Differential gene expression analysis of palbociclib-resistant TNBC via RNA-seq

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Abstract
Purpose The management of triple-negative breast cancer (TNBC) remains a significant clinical challenge due to the lack of effective targeted therapies. Inhibitors of the cyclin-dependent kinases 4 and 6 (CDK4/6) are emerging as promising therapeutic agents against TNBC; however, cells can rapidly acquire resistance through multiple mechanisms that are yet to be identified. Therefore, determining the mechanisms underlying resistance to CDK4/6 inhibition is crucial to develop combination therapies that can extend the efficacy of the CDK4/6 inhibitors or delay resistance. This study aims to identify differentially expressed genes (DEG) associated with acquired resistance to palbociclib in ER− breast cancer cells.
Methods We performed next-generation transcriptomic sequencing (RNA-seq) and pathway analysis in ER− MDA-MB-231 palbociclib-sensitive (231/pS) and palbociclib-resistant (231/pR) cells.
Results We identified 2247 up-regulated and 1427 down-regulated transcripts in 231/pR compared to 231/pS cells. DEGs were subjected to functional analysis using Gene Ontology (GO) and the KEGG database which identified many transduction pathways associated with breast cancer, including the PI3K/AKT, PTEN and mTOR pathways. Additionally, Ingenuity Pathway Analysis (IPA) revealed that resistance to palbociclib is closely associated with altered cholesterol and fatty acid biosynthesis suggesting that resistance to palbociclib may be dependent on lipid metabolic reprogramming.
Conclusion This study provides evidence that lipid metabolism is altered in TNBC with acquired resistance to palbociclib. Further studies are needed to determine if the observed lipid metabolic rewiring can be exploited to overcome therapy resistance in TNBC.

Keywords Palbociclib · TNBC · CDK4/6 inhibitors · Therapy resistance · Metabolism reprogramming

Abbreviations
TNBC Triple-negative breast cancer
KEGG Kyoto encyclopedia of genes and genomes
CDK4/6 Cyclin-dependent kinases 4 and 6
ER Estrogen receptor
PR Progesterone receptor

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Introduction

Triple-negative breast cancer (TNBC) encompasses an heterogenous subtype of breast cancer that is histologically defined by the lack of estrogen receptor (ER), progesterone receptor (PR) and HER-2/neu overexpression [1, 2]. Although this breast cancer subtype only accounts for only 10–20% of all breast cancers, it is associated with poor prognosis due to the high risk of distant recurrence [3]. Because of the lack of hormone receptors and HER-2/neu amplification, management of TNBC has been mainly limited to surgery and chemotherapy. More recently, a subset of TNBC patients are now treated with biomarker-driven therapies such as PARP inhibitors or platinum agents and immune checkpoint inhibitors (Keytruda, Tecentriq) in PD-L1-positive TNBC. Additionally, a new class of potent anti-cancer drugs such as antibody–drug conjugates (Trodelvy) are now FDA approved for TNBC [4]. Despite these promising novel therapies, their ability to improve 5-year relative survival is still limited. Compared with ER-positive (ER+) breast cancer patients, the 5-year relative survival rates for localized, regional and distant TNBC patients are 91%, 65% and 11.5%, respectively, vs 100%, 89.9% and 30.4% [5]. This disparity in survival compared to ER+ breast cancer is largely due to the lack of targeted therapies against TNBC [6]. Thus, effective treatments against TNBC are urgently needed to improve the overall survival of these patients.

