Prostate tumorigenesis in estrogen receptor β-inactivated, prostate targeted fibroblast growth factor 8b-transgenic mice

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Introduction

In the prostate, Esr1 has been suggested to mediate tumor-promoting and Esr2 anti-tumorigenic functions. Esr2 knockout (BERKO) mice have been reported to generate prostate hyperplasia as well as increased proliferation, inflammation and decreased differentiation of epithelial cells in the prostate. Fibroblast growth factor 8 (FGF-8) is a mitogenic, angiogenic and transforming growth factor, that has four isoforms in human (a, b, e, f). The level of FGF8 has been found to be elevated in breast, ovarian and prostate cancer as well as in premalignant prostatic intraepithelial neoplasia (PIN) lesions. Fgf8b-transgenic (Fgf8b-Tg) mice develop advancing stromal and epithelial prostatic changes that slowly progress to mouse PIN (mPIN) lesions and to prostate cancer with mixed features of adenocarcinoma and sarcoma at old age (Elo et al. 2010).

Our objective was to study whether inactivation of Esr2 affects prostate tumorigenesis, inflammation and stromal changes observed in Fgf8b-Tg mice.

Materials and Methods

BERKO mice (Krege et al. 1998) were bred with prostate targeted Fgf8b-Tg mice previously generated by us, to obtain Fgf8b-Tg-BERKO mice bearing two genomic modifications (Fig 1). Prostate histology of over 12-month-old WT, Fgf8b-Tg, BERKO and Fgf8b-Tg-BERKO mice were analyzed. Quantitative RT-PCR (qRT-PCR) and immunohistochemical (IHC) stainings are used to study gene expression and presence of proteins in the prostate.

Results

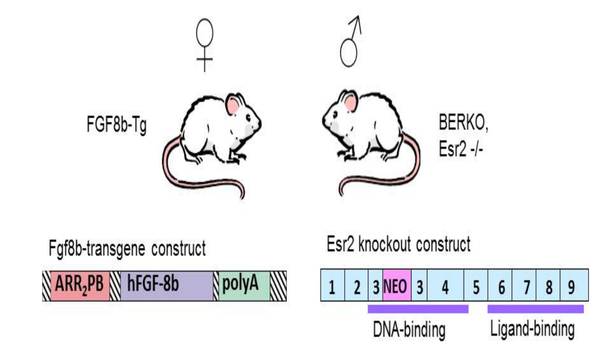

Figure 1. Fgf8b-Tg-BERKO mice (FVB/N strain) were generated by breeding female Fgf8b-Tg mice (Fgf8b+/-) with male BERKO (Esr2-/-) mice, which in the F1 generation gained mice heterozygotes for Esr2 knockout (Esr2+/-) of which half were Fgf8b-Tg-positive (Fgf8b+/-) and half negative (Fgf8b-/-). Next, Fgf8b+/- Esr2+/- female mice were bred with Fgf8b-/- Esr2-/- and Fgf8b-/- Esr2+/- male mice to obtain F2 offspring with Fgf8b+/-, Esr2-/- (Fgf8b-Tg-BERKO); Fgf8b+/-, Esr2+/+ (Fgf8b-Tg); Fgf8b-/-, Esr2-/-(BERKO); Fgf8b-/-, Esr2+/+ (WT); Fgf8b+/-, Esr2+/- and Fgf8b-/-, Esr2-/+ genotypes.

