Chromatin remodelling is involved in resistance to CDK4/6 inhibitors in ER+ breast cancer

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Introduction and Methods

About 75% of breast cancers (BC) express estrogen receptor α (ER+) (1). The cyclin-dependent kinase 4/6 (CDK4/6) inhibitors palbociclib, abemaciclib and ribociclib, in association with endocrine therapy, are the gold standard treatment in ER+/HER2- metastatic BC (2). Preclinical and clinical studies using whole exome (WES) and/or targeted sequencing methodologies have revealed various genomic alterations responsible of resistance to CDK4/6 inhibitors (3).

Despite an undoubted benefit of cyclin-dependent kinase 4/6 inhibitors (CDK4/6i) in estrogen receptor positive (ER+) breast cancer (BC), resistance eventually occurs. However, DNA-Sequencing studies aiming at identification of genomic abnormalities did not comprehensively assess the landscape of resistance, underestimating other biological mechanisms that cause treatment failure.

We explored whether changes in three-dimensional (3D) chromatin landscape, potentially induced by single-nucleotide variants (SNVs) of non-coding regulatory regions, are involved in resistance to CDK4/6i.

We generated ER+/HER2- palbociclib-resistant T47D and MCF7 BC cells (T47D-PR and MCF7-PR), by exposing parental cells to increasing doses of the drug until resistance occurred. In resistant and parental cells, we performed: 1) High-throughput Chromosome Conformation Capture (Hi-C), to investigate 3D chromatin remodelling associated with drug resistance; 2) Whole Genome Sequencing (WGS), to detect SNVs, insertions or deletions (InDels, <50 bp) acquired in PR cells; 3) ATAC-Seq to identify transcriptional factors (TFs) potentially leading to drug resistance and 4) RNA-Seq for gene expression (data not showed). The results indicate that SNVs and chromatin remodelling are involved in resistance to CDK4/6i. Results from our study may help to identify novel therapeutic vulnerabilities in ER+ BC.

Results

1. Generation of palbociclib-resistant ER+ breast cancer cells

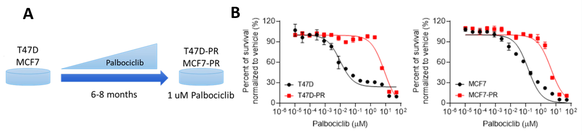
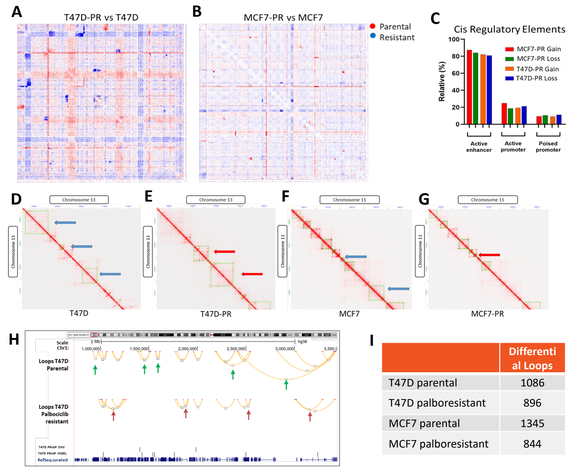


Figure 1: A. Schematic depiction of generation of palbociclib-resistant T47D and MCF7 cells. **B.** Dose-response curves of T47D, T47D-PR, and MCF7 and MCF7-PR cells treated with palbociblib. After 7 days of treatment, cells were counted on a Coulter counter. Each data point represents the percentage of cell viability relative to vehicle-treated controls.

2. High-throughput Chromosome Conformation Capture (Hi-C) reveals 3D chromatin remodelling in palbociclib-resistant cells



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	Differential Loops
T47D parental	1086
T47D palboresistant	896
MCF7 parental	1345
MCF7 palboresistant	844

Figure 2: (A-B). Hi-C Heatmaps of differential chromosomal interactions between palboresistant (PR) and parental cells. In blue and red unique interactions in PR and parental cells, respectively. Each pixel in the heatmaps represents the number of proximally ligated molecules sequenced between those two bins in the genome. **C.** Distribution of CREs in anchors of differential chromosomal interactions. We found 2,189 differential interactions (loops) between T47D and T47D-PR cells (FDR < 0.05): 844 interactions were gained in T47D-PR cells; 1,345 were exclusive for T47D, therefore they were lost in T47D-PR cells. Next, we found 1,982 differential interactions between MCF7 and MCF7-PR cells. Among them, 896 and 1,086 interactions were gained or lost in MCF7-PR cells, respectively. Examples of TADs, shown in green boxes in **(D)** T47D, **(E)** T47D-PR, **(F)** MCF7 and **(G)** MCF7-PR. Blue and red arrows indicate TADs that are lost and gained in PR cells, respectively. In T47D-PR, compared to T47D parental cells, we found 356 gained and 1,406 lost TADs, based on different TAD boundaries. We found 2,033 TADs that were shared between two cells lines. In MCF7-PR, compared to MCF7 parental cells, we found 498 and 783 gained and lost TADs, respectively. Instead, 3,874 TADs were shared between cell lines. **H.** Example of changes in loops structure. Green arrows indicates lost loops, red arrows acquired ones. **G.** Differential loops found.

