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Reverse Engineering Strategy for New Targets Identification

LIDE

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Abstract

The issue of drug resistance in cancer is very similar to the field of infectious disease, either existing before treatment (intrinsic) or generated during therapy (acquired), and highly affected by cell endogenous factors and/or the surrounding environment. Acquired resistance can occur several months after targeted therapy via alternations of drug targets due to genomic mutations of the targets or epigenetic transcriptional/translational changes, for example, a C797S mutation impairs AZD9291 binding to the EGFR kinase domain. To overcome this, novel, well-designed chemical compounds targeting new mutations are urgently needed, as well as the identification of potential new oncogenes/susceptible genes driving drug resistance.

In consideration of the heterogeneity of primary tumor tissues, LIDE has established a functional cost-effective in vitro and in vivo screening method. This “reverse engineering” strategy for targets identification starts by leveraging patient-derived conditional reprogramed cancer (CR) cell lines and our IO-FIVE (Immuno-Oncology drugs Five In Vivo Efficacy) test. Basically, pro-siRNA library or CRISPR/sgRNA library is carried out in CRs for candidates hunting, filtered against bioinformatic scanning from patient datasets, in combination with drug-response evaluation across multiple clinical biopsies in mice cancer trials. Finally, potential targets are validated in vivo by CRISPR, shRNA silence or targeted compounds. This has been validated against known factors (EGFR C797S, HER2/MET amplification, RAS and MAPK activation)[1], but also let to the discovery of epigenetic factors, protein translation kinases, mitochondrial/ peroxisomal transporters and cell membrane lectins that may contribute to AZD92921 resistance in vivo. And one previously unknown acyltransferase member has been identified as key driver gene for pan-cancer development. This is a promising therapeutic target given patients with high expression level of this enzyme have had poor survival outcomes, resultant from uncontrolled tumor growth and immunotherapy resistance.

Materials and Methods

Cancer cells: Commercial cancer cell lines, e.g. A375, K562, MDA-MB-231, were from either ATCC, China Cell Bank (Shanghai) or our collaborators. Human biopsies, fresh human surgery tumor tissues were from collaborated hospitals (HMEC approval). Immune deficient mice (e.g. Balb/c Nude or NCG) were used to grow the primary tumor tissues.

Human conditionally reprogrammed (CR) cell lines reconstruction[2]: The tumor tissue, clinically resistant or sensitive to medicine, was cut into 1~3 mm^3 fragments, suspended in digestion solution, 37℃ for 1.5 hours. Single cells were collected through 70uM strainer, cultured with OncoVee™ Conditional Reprogramming Cell Culture Kit, to generate stable cell lines.

Pro-siRNA or CRISPR *in vitro* and *in vivo* screening: commercial Pro-siRNA library and CRISPR/CAS9-sgRNA lentivirus library were transduced into cancer cell lines, respectively. CellTiter Glo (Promega) assay was the readout of Pro-siRNA in vitro proliferation screening for individual gene silence. Pooled CRISPR lentivirus transduced cell lines (MOI=0.1) was selected by puromycin and expanded before target drugs treatment *in vitro*, or transferred into immune deficient mice then dosed with target drugs (e.g. AZD9291, 5mg/kg p.o., 3 weeks). Tumors were collected at the endpoint for genomic DNA isolation, and gRNA abundance was analyzed by deep-sequence.

Individual gene knock down/out by shRNA and CRISPR/cas9: wild-type or cas9-transduced cancer cell lines, were infected by target gene shRNA virus or sgRNA virus, selected by puromycin before expansion. Gene modified cancer cells validated by WB, could be used in vitro functional proliferation assay upon drug treatment, or in vivo implantation into immune deficient mice with or without drug treatment.

