Claudio Diema
Dr. sc. hum.

**Analysis of differential protein expression in prostate carcinoma cell lines versus cells from non-cancerous prostate**

Geboren am 16.06.1976 in General Deheza, Cordoba, Argentinien
Diplom der Fachrichtung Chemie am 21.03.2000 an der Chemie Fakultät Cordoba, Argentinien

Promotionsfach: DKFZ (Experimentelle Pathologie)
Doktorvater: Herr Prof. Dr. Hermann-Josef Gröne

The molecular pathology of prostate cancer (PCa) is complex and the pathway and the acquired molecular defects responsible for PCa initiation, development and progression are still largely unknown. Tumors of the prostate have an unpredictable behaviour and in clinically detected cancer it is not possible to assess tumor aggressiveness. Prostate specific antigen (PSA) is currently the only clinically used biomarker for detection of PCa, but its specificity is low and there is a need for additional biomarkers for early PCa detection and for prediction of prognosis.

The aim of this work was to find possible biomarkers for unambiguous detection and reliable differentiation of various stages of prostate cancer. First we established a protocol which allowed us to perform nuclei isolation of cell lines with a purity of 90-95%. The purity was checked by western blot analysis and by reverse phase microscopy. Extracts of proteins obtained from nuclei of the three cell lines BPH-1, DU145 and PC3 were separated by high resolution two-dimensional polyacrylamide gel electrophoresis (2D-PAGE). Proteins were visualized by using a destructive silver stain protocol for analytical gels and colloidal coomassie or silver stain protocols compatible with mass spectrometry for preparative gels.

We analysed the protein expression profile of PCa in order to identify proteins with decreased or increased expression in two prostate cancer cell lines DU145 and PC3 in comparison to the benign control cell line BPH-1. Image analysis of 2D-PAGE allowed us to detect 30 protein spots with significantly different expression levels; 18 spots were down- and 12 spots up- regulated at least 2.5 fold compared with the BPH-1 cell line. These proteins were identified by MALDI peptide mass fingerprinting. Database searches were performed against the NCBInr database using the ProFound and Mascot search algorithms. The results were validated by immunoblots assay for those proteins where antibodies were commercially available. The proteins calreticulin, glucosidase II, PCAF, nucleophosmin, prohibitin and protein disulfide isomerase showed the same differential expression behaviour performing two independent methodologies, namely immunoblotting and image analysis of 2D-PAGE.

Furthermore we expanded our studies by the analysis of the posttranslational modification SUMOylation. SUMO (small ubiquitin-related modifier) represents a class of ubiquitin-like proteins that is conjugated, like ubiquitin, by a set of enzymes to cellular regulatory proteins, including oncogenes and tumor suppressor genes that play key roles in the control of cell growth, differentiation and apoptosis. The SUMOylated substrates involved are expected to be important in the course of tumorigenesis and, accordingly, altered in human cancer. For this analysis we produced and purified specific antibodies to the SUMO protein family. We demonstrated the presence of SUMOylated proteins by immunoblot analysis of 1D and 2D gels using the anti-SUMO-1-NT antibody. However, SUMO-1 related peptides could not be identified by mass spectrometry most probably due to the difficulty of identification of SUMO proteins in cell lines without enrichment or concentration procedures.

In conclusion, we identified and confirmed by two independent and orthogonal methods differential protein expression levels of 6 proteins between prostate cancer cell lines of different stages and non-cancerous prostate cell line. Thus, these proteins are promising biomarker candidates which may have the potential to become diagnostic or prognostic biomarkers for PCa.