Abstract title

High-dimensional arrayed CRISPR screens to deliver mechanistic insights and prioritise targets in ER+ breast cancer.

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Abstract:

Three-quarters of breast cancers are Estrogen Receptor-positive (ER+). Endocrine therapies are a backbone therapy for ER+ breast cancer patients, and the combination of fulvestrant (Estrogen degrader) with palbociclib (CDK4/6 inhibitor) is a standard-of-care (SoC) therapy in advanced ER+ breast cancer. However, the emergence of resistance remains a challenge that limits clinical response to the SoC. To identify novel mechanisms of resistance or sensitisation to fulvestrant and its combination with palbociclib, we performed genome-wide pooled CRISPR screens in 4 ER+ breast cancer cell lines as part of an integrated CRISPR screening pipeline for target discovery. A set of 85 hit genes were progressed to follow up high throughput validation using an arrayed CRISPR platform to provide detailed mechanistic insights and annotate the impact of gene knockout (KO) on key pathways in breast cancer (ER transcription, cell cycle, PI3K-mTOR, Myc etc). To this end, we established a new workflow that couples arrayed CRISPR screening to a high throughput qPCR assay using the Fluidigm BioMark HD platform, enabling us to profile the expression of 24 genes across 1000s of samples. The combined CRISPR-qPCR screen exhibited high sensitivity and successfully identified expected modulation of ER biomarkers expression by both fulvestrant and ESR1 KO. Using this approach, together with high content microscopy CRISPR screens assessing PI3K, mTOR and MAPK signalling, we generated a broad and integrated biomarker dataset that is providing insights into how genes modulate response to fulvestrant, palbociclib or their combination across cell lines. Collectively, these CRISPR datasets are helping to prioritise novel candidate targets and biomarkers for the drug discovery pipeline with the long-term goal of improving the SoC for ER+ breast cancer patients.