Results

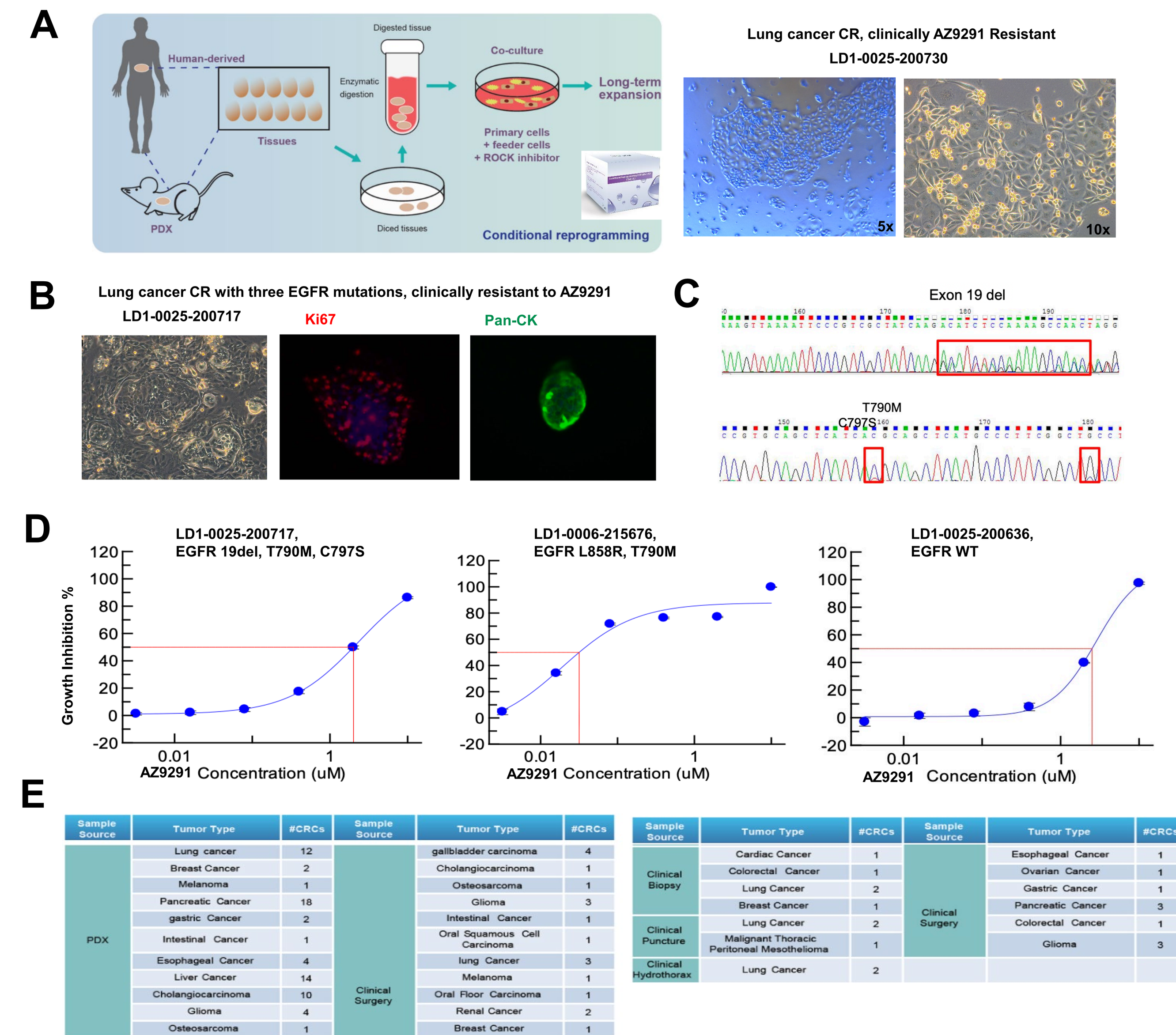

Figure 1. Conditional reprogrammed cells reconstruction

A. Schematic of conditional reprogrammed (CR) cell generation by using human biopsies or PDX tissues(left). Images of LD1-0025-200730 lung cancer CR (right). **B.** Morphology, Ki67 and Pan-CK staining of LD1-0025-200717 lung cancer CR cell. **C.** Sanger sequencing for EGFR of LD1-0025-200717 CR cells. **D.** Growth inhibition curve of LD1-0025-200636 (EGFR WT) cell line, LD1- 0006-215676 (EGFR L858R/T790M) cell line and LD1-0025-200717 (EGFR 19del, T790M & C797S mutant) cell line. **E.** Current LIDE CR cell line library.

