NADPH Oxidases in Prostate Cancer

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Background
NADPH-Oxidases (Nox) are a family of membrane bound enzymes which reduce molecular oxygen to superoxide using NADPH as electron donor. Nox enzymes generate reactive oxygen species (ROS) in a regulated manner in various cells and tissues in response to growth factors, cytokines and calcium signals. This implies important biological functions for ROS, such as proliferation, differentiation and apoptosis. However, besides its various physiological functions, ROS are also known to play crucial roles in pathological processes as various investigations revealed ROS to cause molecular damage such as DNA mutations, lipid peroxidation and protein oxidations. Cumulative studies correlate overexpression of Nox and increased ROS with various cancer types. In distinct prostate cancer cell lines, literature shows contradictory results concerning the expression levels of Nox isoforms. The aim of our study is to identify the predominant Nox isoform and characterize its role in selected prostate cancer cell lines. We also investigate a potential functional interaction of Nox4 with a cellular transcription factor belonging to the superfamily of SCAN domain containing zinc finger transcription factors. We hypothesize that the functional interaction of Nox4 with transcriptional regulators, initially revealed in a yeast two-hybrid screen, may shed new light on the well-known ability of Nox4 to influence the expression of a large set of cellular genes, by mechanisms which are only incompletely understood.

Methods
Quantitative real-time pcr and western blot analysis were used to clarify expression of Noxes in prostate epithelial cancer cell lines LNCaP, VCaP, DU145 and PC3. Protein knock-down was achieved by lentiviral infection. Consequences of protein knock-down were determined by several staining methods followed by FACS analysis: Propidium-Iodide and AnnexinV were used for determination of apoptosis, necrosis and cell cycle. BrdU staining was performed for analysis of proliferation and CM-H2DCFDA was used for ROS detection. ROS production was also measured by chemoluminescence. Morphological changes were observed by immunofluorescence staining methods and subsequent confocal microscopy.

Results
We identified low amounts of Nox1 only in DU145 whereas none of the investigated prostate cancer cell lines expresses Nox4. Nox5 is present in low amounts in LNCaP but to a much higher degree in PC3. Based on this data we selected Nox5 in PC3 cells for further analysis. Nox5 knock down leads to a significant reduced ROS production in prostate cancer cells.
Cell proliferation of Nox5 depleted cells is markedly reduced compared to control cells. So far we found no striking differences in apoptosis and necrosis, but slight changes in cell cycle distribution were observed. In the near future we will further characterize the function of Nox5 in prostate cancer cells.

A yeast two hybrid screen revealed a member of the ZSCAN family of transcription factors as potential interaction partner of Nox4. We found Nox4 to be located not only in membrane structures of the cytosol but also within the nuclear membrane. If there is any interaction between those two proteins, we assume that they coact within or closely related to the inner side of the nuclear membrane. Therefore we are looking forward to further examine the interplay between Nox4 and transcriptional regulators.