ImmunoTools *special* Award 2014

Dimitrios Chioureas, PhD student

Supervisor: Dr. Theocharis Panaretakis

Karolinska universitetssjukhuset Solna, Department of Oncology-Pathology, CCK R8:03, via varumottagningen, Karolinska vägen 8, 171 64 Solna, Sweden

The role of exosomes in the prostate cancer development.

Project plan

Predictive marker studies on prostate cancer cell-derived exosomes

Background

Prostate cancer (PC) is the most commonly diagnosed cancer in men and a leading cause of cancer related deaths worldwide. Even though the organ-confined prostate cancer has become curable, the overall death toll remains high because of relapse and the development of metastatic, castration resistant prostate cancer (CRPC) that remains incurable. There are currently no specific biomarkers that will prognose whether CRPC patients will respond to therapy. We are planning to address this major clinical problems by implementing the knowledge gained from cancer exosome biology into the discovery of biomarkers for CRPC.

Exosomes are endosome-derived vesicles actively secreted by virtually all cell types under normal and importantly, under pathological conditions (Kharaziha, Ceder et al. 2012). These 30-100 nm vesicles float on a sucrose gradient at a density between 1.13-1.19 g/ml and can be visualized by transmission electron microscopy (**Figure 1,** transmission electron microscopy of prostate cancer cell line, PC3-derived exosomes). Exosomes are enriched to a conserved set of proteins characteristic of their secretory pathway such as tetraspanins (CD9, CD63), ESCRT proteins, clathrins and Rabs (e.g. Rab5 and Rab27). It is also known that exosomes are enriched in functional messenger RNA (mRNA) and microRNA (miRNA) (Valadi, Ekstrom et al. 2007; Taylor and Gercel-Taylor 2008; Nilsson, Skog et al. 2009; Rosell, Wei et al. 2009). Cancer cells have been shown to secrete elevated levels of exosomes which may be perceived as the tumor's fingerprint (a combination of proteins, mRNA and miRNA). Importantly, uptake of cancer cell derived exosomes by recipient cells has been suggested to: i) promote inter-cellular communication by transferring intra-exosomal proteins and RNA; ii) modulate immune responses; iii) preparing the pre-metastatic niche by molding the tumor microenvironment (Kharaziha, Ceder et al. 2012).

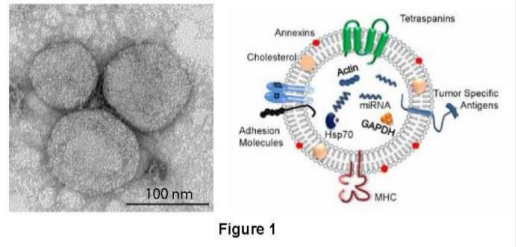

Figure 1

Aim of the study

In the proposed research plan, we aim to characterise the molecular composition of exosomes secreted from CRPC cells that are sensitive or resistant to therapy. The ultimate goal of this research plan is to discover the urgently needed predictive biomarkers and to develop novel therapeutic strategies that can be tailored to individual CRPC patient's needs.

Project pan: Discover novel, multiplex biomarkers for CRPC that will predict therapeutic response

The first line treatment for CRPC patients is taxotere (Tax). Taxanes (e.g. taxotere and its derivatives) exert their cytotoxic effects by stabilizing microtubules and thereby disrupting important cellular functions such as cell division, protein localisation, cell signaling and inhibition of anti-apoptotic proteins. We generated prostate cancer cell lines (DU145, LNCaP and PC3) that have acquired resistance to Tax after prolonged incubation with this drug to a final concentration of 100 μM. We isolated and molecularly profiled exosomes from DU145 cells that are sensitive (DU145-S) or stably resistant to 100μM Tax (DU145-R). We performed the proteomics analysis on the parental cell lines and comparative analysis between DU145-R and DU145-S which revealed several proteins that are differentially enriched. Several exciting proteins were identified and confirmed by western blotting in DU145-S and DU145-R cells and exosomes, and are now examined in CRPC plasma samples. The functional importance of these proteins in mediating resistance and in promoting metastasis is under investigation.

In the present project we would like to focus our attention on novel drugs that target the the among others, the androgen receptor. There are currently no clinically useful markers that will predict prior to the initiation of treatment whether there the patients will benefit from these particular treatments. We have established the prostate cancer cell lines (LNCaP, Du145 and PC3) that are resistant to the selcted anti-cancer drugs. We will examine the amount and size of exosomes secreted by sensitive and resistant cell lines by nanoparticle tracking analysis and protein concentration measurements. We will perform immunophenotyping by flow cytometry and western blot analysis and transmission electron microscopy to confirm the purity and quality of the isolated exosomes. The protein content of the isolated exosomes will be identified by performing comparative proteomics analysis on the sensitive and resistant cell derived exosomes. This part of the project is critical since it will reveal potential biomarkers but also proteins that may regulate resistance to therapy. The identified putative predictive biomarkers will be validated by western blotting the exosomes isolated from the resistance cell line. The validated putative biomarkers will be further investigated for their predictive capacity in the plasma of CRPC patients by western blotting and/or flow cytometry.

Expected results

The exploitation of resistant CRPC-derived exosomes will lead to the discovery of biomarkers as of surrogate markers for evaluation of efficacy of novel and commonly used anti-cancer drugs. Ultimately, exploitation of the knowledge gained from this research proposal will lead to the development of automated detections assays that hopefully will accurately and reproducibly predict response to therapy.

Utilisation of the aforementioned antibodies

The aforementioned antibodies from immuno tools will be instrumental for all our studies on isolated exosomes from in vitro and ex vivo samples. They are part of the comprehensive exosome characterisation protocol that we are using in the lab and involves the flow cytometric immunophenotyping of our exosomes with classical exosomal markers and some more cancer specific. This assay allways preceeds the molecular profiling that will performed in an attempt to identify novel, predictive biomarkers for therapeutic response to docetaxel and other novel and commonly used anti-cancer agents.

Involvement in public activities

We are involved in a number of activities that aim to spread information of the knowledge gained from our research to the public. We are also actively organising seminar series, meetings, national and interantional networks that will promote collaborations and interactions between fellow researchers and public health organisations. For example we have been organising the national cell death network, the european cell death organisation, the exosome club and we have applied for the organisation of a nobel symposium on exosomes.

ImmunoTools *special* AWARD for **Dimitrios Chioureas** includes 25 reagents

FITC - conjugated anti-human CD9, CD47, CD80, CD86, CD95,

PE - conjugated anti-human CD9, CD20, CD80, CD95, CD147, TNFa,

APC - conjugated anti-human CD9, CD20, CD63, CD147, IL-6, Control-IgG1, Control-IgG2a, Control-IgG2b,

human CD147 (sEMMPRIN) - ELISA-set for 96 wells, human TNFa – ELISA-set for 96 wells (each 3 reagents) DETAILS more AWARDS