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A Functional Genetic Screen Identifies the Phosphoinositide 3-kinase Pathway as a Determinant of Resistance to Fibroblast Growth Factor Receptor Inhibitors in *FGFR* Mutant Urothelial Cell Carcinoma

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Abstract

Activating mutations and translocations of the *FGFR3* gene are commonly seen in urothelial cell carcinoma (UCC) of the bladder and urinary tract. Several fibroblast growth factor receptor (FGFR) inhibitors are currently in clinical development and response rates appear promising for advanced UCC. A common problem with targeted therapeutics is intrinsic or acquired resistance of the cancer cells. To find potential drug targets that can act synergistically with FGFR inhibition, we performed a synthetic lethality screen for the FGFR inhibitor AZD4547 using a short hairpin RNA library targeting the human kinome in the UCC cell line RT112 (*FGFR3-TACC3* translocation). We identified multiple members of the phosphoinositide 3-kinase (PI3K) pathway and found that inhibition of *PIK3CA* acts synergistically with FGFR inhibitors. The PI3K inhibitor BKM120 acted synergistically with inhibition of FGFR in multiple UCC and lung cancer cell lines having *FGFR* mutations. Consistently, we observed an elevated PI3K-protein kinase B pathway activity resulting from epidermal growth factor receptor or Erb-B2 receptor tyrosine kinase 3 reactivation caused by FGFR inhibition as the underlying molecular mechanism of the synergy. Our data show that feedback pathways activated by FGFR inhibition converge on the PI3K pathway. These findings provide a strong rationale to test FGFR inhibitors in combination with PI3K inhibitors in cancers harboring genetic activation of *FGFR* genes.

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Fibroblast growth factor receptors (*FGFRs*) are activated in a subset of urothelial cell carcinoma (UCC), most commonly by *FGFR3* mutation or overexpression of *FGFR1* [1]. Clinical trials with FGFR inhibitors (FGFRi) are currently ongoing in UCC. Initial results with BGJ398 showed encouraging response rates [2], though information on the durability of these responses is currently lacking. Akin to other molecularly-targeted therapies, resistance is likely to be a major concern. Resistance to FGFR inhibition was observed *in vitro* (Fig. 1A, Supplementary materials and methods): the *FGFR-TACC3* translocated cell line RT112 responded initially, but cells quickly adapted to AZD4547, an inhibitor of FGFR1, FGFR2, and FGFR3 (weaker activity against FGFR4). Resistance to targeted therapies often develops through feedback activation of additional signaling pathways: one such example is synergy between FGFRi with

