Real-World Clinical Genomics Study of HR+/HER2- Metastatic Breast Cancers Treated by CDK4/6i plus Endocrine Therapies Revealed a Drug Resistant Tumor Segment Characterized by ER Independence

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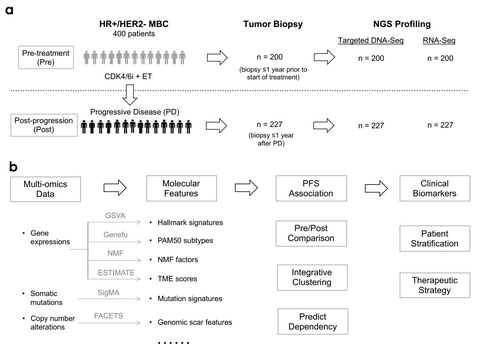
INTRODUCTION

CDK4/6 inhibitors (CDK4/6i) plus endocrine therapies (ET) are the standard-of-care for hormone receptor–positive/human epidermal receptor 2–negative metastatic breast cancer (HR+/HER2− MBC). However, drug resistance remains a major unmet need. Investigations of drug resistance mechanisms has been hampered by a dearth of tumor molecular profiling data from the post-progression setting. To address this challenge, we have conducted a real-world clinical genomics study to better understand the molecular mechanism of CDK4/6i resistance and to stratify patients based on integrated multi-omics profiles.

METHODS

We retrospectively analyzed a multi-omics dataset of 400 HR+/HER2- MBC patients who received CDK4/6i plus ET and developed progressive disease (PD) from the de-identified Tempus database. Pre-treatment and post-progression biopsies were taken ≤ 1 year prior to starting the CDK4/6i treatment or following PD respectively. Tempus xT next-generation sequencing (DNA-seq of 648 genes) and RNA sequencing assays were performed on 427 tumor FFPE samples, including 200 pre-treatment, 227 post-progression and 27 longitudinal pairs.

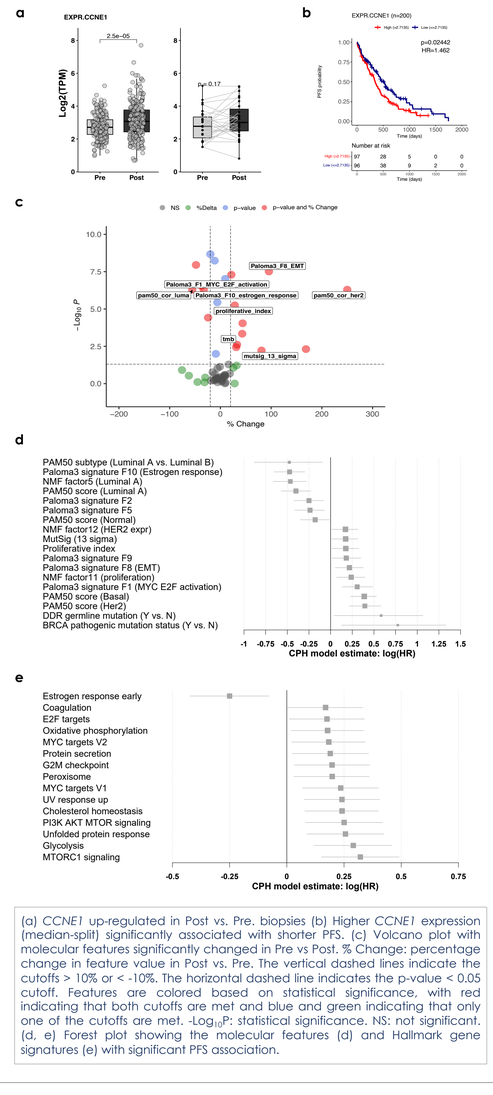
Figure 1. Overview of Study Design & Analysis



(a) Overview of cohort and dataset. (b) Data analysis workflow. PFS: Progression Free Survival. GSVA: Gene Set Variation Analysis. NMF: Non-negative Matrix Factorization. TME: tumor microenvironment. SigMA: Signature Multivariate Analysis.