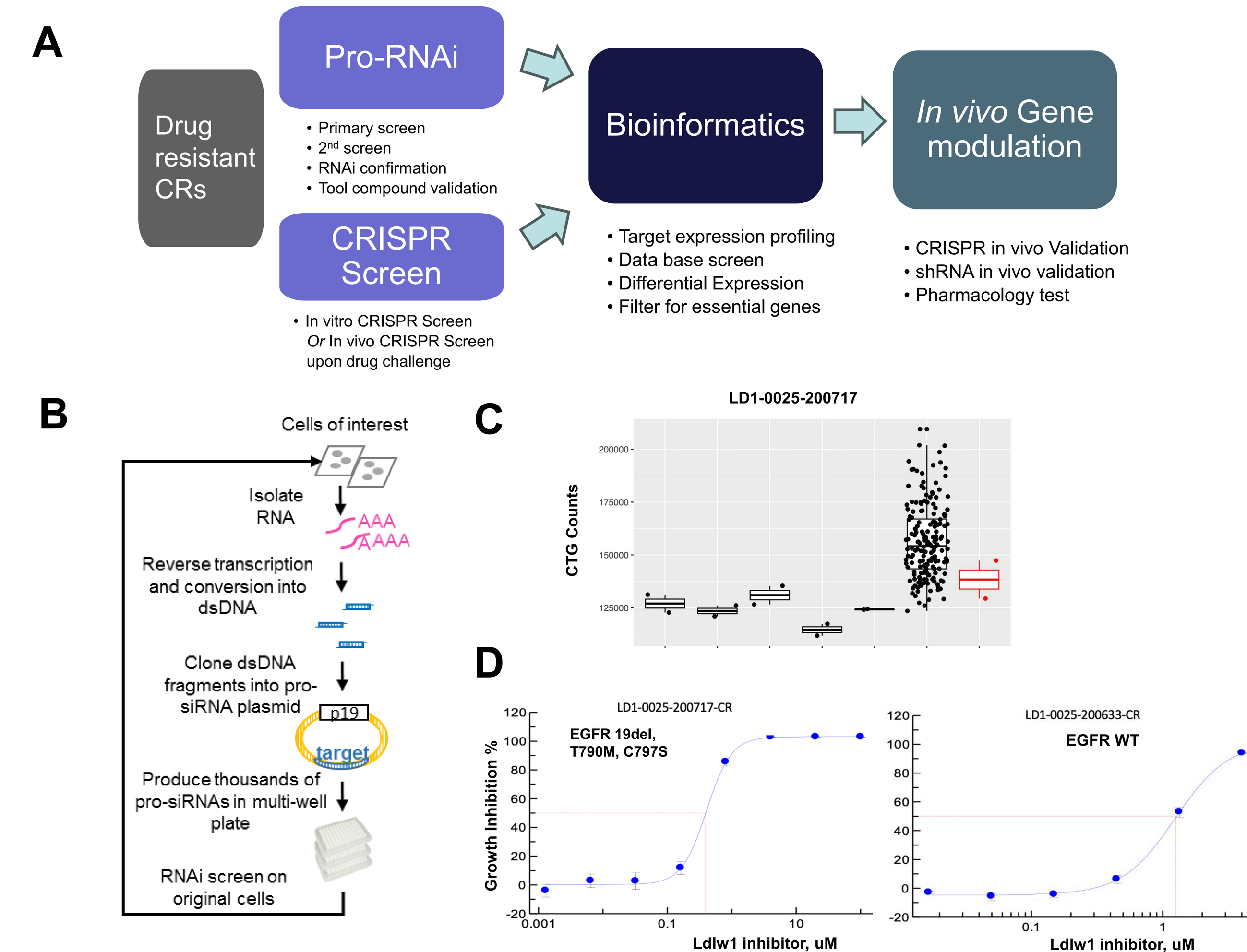

Figure 2. New targets identification by reverse engineering strategy.

