Crucial Role of Dickkopf-3 in Prostate Morphogenesis *in vitro* and *in vivo*

Introduction and Objective: To investigate the role of the tumour suppressor Dickkopf-3 in prostate acinar formation *in vitro* and *in vivo*.
Materials and Methods: To establish Dkk-3 depleted RWPE-1 cell lines (RWPE-1/D3-sh cells), RWPE-1 cells were stably transfected with Dkk-3-targeting pSM2-shRNAmir (Open Biosystems). The cells transfected with non-targeting vector (RWPE-1/NS-sh) were used as a control. For 3D acinar morphogenesis assays, either RWPE-1/NS-sh or RWPE-1/D3-sh cells were plated on a thin-layered bed of Matrigel on 8-chamber glass slides and cultured with Keratinocyte SFM containing 2% Matrigel. For analysis of Dkk-3 knockout mice and their wild-type littermates, prostates were dissected from 6-8 week-old mice, fixed with 4% paraformaldehyde and embedded in paraffin. Sections were analysed by H&E and immunofluorescent staining.
Results: RWPE-1/D3-sh cells showed more than a 90% reduction in Dkk-3 mRNA and protein expression, compared to both parental and RWPE-1/NS-sh cells. There were no apparent morphological differences between the cell lines when cultured in 2D. However, in 3D assays, while RWPE-1/NS-sh cells underwent normal acinar morphogenesis with spherically arranged polarisation, RWPE-1/D3-sh cells formed disorganised cell aggregates. Immunofluorescent staining for phosphotylated histone H3 revealed increased cell division in Dkk-3 depleted acini. Simultaneous staining for beta4-integrin and beta-catenin suggested that partitioning of basal and lateral membranes in Dkk-3 depleted acini was not affected. However, gene reporter assays and analysis of target gene expression indicated changes in Wnt and TGF signalling in RWPE-1/D3-sh cells. These results indicate that Dkk-3 knockdown results in changes in key cell signalling pathways, abnormal cell division and disorder of apical membrane integrity, leading to the disruption of acini. Analysis of prostates from mice showed an increased Ki-67 index in all lobes of Dkk-3 knockout mice, compared to wild type, consistent with the results in the *in vitro* 3D morphogenesis assay. H&E and immunofluorescent staining for ZO-1 and E-Cadherin revealed subtle changes in cellular structure during prostate development.
Conclusions: These results suggest that Dkk-3 controls cell proliferation and polarisation to secure organised acinar structure of prostate epithelial cells, and suggest that loss of Dkk-3 expression contributes to prostate cancer development due to aberrant cell proliferation and impaired cellular structure.