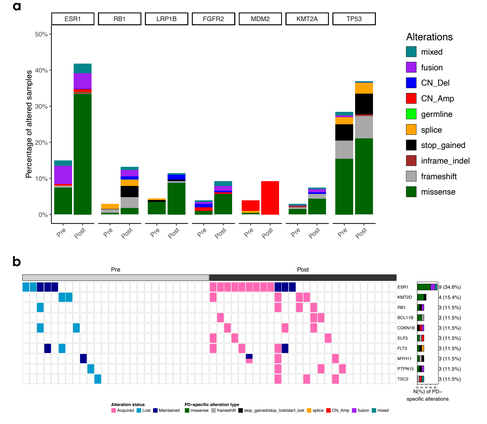
RESULTS

Figure 2. Molecular Features Associated with Disease Progression



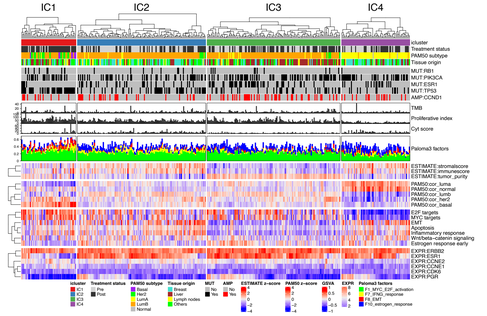
(a) *CCNE1* up-regulated in Post vs. Pre. biopsies (b) Higher *CCNE1* expression (median-split) significantly associated with shorter PFS. (c) Volcano plot with molecular features significantly changed in Pre vs Post. % Change: percentage change in feature value in Post vs. Pre. The vertical dashed lines indicate the cutoffs > 10% or < -10%. The horizontal dashed line indicates the p-value < 0.05 cutoff. Features are colored based on statistical significance, with red indicating that both cutoffs are met and blue and green indicating that only one of the cutoffs are met. -Log_{10}P: statistical significance. NS: not significant. (d, e) Forest plot showing the molecular features (d) and Hallmark gene signatures (e) with significant PFS association.

Figure 3. Genomic alterations in *ESR1* & *RB1* are most significantly increased post-progression



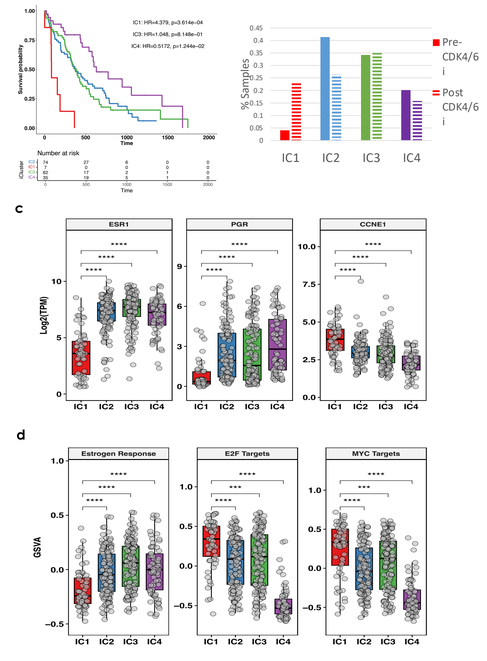
(a) Pre vs. Post comparison identified 7 genes with significant changes in genomic alteration frequencies (fisher exact test, $p<0.05$), with *ESR1* and *RB1* having the most significant increases. Colors represent different types of genomic alterations. (b) *ESR1* and *RB1* are also among the top genes with frequent PD-specific mutations among patients with paired Pre/Post samples. The number and percentage of the patients with PD-specific mutations for each gene were shown at the right side.

