56 Generation of prostate basal stem cell lines from transgenic mice - proof of principle of inducible ex vivo gene deletion

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Introduction & Objectives: The long-term propagation of basal prostate stem cells ex vivo has been very difficult in the past. Our development of methods to enrich and expand these rare cells allows more detailed research regarding their function in prostatic disease. We wondered whether the new methods are useful for enrichment of stem cells from inducible transgenic mice and their subsequent genetic ex vivo manipulation.

Materials & Methods: Tamoxifen inducible-Cre transgenic mice were crossed with Rosa26-eYFP transgenic Reporter mice. Prostate basal stem cell lines out of these mice (Cre/Rosa-eYFP) were generated by our previously described methods to enrich and amplify basal prostate stem cells ex vivo (microdissection of the prostate, enzymatic digestion, MACS EpCAM enrichment, stem cell culture using experimentally defined combinations of culture flask surface and serum-free growth factor conditioned media).

Results: The generated basal prostate stem cell line from double transgenic mice was treated with various tamoxifen regimens to induce gene deletion at a self-defined time point. Treatment with dihydrotamoxifen in vitro (500nM and 2µM) lead to successful deletion of the loxP flanked STOP sequence, thus expression of the EYFP (Enhanced Yellow Fluorescent Protein) was enabled in these cells. Flow cytometry results subsequently detected positive EYFP fluorescence (45% with 500nM, 51% with 2µM) while viability of basal prostate stem cells could be preserved as measured by propidium iodide co-staining.

Conclusions: This proof of principle of genetic alterability in ex vivo enriched and amplified primary prostate basal stem cells reveals the usefulness of our developed techniques in future basic research projects to investigate essential pathways in aetiology of prostate cancer and prostatic diseases.