50 The role of SPOP & BRCA-1 in the regulation of estrogenic activity in prostate stem cells

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Introduction & Objectives: Speckle-type POZ Protein (SPOP) is the most frequently mutated gene in primary prostate cancer. The overall risk of prostate cancer has been reported up to 3.8-fold for men who carry BRCA1 mutations. Both SPOP and BRCA1 act as E3 ubiquitin ligases, each targeting several proteins, including estrogen receptor-α (ER-α), for proteasome-mediated degradation. In addition, the SPOP mutation in endometrial cancer impairs ER-α degradation and increases pro-oncogenic estrogen activity. Previously our laboratory identified ER-α and estrogen receptor-β (ER-β) in prostate stem cells (PSC). Estrogen's effect on PSC is enacted via ER-α and ER-β, where the binding of estrogen to ER-α stimulates PSC to self-renew whereas estrogen activation of ER-β inhibits PSC self-renewal, acting as a "brake" for PSC homeostasis. Further evaluation revealed that ER-α down-regulates ER-β in PSC thus forming a tight regulatory loop for controlling PSC self-renewal by estrogens. As such, increased PSC ER-α levels lead to reduced ER-β which, in turn, removes the "brake" to enable an increase in PSC self-renewal. Importantly, estrogen exposure has also been shown to promote prostate carcinogenesis. The objective of this study was to determine if SPOP & BRCA1, via ER-α selective E3 ligase activity, regulate PSC ER-α levels and whether this affects PSC proliferation.

Materials & Methods: Primary cultures of normal human prostate epithelial cells (PrEC) were grown in a prostasphere-based, bromodeoyuridine (BrdU) label-retention assay that permits identification of initiating stem cells within the PS. PrEC were subjected to ER-α selective E3 ligase knockdown with siRNA transfection (SPOP and/or BRCA1), confirmed by RT qPCR. Fluorescence immunocytochemistry (ICC) was used to visualize ER-α & ER-β protein levels in BrdU labeled stem cells. ICC data was analyzed with image quantification software.

Results: SPOP knockdown was achieved with mean 29% (8-54%) efficiency in PrEC and prostaspheres. BRCA1 knockdown was achieved with mean 21% (9-36%) efficiency in PrEC and prostaspheres. ICC revealed a statistically significantly elevated level of ER-α in BrdU label-retaining stem cells of both SPOP and BRCA1 knockdowns compared to scramble siRNA controls. ICC also revealed a statistically significantly suppressed level of ER-β in BrdU label-retaining stem cells of both SPOP and BRCA1 knockdowns compared to scramble siRNA controls.

Conclusions: The results indicate that both SPOP and BRCA1, via ER-α selective E3 ligase activity, regulate ER-α levels which in turn affects ER-β levels in PSC. Results also indicate that these alterations lead to changes in the ability of PrEC to form PS, indicating changes in PSC self-renewal capacity. We propose that functional SPOP & BRCA1 mutations may lead to higher stem cell ER-α expression, which may be pro-oncogenic in primary prostate cancer.