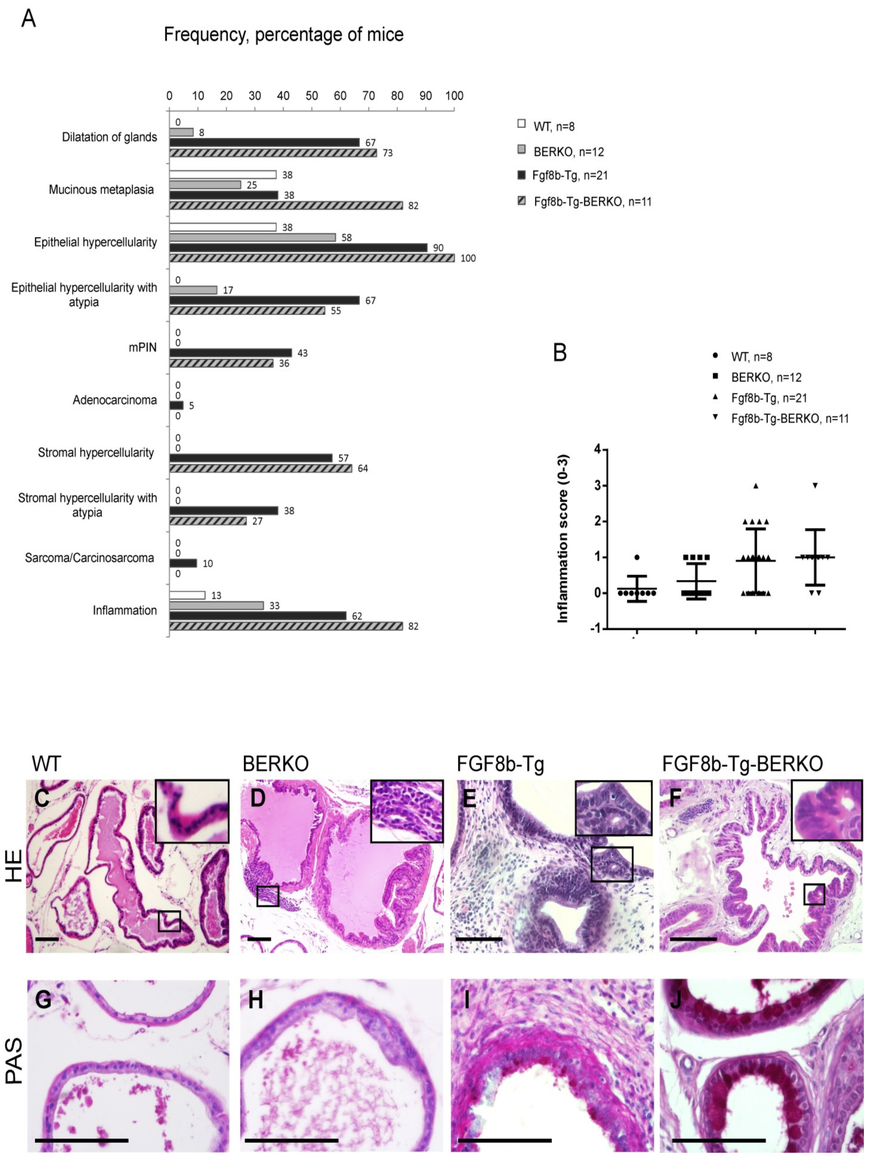

Figure 2. A) Frequency of histological changes in the prostates of 10-14-month-old WT, BERKO, FGF8b-Tg and FGF8b-Tg-BERKO mice. B) Inflammation score in the mouse prostates evaluated in the scale from 0 to 3. Mean value and standard deviation are shown. C) Normal histology of a 12.5-month-old WT mouse VP. D) Epithelial hypercellularity and inflammation in a 14-month-old BERKO mouse VP. E) mPIN stromal hypercellularity and inflammation in the VP of a 12-month-old FGF8b-Tg mouse. F) Mucinous metaplasia in the VP of a 12,5 –month-old mouse. G-H) PAS stain in the VP of WT, BERKO, FGF8b-Tg and FGF8b-Tg-BERKO mice.