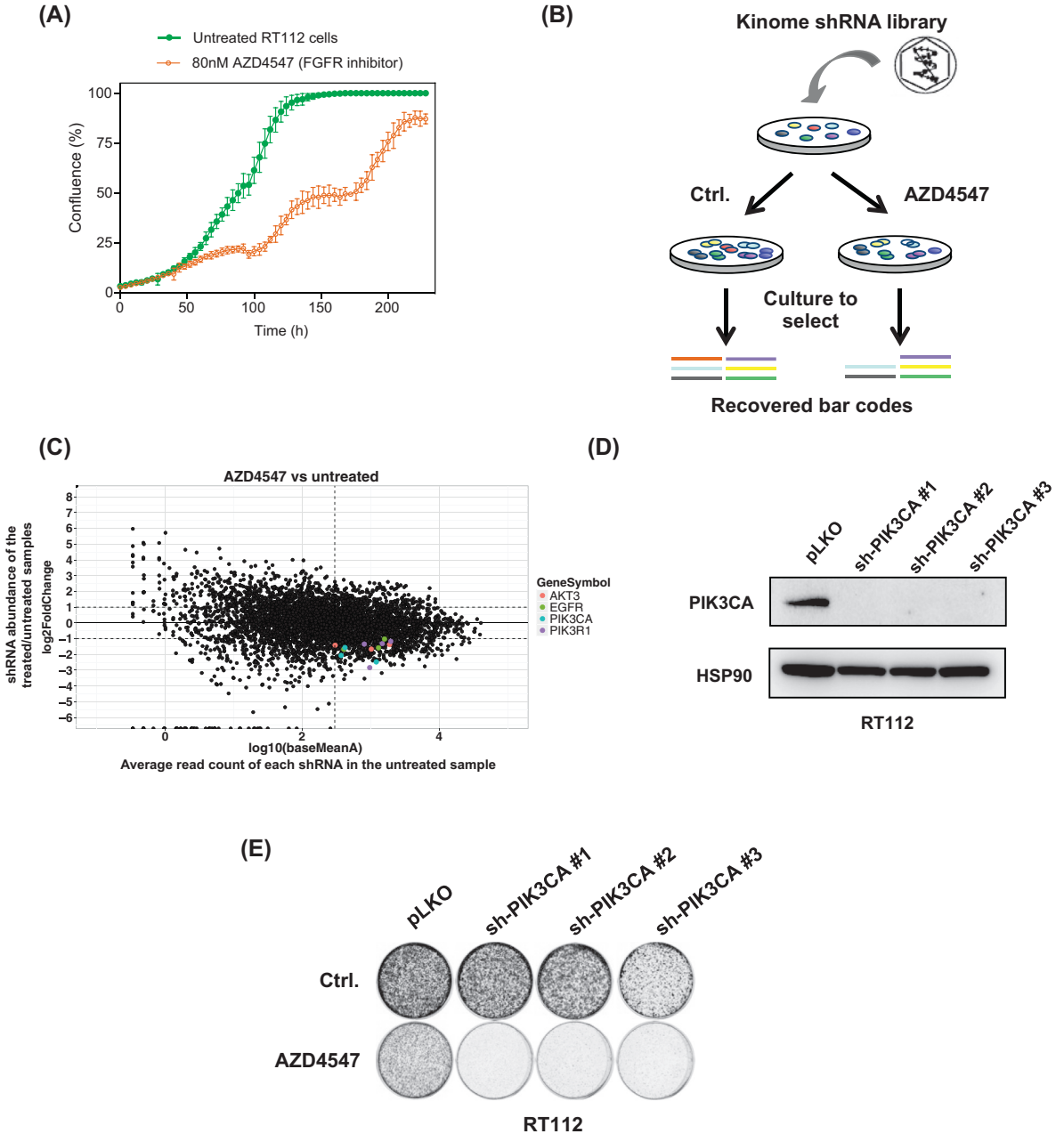

Fig. 1 – PIK3CA suppression enhances sensitivity to fibroblast growth factor receptor (FGFR) inhibition in UCC. (A) RT112 cells were treated with AZD4547 (80 nM) and viability was followed using an incucyte assay. Error bars represent four biological replicates. (B) Outline of synthetic lethality short hairpin RNA (shRNA) screen for enhancers of AZD4547 sensitivity. Human kinome shRNA library polyclonal virus was produced to infect RT112 cells, which were then left untreated (Ctrl.) for 10 d or treated with 30 nM AZD4547 for 14 d. After selection, shRNA inserts from both arms were recovered by polymerase chain reaction and their abundance was quantified by deep sequencing. (C) Representation of the relative abundance of the shRNA barcode sequences from the shRNA screen. The y-axis shows log2 of the fold change of shRNA abundance of the treated and untreated samples. The x-axis indicates the log10 of the average read count of each shRNA in the untreated sample. *shPIK3CA*, *shPIK3R1*, *shAKT3*, and *shEGFR* identified as the top hit according to the presence of at least three independent shRNAs in three biological screen replicates. (D) The level of knockdown of *PIK3CA* by three different shRNAs was measured by PIK3CA protein levels by western blot. HSP90 protein expression is used for normalization. (E) The functional phenotypes of independent *shPIK3CA* vectors are indicated by colony formation assay in 50 nM AZD4547. The cells were fixed, stained, and photographed after 10 d.

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