Figure 4. Integrative clustering analysis identified molecularly distinct subgroups IC1-4



Integrative clustering analysis using iClusterPlus[1] on selected multi-omics features representing different aspects of breast cancer biology identified four clusters, IC1-4. These features include gene expressions, hallmark signatures (GSVA), PAM50 subtype scores, ESTIMATE scores for tumor microenvironment, projection to Paloma3 NMF factors[2], gene-level genomic alteration status and other tumor characteristics. icluster: integrative cluster. Cyt score: cytolytic activity score. TMB: tumor mutation burden. Treatment status: Pre/Post.

Figure 5. Emergence of ER independent tumors post-progression on CDK4/6i+ET

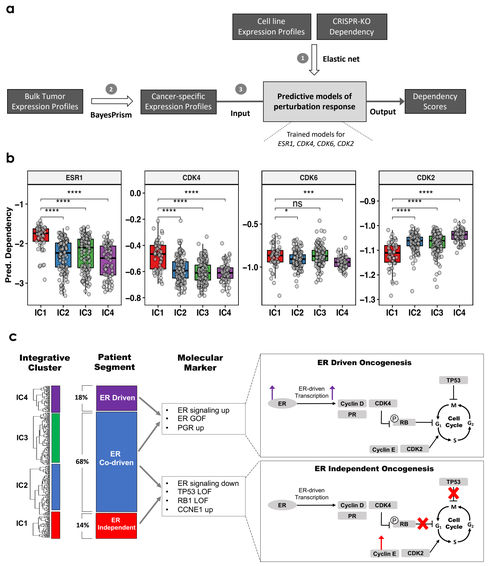


(a) KM plot comparing the PFS of patients classified into the four IC clusters at baseline. (b) Changes in the prevalence of IC clusters Pre vs. Post. (c, d) Distributions of *ESR1*, *PGR* and *CCNE1* gene expressions (c) and Hallmark signature scores (d) vs. IC clusters. ns: p>0.05; *: $p\leq0.05$; **: $p\leq0.01$; ***: $p\leq0.001$; ****: $p\leq0.0001$. (e) Distributions of *TP53*, *ESR1*, *RB1*, *GATA3* mutation statuses vs. IC clusters.

REFERENCES

1. Mo, Q., et al. Pattern discovery and cancer gene identification in integrated cancer genomic data. Proc Natl Acad Sci U S A 110, 4245-4250 (2013).
2. Freeman-Cook, K., et al. Expanding control of the tumor cell cycle with a CDK2/4/6 inhibitor. Cancer Cell 39, 1404-1421 e1411 (2021).

Figure 6. Therapeutic dependency of ER-independent tumors predicted by Machine Learning models



(a) We developed elastic net models trained on CRISPR loss-of-function knockout screen data in cell lines to predict drug target gene dependency using tumor gene expression profiles. (b) Predicted dependency on *CDK2* increased (lower score) in IC1 along with decreased dependency on *ESR1* and *CDK4*. (g) HR+/HER2- MBC patients may be stratified into three segments with different dependency on ER signaling as the oncogenic mechanism, which suggests differentiated therapeutic strategy.

CONCLUSIONS

- Our real-world clinical genomics study confirmed a comprehensive list of molecular markers associated with disease progression under the CDK4/6i plus ET treatment and estimated prevalence for these markers in the post-progression setting.
- Integrative clustering analysis identified a subset of aggressive tumors (IC1) with estrogen independence characteristics. IC1 increased in prevalence from 4.3% pre-treatment to 23.0% post-progression. It is also enriched in *TP53* and *RB1* mutations and associated with *CCNE1* up-regulation.
- HR+/HER2- mBC patients may be stratified into three segments – ER driven, ER co-driven and ER independent.
- Machine learning analysis suggested the therapeutic strategy of targeting *CDK2* against the ER dependent tumor segment, echoing an earlier study[2].