**ELL2 and EAF2 co-regulation of AKT in prostate cancer cells**

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**Abstract**

Elongation factor for RNA polymerase II 2 (ELL2) and ELL-associated factor 2 (EAF2) are two functionally related androgen responsive gene-encoded proteins with prostate tumor suppressor characteristics. EAF2 and ELL2 have both been shown to be down-regulated in advanced prostate cancer, and mice with either Eaf2 or Eill2 deficiency developed murine prostatic intraepithelial neoplasia (mPIN), increased cellular proliferation and increased vascularity. Functional studies have revealed that EAF2 and ELL2 can bind to each other and have similar roles in regulating cell proliferation, angiogenesis and prostate homeostasis. Here, cell line experiments showed that knockdown of EAF2 or ELL2 induced an increase in proliferation and migration in C4-2 and 22Rv1 prostate cancer cells. Concurrent knockdown of EAF2 and ELL2 increased proliferation and migration similarly to the loss of EAF2 or ELL2 alone. Mice with homozygous deletion of Eil2 or heterozygous deletion of Eaf2 developed mPIN lesions characterized by increased epithelial proliferation, intraductal microvesSEL density, and infiltrating intraductal CD3-positive T-cells compared to wild-type controls. Mice with combined heterozygous deletion of Eaf2 and Eil2 developed mPIN lesions that were similar to those observed in mice with deficiency in Eaf2 or Eil2 alone. These results suggest that EAF2 and ELL2 have similar functions and are likely to require each other in their regulation of prostate epithelial cell proliferation and migration in prostate cancer cells as well as their tumor suppressive properties in the murine prostate.

**Figure 1.** Cell proliferation in EAF2- and ELL2-deficient prostate cancer cells.

**Figure 2.** Cell migration in EAF2- and ELL2-deficient prostate cancer cells.

**Figure 3.** Western blotting analysis of C4-2 cells transfected with nontargeted siRNA. (A) EAF2, (B) ELL2 as mean ± standard error of the mean from 3 to 6 mice per group. **P < 0.01, ***P < 0.001.

**Figure 4.** Combined loss of Eaf2 and Eil2 induced murine prostatic intraepithelial neoplasia in the mouse model

**Figure 5.** Effects of combined Eaf2 and Eil2 loss on epithelial proliferation in the C57BL/6J mouse prostate at age 24 mos.

**Figure 6.** Effects of combined Eaf2 and Eil2 loss on CD3-positive microvesSEL density in the C57BL/6J mouse prostate at age 24 mos.

**Figure 7.** Effects of combined Eaf2 and Eil2 loss on CD3-positive T-cells in the C57BL/6J mouse prostate at age 24 mos.

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**Concurrent EAF2 and ELL2 loss phenocopies individual EAF2 or ELL2 loss in prostate cancer cells and murine prostate**

**Figure 2.** Cell migration in EAF2- and ELL2-deficient prostate cancer cells.

**Figure 3.** Western blotting analysis of C4-2 cells transfected with nontargeted siRNA. (A) EAF2, (B) ELL2 as mean ± standard error of the mean from 3 to 6 mice per group. **P < 0.01, ***P < 0.001.