3.Whole Genome Sequencing (WGS) identified acquired genomic alterations in palbociclib-resistant cells

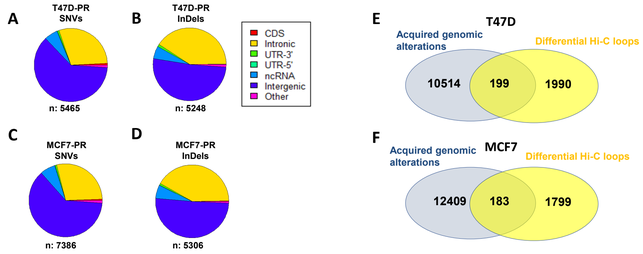


Figure 3: A-D. WGS acquired mutations distribution of T47D-PR and MCF7-PR cells. In T47D-PR cells, compared to parental T47D cells, we found 5,465 acquired SNVs **(A)** and 5,248 InDels **(B)**. 3,374 (61.7%) and 1,610 (29.4%) SNVs occurred at intergenic and intron genomic regions, respectively; 2,695 (51.3%) and 2,137 (40.7%) InDels occurred at intergenic and intron regions, respectively. In MCF7-PR cells we found 7,386 acquired SNVs and 5,206 InDels: 4,602 (62.3%) and 2,102 (28.5%) SNVs occurred at intergenic and intron regions **(C)**; 2,629 (50.5%) and 2,165 (41.6%) InDels occurred at intergenic and intron loci, respectively **(D)**. **E-F.** Venn diagram indicating the number of differential loops containing at least one acquired alteration in T47D and MCF7 respectively.

4. ATAC-Seq identified FOS family members among drivers of chromatin remodelling in palbociclib-resistant cells

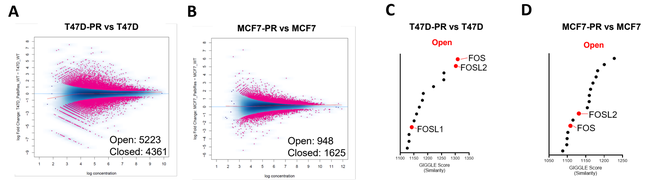


Figure 4: A. ATAC-Seq results in T47D-PR vs T47D and **(B)** MCF7-PR vs MCF7 cells. Setting a cut-off of LogFC <-1 and >1 we found **(A)** 5,223 and 4,361 newly closed and open regions, respectively; **(B)** 948 and 1,625 newly closed and open regions, respectively. **(C)** Similarity scores between open regions and datasets of ChIP-Seq for transcription factors in T47D-PR and **(D)** MCF7-PR cells (Toolkit for Cistrome Data Browser) for open regions. FOS family members labelled (red).

5. ERα cistrome is reprogrammed in palbociclib-resistant cells

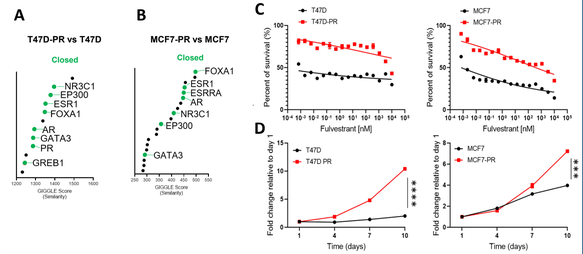


Figure 5: A. Similarity scores between closed regions and datasets of ChIP-Seq for transcription factors in T47D-PR and **(B)** MCF7-PR cells (Toolkit for Cistrome Data Browser). ERα and ERα-binding partners are labelled (green). **C.** Dose response curves of of T47D, T47D-PR (left), MCF7 and MCF7-PR (right) cells treated with fulvestrant. **D**. Growth of T47D, T47D-PR (left), MCF7 and MCF7-PR (right) cells in E2-deprived media (*** p<0.001; **** p<0.0001).

Conclusions

- Genomic alterations in non-coding regulatory regions and chromatin remodelling are involved in resistance to CDK4/6i in ER+ breast cancer models.
- FOS family members might be responsible of transcriptional reprogramming associated with resistance.
- This phenomenon could cause a reduced dependency on ERα transcriptional activity, ultimately leading to antiestrogen resistance.
- Further investigation of mechanisms of chromatin remodelling might identify novel therapeutic vulnerabilities in the context of CDK4/6 inhibitors resistance.

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