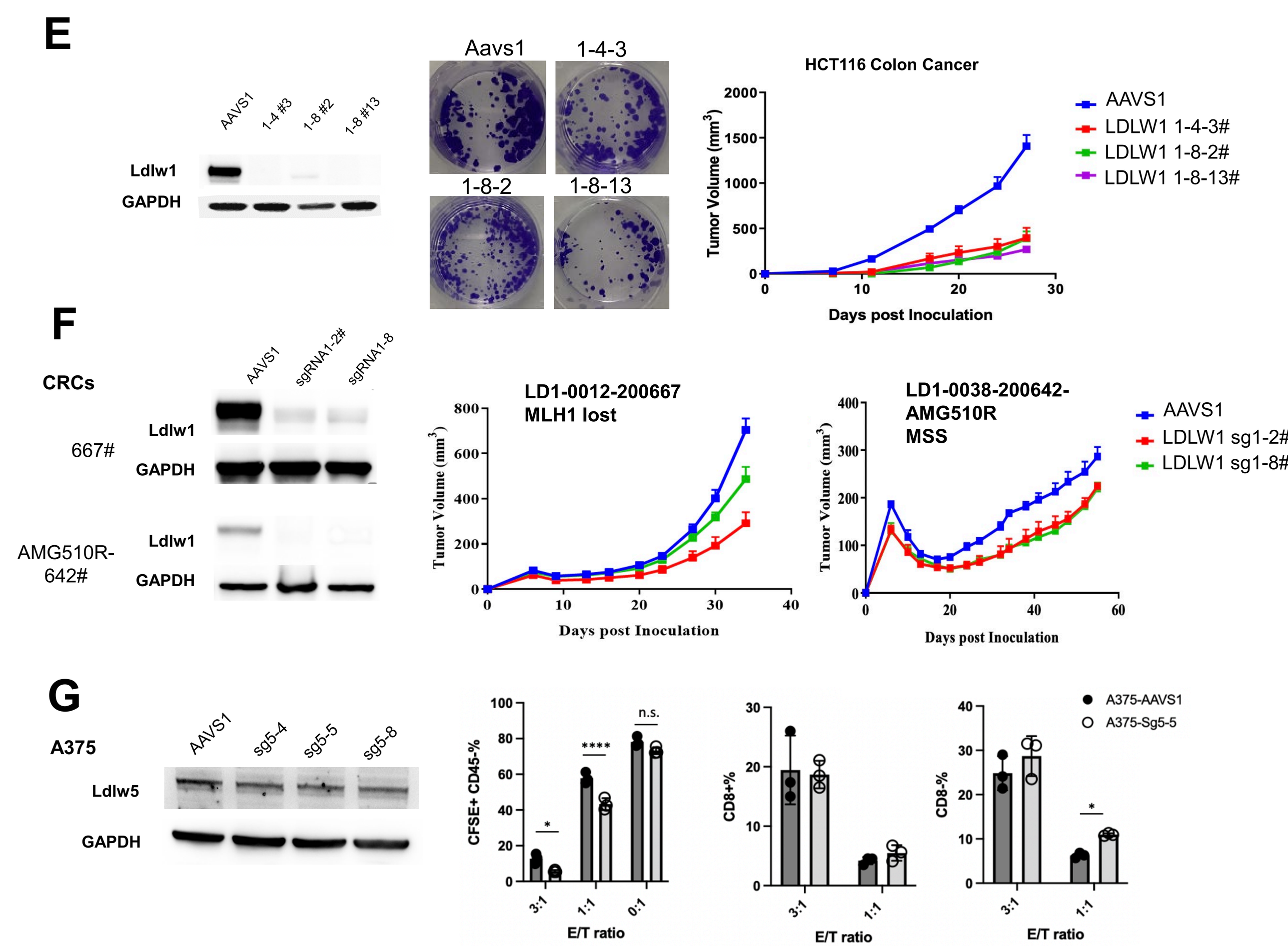

Figure 2(continued) New targets identification by reverse engineering strategy

A. Schematic of reverse engineering strategy for targets discovery by using (drug-resistant) conditionally reprogrammed CR cells. **B.** Platform for efficient pro-siRNA pull-down and functional screening[3]. **C.** CellTiter-Glo assay of LD1-0025-200717 CR after self-Pro-siRNA library transduction. Each dot represents single pro-siRNA. **D.** The growth inhibition curve of Ldlw1 inhibitor for LD1-0025-200717(EGFR 19Del T790M C797S) and LD1-0025-200633(EGFR WT) CRs. **E.** In vitro tumor colony form assay and in vivo growth curve of Hct116 cell after Ldlw1-KO. **F.** in vivo growth curve of LD1-0012-200667(MLH1 lost) and AMG510 Resistent LD1-0038-200642 (MSS) after Ldlw1-KO. **G.** *Ldlw5* impaired A375 melanoma cells were sensitive to primary human T cells.

