4EBP1 allows luminal prostate cancer cells to fly under the radar

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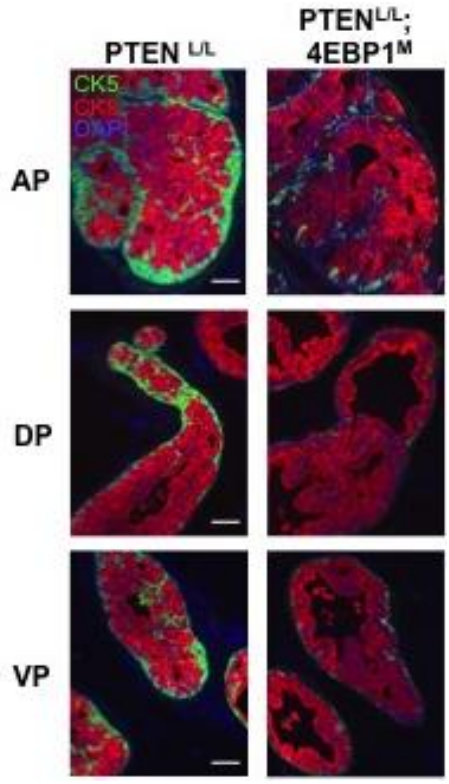

PTEN L/L prostate luminal epithelial cells can form tumors which are insensitive to inhibition of EIF4E activity in vivo. Representative immunofluorescence images of the anterior (AP), dorsal (DP), and ventral (VP) prostate in PTEN L/L prostate cancer mice with and without induction of the 4EBP1M transgene which inhibits eIF4E activity in a tissue specific manner in vivo. Basal epithelial cells (green, CK5+) are sensitive to inhibition of eIF4E activity, while luminal epithelial cells (red, CK8+) are resistant.

Image provided by Dr. Andrew Hsieh.

Whole-genome sequencing of various cancers has heralded a new era of targeted therapies and provides the tantalizing prospect of personalized medicine. These sequencing studies revealed that the PI3K-AKT-mTOR pathway is commonly altered in cancer and thus major efforts have been devoted to develop and test PI3K-AKT-mTOR inhibitors. Most, if not all, advanced prostate cancers harbor alterations in this pathway. While PI3K-AKT-mTOR pathway inhibitors have shown promise in preclinical studies, drug resistance often develops. However, it remains unknown if all types of epithelial cells present in normal or cancerous prostate glands are equally sensitive to such inhibitors. A new Fred Hutch study led by Dr. Andrew Hsieh (Human Biology and Clinical Research Divisions) leveraged a combination of a new prostate cancer mouse model, a clinical trial and detailed molecular studies to reveal that the protein synthesis inhibitor 4EBP1 is critically involved in prostate cancer initiation, progression and drug resistance. This study was recently published in the journal *Science Signaling*.

The study began with an intriguing observation from a previous preclinical trial of an ATP-site mTOR inhibitor (MLN0128) in mice that develop prostate cancer due to prostate-specific deletion of the tumor suppressor PTEN ($PTEN^{L/L}$ mice). The prostate gland is composed of two distinct epithelial cell types: luminal cells ($CK8^+$) and basal cells ($CK5^+$). Dr. Hsieh and his collaborators at UCSF observed tumors that resisted an 8-week course of treatment with MLN0128 were enriched in $CK8^+$ luminal cells, relative to $CK5^+$ basal cells. To determine the molecular basis for this differential sensitivity to mTOR inhibitors, the authors queried the protein abundance of key components of the protein synthesis machinery, including eIF4A, eIF4E, eIF4G and 4EBP1. Western blotting of fluorescence activated cell sorting (FACS)-sorted basal or luminal cells obtained from $PTEN^{L/L}$ mice showed that 4EBP1, an inhibitor of cap-dependent translation, was the only component of this machinery analyzed found to be enriched in both in normal and $PTEN^{L/L}$ luminal cells compared to basal cells. Importantly, this increased 4EBP1 expression was likely functional as luminal cells exhibited the lowest rates or protein synthesis, as demonstrated by ^{35}S methionine incorporation assays on FACS-sorted cells.

Because 4EBP1 functions primarily by inhibiting the mTOR-regulated oncogene eIF4E, the authors sought to determine the role of 4EBP1 *in vivo*. To this end, they developed a novel genetic mouse model, wherein a doxycycline-inducible expression of a mTOR-insensitive mutant (4EBP1M) was combined with the $PTEN^{L/L}$ model. Said Dr. Hsieh: "Historically it has been difficult to inhibit protein synthesis in a tissue specific inducible manner. We overcame this hurdle by developing a new prostate cancer mouse model called the $PTEN^{L/L}$; 4EBP1M mouse. Here, PTEN loss leads to prostate cancer, and the addition of doxycycline to the drinking water induces a transgene, which can inhibit protein synthesis *in vivo*. Using this model we demonstrate that eIF4E mediated translation is essential for prostate cancer initiation and progression." Expression of 4EBP1M markedly decreased both the development and maintenance of prostate cancer, concomitant with an increase in apoptosis. Strikingly, 4EBP1M expression led to an enrichment of luminal cells, suggesting basal cells, but not luminal cells, require eIF4E for growth and survival. These findings along with *in vitro* molecular studies provide a mechanistic rationale for why tumor prone luminal epithelial cells are uniquely resistant to PI3K pathway inhibitors.

Finally, the authors sought to determine whether increased 4EBP1 abundance also occurs in prostate cancer patients. Comparison of pre-treatment with post-treatment prostate cancer tissue from an ongoing clinical trial with a PI3K inhibitor (BKM120) revealed an enrichment of $CK8^+$ luminal cells that expressed high levels of 4EBP1. In conclusion, this study showed that cell type-specific expression of the tumor suppressor 4EBP1 in luminal prostate cancer cells primes cells for either sensitivity or resistance to PI3K-AKT-mTOR inhibitors and may have utility in predicting drug

responses in patients. "Our laboratory has discovered that protein synthesis rates of specific prostate epithelial cell types can tune them for resistance to an emerging line of PI3K pathway inhibitors," said Hsieh. Reflecting on the meaning of these findings, Hsieh said, "Many questions remain. If low protein synthesis leads to drug resistance, will more potent inhibitors of protein synthesis lead to drug sensitivity? What are the downstream translationally regulated transcripts that direct the therapeutic response and drug resistance?"

Hsieh AC, Nguyen HG, Wen L, Edlind MP, Carroll PR, Kim W, Ruggero D. 2015. Cell type-specific abundance of 4EBP1 primes prostate cancer sensitivity or resistance to PI3K pathway inhibitors. *Sci Signal*. 8(403):ra116. doi: 10.1126/scisignal.aad5111.

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