IDENTIFICATION OF POTENTIAL BIOMARKERS FOR PROSTATE CANCER IN TEARS AND SERUM USING PROTEOMIC APPROACHES

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Introduction:

Prostate cancer (CaP) has the second highest mortality rate of any cancer in men [1]. The current blood test to diagnose CaP, testing for Prostate-Specific Antigen (PSA), is not able to effectively differentiate between benign prostatic hyperplasia (BPH), non-aggressive and aggressive CaP. The best prognostic indicator, the Gleason score, requires biopsy and quantification is difficult. Therefore, finding effective biomarkers that can differentiate between these states of prostatic disease are important for CaP diagnosis, prognosis and monitoring disease progression and treatment efficacy.

Background:

Proteomic studies investigate the complement of proteins expressed by a genome at a particular point in time and so can be used to identify proteins differentially expressed by that genome under pathophysiological conditions, potential biomarkers. A large proportion of proteomic studies isolate biomarkers from bodily fluids and the main advantage of this sample type over tissue is the less invasive collection. The tear is a body fluid that has long been investigated for biomarkers related to ocular diseases, however more recently tears have been the source of potential biomarkers for other diseases such as diabetes [2] and breast cancer [3]. Tear secretion is regulated by androgens, which are related to the health of the prostate gland. It is therefore possible there maybe changes in tear composition associated with CaP. Serum is an important biomarker reservoir. It perfuses every body organ and tissue and as a result the serum proteome reflects the physiological and pathophysiological state of the individual at the moment of blood withdrawal. The two most common post-translational protein modifications (PTMs), phosphorylation and glycosylation, are increasingly regarded as being associated with tumourigenesis [4]

Purpose:

In this study, our objective is to analyse the tear and serum proteomes from CaP patients using a combination of standard two-dimensional gel electrophoresis (2D-E) and 2D difference gel electrophoresis (DIGE) in the hunt for biomarkers of the disease as well as produce the first 2-D map of total, phospho- and glyco- protein profiles of tears.

Methods:

Tear samples from 45 subjects were collected from healthy controls (n=15), BPH (n=15) and CaP (n=15) patients. All samples were analysed using 2D-E. The gels were stained with three different dyes: Pro-Q diamond for phosphoproteins; Pro-Q Emerald for glycoproteins; and Sypro-Ruby for total protein. Each set of protein profiles

was analysed using Progenesis SameSpots to detect any significant differences in protein spot abundance. The glyco- and phosphoprotein profiles were mapped against the total protein profile to examine PTMs of the tear proteins using Progenesis SameSpots. Protein spots that were glycosylated and/or phosphorylated were identified by mass spectrometry. Other protein spots that have not been identified in published 2D gel papers were also selected for identification.

Serum samples collected from CaP patients (n=15) at different stages of disease, or from BPH patients (n=5) were depleted using ProteoPrep immunoaffinity albumin and IgG depletion kit (Sigma-Aldrich, Inc. USA). The depleted serum proteins were separated using 2D DIGE. The gels were then analyzed using Progenesis SameSpots (Nonlinear dynamics, UK) to detect differences in protein profiles between the different pathological states.

Results:
Comparison of the tear total-, phospho- and glycoprotein profiles identified the major tear proteins Lipocalin and Cystatin S as both glycosylated and phosphorylated for the first time. This study also provides the first evidence that Dermicidin, an anti-microbial protein, is present in tears.
No single protein spot was significantly altered in abundance in CaP compared with BPH tear samples suggesting that 2DE analysis of tears may not be the optimal method for detection of CaP biomarkers.
Three proteins not previously associated with CaP were identified in serum as potential biomarkers to differentiate CaP from BPH. A further five proteins previously reported as potential CaP biomarkers were also identified as such in the present study.

Conclusions:
Although 2DE analysis of the tear proteome of CaP patients did not generate any potential biomarkers, a new tear protein Dermicidin was identified. Importantly, the mapping of phosphor-, glyco- to total protein profiles has aided in the understanding of the PTMs of tear proteins, an important source of potential biomarkers for various pathophysiological conditions.
Three novel potential serum biomarkers for CaP and five that have been previously reported were identified in the present study. Analysis of a greater number of samples using ELISA is currently underway to verify their abundance change and their usefulness in the clinical setting.

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