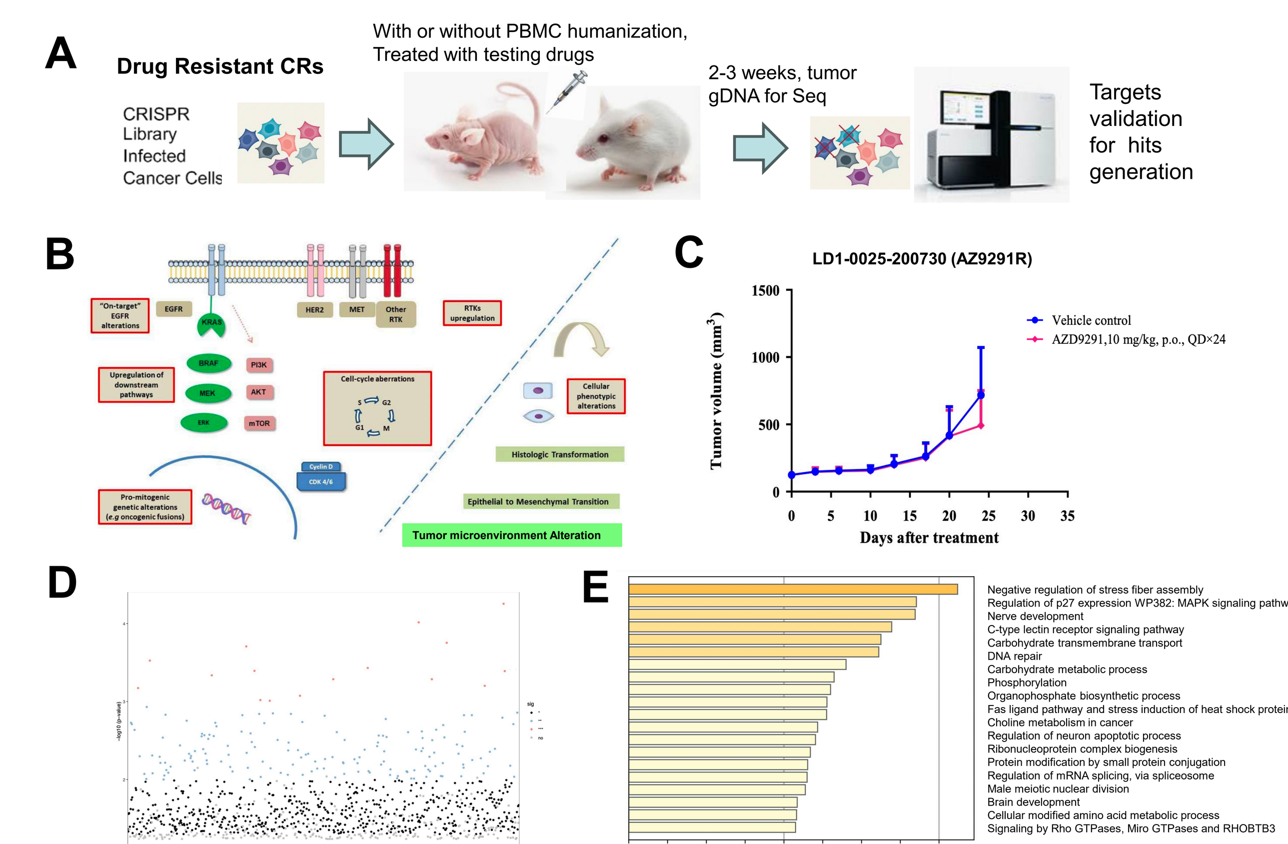

Figure 3. In vivo CRISPR screening for drug-resistant genes

A. Schematic of *in vivo* CRISPR screening in patients-derived drug-resistant CRs for targets identification. **B.** Overview of mechanisms of resistance to AZD9291/ Osimertinib[4]. **C.** In vivo tumor growth curve of sgRNA library lentiviral infected LD1-0025-200730 CR upon AZD9291 treatment. **D.** Bubble plots show the top 1000 enriched genes(potential AZD9291 resistant) identified by in vivo CRISPR screen. **E.** Bar graph of enriched pathways/clusters in top 100 genes by Metascape, colored by p-values

Figure 4. IO-FIVE (Immuno-Oncology Fast In Vivo Efficacy test) for cancer therapeutic targets discovery.

A. Schematic of functional IO-FIVE assay. **B.** CellTiter-Glo (ATP) quantification and FACS (CD45+SSC-A^{low}%) analysis of two adult acute myeloid leukaemia (AML) patients after daratumumab, Sintilimab and venetoclax treatment in mice IO-FIVE. **C.** Cell phenotyping (CD45, CD33, CD38, CD3, CD8) of the two AML patients by FACS. **D.** Heatmap of differential genes in the two AML patients by RNA-Seq(left), Histogram of cancer(CD34, BTK, LDLW11, NT5E), TME and immune regulatory genes(TGFBR2, HAVCR2, CD8A, CD274, PDCD1, TCF7, Bcl6) between two samples. **E.