostate cancer cells. The biochemical pathway involved in androgen induced ROS production in the prostate cells, however, remained unknown.
Method: DNA microarray, qRT-PCR, cell culture and cellular polyamine level determination by standard HPLC were carried out using androgen dependent LNCaP human prostate cancer cells grown with or without androgen analog R1881. Prostate tumor development in TRAMP animals was determined by tumor palpation and confirmed by micro-PET and micro-CT imaging.
Results: Our DNA microarray data validated by qRT-PCR results and confirmed by cellular polyamine and acetyl polyamine level determination demonstrate that R1881 treatment induces an overexpression of spermidine/spermine acetyl transferase (SSAT) mRNA by over fifty fold with a concomitant increase in SSAT activity in androgen dependent LNCaP human prostate cancer cells. Catabolism of polyamines spermidine and spermine produced in large excess by both normal and malignant prostate cells occurs through acetylation by SSAT followed by oxidation by acetyl polyamine oxidase (APAO) that generates H_2O_2 (ROS). Downregulation of SSAT gene expression by siRNA blocks R1881 induced peroxide production in LNCaP cells.



MDL significantly delays prostate tumor development in TRAMP mice.

We have identified a specific APAO inhibitor N1,N4-bis(2,3-butadienyl)-1,4-butanediamine (MDL) that blocks R1881 induced ROS production in LNCaP cells. MDL given 25 mg/kg i.p. once in two weeks is well tolerated by TRAMP animals without any overt sign of systemic toxicity. At this dose, MDL completely inhibits APAO activity in mice and reduced oxidative stress in the prostate *in vivo*. MDL treatment significantly increased overall survival and delayed time to prostate tumor development by over ten weeks.
Conclusion: Our results demonstrate that polyamine oxidation is one of the major causes of androgen induced oxidative stress in prostate cancer cells. To the best of our knowledge, this is the first report of an enzyme inhibitor MDL that blocks androgen induced oxidative stress specifically in the prostate and prevents spontaneous prostate tumor development.