

Supplemental material and methods

Mice strains

Mouse strains used in this study are: 1) BL6 mice were maintained in Owen Witte's lab in UCLA; 2) B6;129-Kras^{tm5Tyj}/J mice were originally derived from Tyler Jacks' lab (1), and the colony was maintained in Sanaz Memarzadeh's lab in UCLA. This strain carries a point mutation (G12D), and is flanked by loxP sites. The constitutively active Kras(G12D) oncogenic protein was expressed under the presence of Cre protein (1).

Plasmids

The open reading frame of the Cre gene was cloned into the lentivector FUCRW (2), in which RFP expression is constitutively active and regulated by the CMV promoter. FUCRW-mAkt-Ires-Cre, FUCGW-AR, and FUCRW-mAkt vectors were prepared as previously described (2, 3).

Immunohistochemistry and Western blot analysis

Tumor tissues derived from the prostate regeneration assays were recovered by surgical resection of the host kidney. Transillumination or fluorescent images were taken with a dissecting microscope. Grafts were fixed in 10% buffered formalin and preserved in 70% ethanol before embedded in paraffin. Sections of tumor tissues were subjected to hematoxylin and eosin (H&E) and immunohistochemical (IHC) staining. Immunohistochemistry (IHC) was performed as described previously (4). The primary antibodies phospho-Erk (1:50, Cell Signaling), AR (1:200, Santa Cruz Biotechnology), CK5 (1:1000, Covance), CK8 (1:1000, Covance), E-Cadherin (1:250, BD Transduction Laboratories), Vimentin (1:250, Abcam), p63 (1:150, Santa

Cruz Biotechnology), phospho-AR (1:50, Imgenex), Cyclin D1 (1:25, Cell Signaling), EZH2 (1:100, BD Transduction Laboratories), and tri-methyl-Histone H3 (Lys27) (1:100, Cell Signaling) were used in the study. For Western analysis, total Erk (1:1000, cell Signaling, #9102), Phospho-Erk (1:1000, cell Signaling, #9101), EZH2 (1:1000, Cell signaling #3147), tri-methyl-Histone H3 (Lys9) (1:500, Millipore, 07-442), tri-methyl-Histone H3 (Lys27) (1:1000, Cell Signaling #9733), and Beta-Actin-Peroxidase (1:10,000, Sigma-Aldrich, #A3854) were used.

References

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