** Overall survival of cancer patients with high or low expression level of ldlw11 gene. **F.** Ldlw11 knock down suppresses K562 or A375 cancer in vivo development. **G.** In vivo tumor growth curve of LD1-0025-200730 CR after ldlw11 silence. **H.** Ex vivo FACS analysis after ldlw11 silence in LD1-0025-200730 model.

Summary and Conclusion

- 100 conditional reprogrammed (CR) cell lines have been successfully constructed for various functional assays.
- CR based “reverse engineering” strategy is a powerful tool for targets identification by using pro-siRNA or/and CRISPR/sgRNA library.
- Patient biopsies based IO-FIVE (Immuno-Oncology Fast In Vivo Efficacy test) mice trial is one cost-effective method for novel cancer driver gene discovery.

References

1. Leonetti A, et al. Resistance mechanisms to osimertinib in EGFR-mutated non-small cell lung cancer. Br J Cancer. 2019..
2. Liu X, et al. ROCK inhibitor and feeder cells induce the conditional reprogramming of epithelial cells. Am J Pathol. 2012 Feb;180(2):599-607.
3. Huang L, et al. Production of highly potent recombinant siRNAs in Escherichia coli. Nat Protoc. 2013 Dec;8(12):2325-36.
4. Gomatou G, et al. Osimertinib Resistance: Molecular Mechanisms and Emerging Treatment Options. Cancers (Basel). 2023 Jan 30;15(3):841.