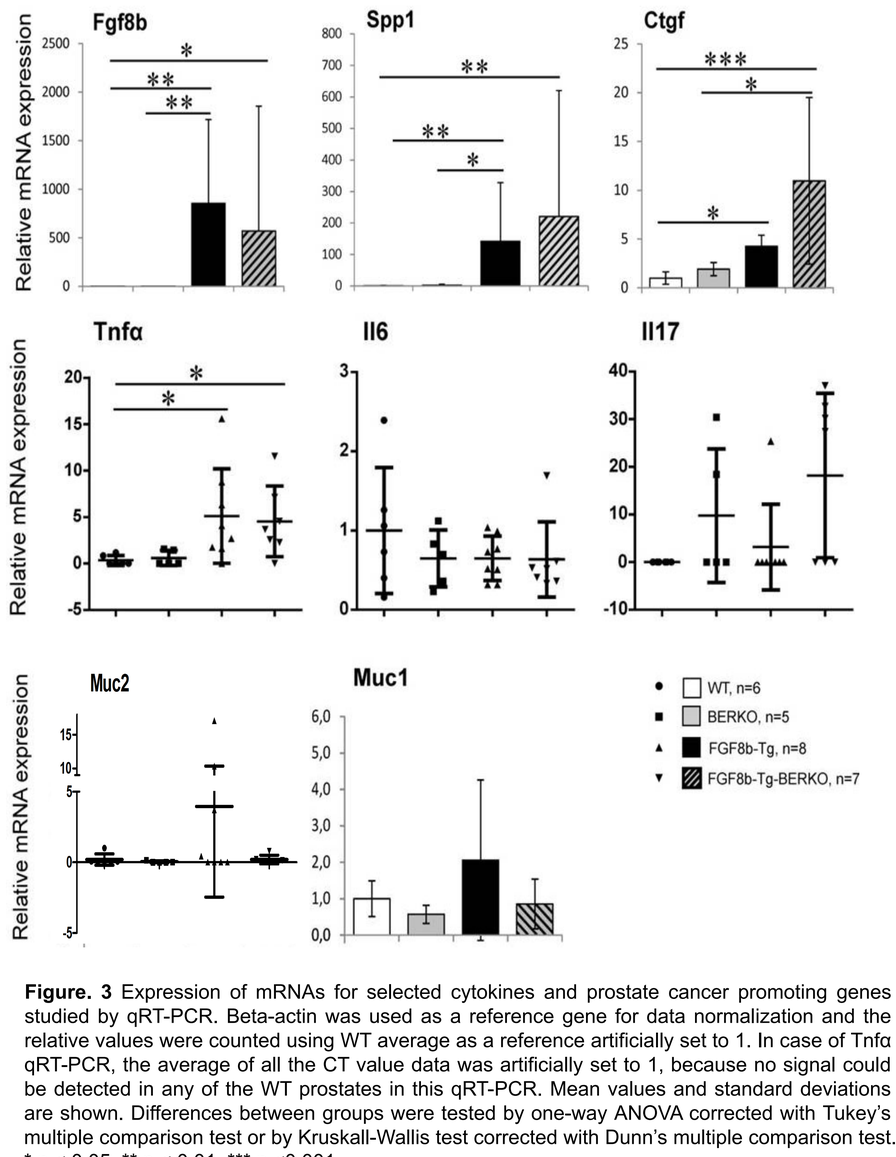

Figure. 3 Expression of mRNAs for selected cytokines and prostate cancer promoting genes studied by qRT-PCR. Beta-actin was used as a reference gene for data normalization and the relative values were counted using WT average as a reference artificially set to 1. In case of Tnfα qRT-PCR, the average of all the CT value data was artificially set to 1, because no signal could be detected in any of the WT prostates in this qRT-PCR. Mean values and standard deviations are shown. Differences between groups were tested by one-way ANOVA corrected with Tukey's multiple comparison test or by Kruskall-Wallis test corrected with Dunn's multiple comparison test. * p < 0,05, ** p < 0,01, *** p <0,001.

Conclusion

- Prostates of one-year-old Fgf-8b-Tg mice contained similar changes as previously reported, including stromal aberrations, mPIN lesions, inflammation and, in some cases, cancer .
- The prostates of one-year-old BERKO mice contained mild epithelial hypercellularity and inflammation, but no neoplastic changes (Fig 2).
- Prostate phenotype of Fgf8b-Tg-BERKO mice was mostly similar to that of the Fgf8b-Tg mice. However, mucinous metaplasia was statistically significantly ($p = 0.013$) more frequent in the prostates of Fgf8b-Tg-BERKO mice than in the Fgf8b-Tg mice (Fig 2). However, gene analysis by qRT-PCR indicated that in Fgf8b-Tg mice, both Muc1 and Muc2 has higher expression compared to Fgf8b-Tg-BERKO mice (Fig 3).
- Inflammation and stromal and epithelial hypercellularity were more frequent in the prostate of Fgf8b-Tg-BERKO mice than in the prostate of Fgf8b-Tg mice (Fig 2). Although there was no statistically significant difference between these two groups, Fgf8b-Tg-BERKO mice showed tendency to higher Il17 levels compared to other groups (Fig 3).
- The qRT-PCR results showed that the expression of mRNA for Fgf8b and the genes previously found to be upregulated in the prostates of Fgf8b-Tg mice, osteopontin (Spp1) and connective tissue growth factor (Ctgf) were also upregulated in Fgf8b-Tg-BERKO prostates (Fig 3).
- All in all, our results suggest that Esr2 may have a role in the differentiation of prostatic epithelium and in protection from inflammation but they do not support the idea of a tumor-suppressive role for Esr2.