Aberrant activation of the cyclin D1-CDK4/6-retinoblastoma (Rb) pathway is hallmark of breast cancer that led to the development of CDK4/6 inhibitors [7–11]. Three selective CDK4/6 inhibitors (palbociclib, abemaciclib, and ribociclib) have been FDA approved for the treatment of metastatic ER-positive (ER+) breast cancer patients in combination with endocrine therapy given their proved ability to increase progression-free survival [12]. TNBC was initially considered a poor candidate for CDK4/6 inhibition given that Rb loss and high cyclin E expression are often observed in these tumors [13, 14]. However, recent studies have demonstrated that TNBC expressing the Rb protein are sensitive to CDK4/6 inhibition providing a strong rationale to extend the use of CDK4/6 inhibitors to TNBC [15–20]. As an example, simultaneous inhibition of CDK4/6 and PI3K was shown to be highly effective both in vivo and in vitro against various Rb-positive preclinical TNBC models, including patient derived xenografts (PDX) [18]. Additionally, the triple combination of CDK4/6, PI3K and immune-checkpoint blockage demonstrated long-lasting anti-tumor activity against TNBC in vivo [18]. Another important study demonstrated that CDK4/6 inhibition blocks the tumor metastasis potential of multiple preclinical models of TNBC without affecting primary tumor growth [19]. Taken together, these observations provide strong rationale for the potential use of CDK4/6 inhibitor for the treatment of TNBC. Consequently, multiple trials aiming to evaluate the utility of CDK4/6 as single agents or in combination with other targeted therapies against TNBC are currently ongoing (ClinicalTrials.gov; NCT03090165, NCT02978716, NCT03979508, NCT03519178).

CDK4/6 inhibition is emerging as a promising therapeutic approach against TNBC; however, we anticipate that resistance will arise and become a significant clinical challenge for TNBC patients given the known development of resistance observed in nearly all ER+ patients receiving this therapy [21]. Thus, the identification of mechanisms of resistance to CDK4/6 blockage will be crucial for the further development of this treatment modality in TNBC patients. The major goal of this study was to identify actionable targets and pathways driving resistance to CDK4/6 inhibition in TNBC through transcriptomic analysis. Using MDA-MB-231 cells as a model of TNBC, we performed next-generation transcriptomic RNA sequencing (RNA-seq) on palbociclib-resistant MDA-MB-231 (231/pR) and their isogenic palbociclib-sensitive counterpart (231/pS). Differential gene expression and pathway analysis indicated that resistance to palbociclib involves many canonical pathways including aryl hydrocarbon receptor, immune responses and signal transduction pathways such as PI3K, PTEN and mTOR signaling. Notably, our analysis showed that cholesterol and lipid biosynthesis were uniquely enriched in palbociclib-resistant cells compared to palbociclib-sensitive. These studies identified novel druggable candidates and pathways that may be able to prevent or alleviate resistance to palbociclib in TNBC patients.

Materials and methods

Cell culture, generation of palbociclib-resistant cells

MDA-MB-231 (HTB-26) cells were purchased from the American Type Culture Collection (ATCC) and maintained at 37 °C with 5% CO₂. MDA-MB-231 cells were cultured in IMEM (Corning) supplemented with 10% fetal bovine serum (FBS, Invitrogen). MFM-223 cells were purchased from SIGMA and maintained at 37 °C with 5% CO₂. MFM-223 cells were cultured in MEM (Corning) supplemented with 10% fetal bovine serum (FBS, Invitrogen). Palbociclib-resistant MDA-MB-231 and MFM-223 cells were established by culturing in media containing palbociclib (0.1–4 μM). Drug was replenished every 3 days. Cells were subcultured every 1–2 weeks with 25% increments in drug concentration. The resistant cells were established after
4–6 months and maintained in the presence of 1 μM palbociclib. Cells were authenticated by the short tandem repeat (STR) assay (Genetica).

RNA extraction and next-generation sequencing

Total RNA was extracted from 231/pS and 231/pR cells using the RNeasy kit (Qiagen) following the manufacturer’s instructions (three independent replicates per cell line). Libraries were prepared simultaneously using the TruSeq Stranded mRNA LT Sample Prep Kit- Set A (Cat# RS-122-2101) with poly-A enrichment. Sequencing was performed on the University of Louisville Center for Genetics and Molecular Medicine’s (CGeMM) Illumina NextSeq 500 using the NextSeq 500/550 1 × 75 cycle High Output Kit v2 (Cat# FC-404-2005). A second run was performed on all samples to achieve an average of 45 million reads per sample.

DEG analysis

The resulting samples were downloaded from Illumina’s BaseSpace [22] (https://basespace.illumina.com/). Sequences were directly aligned to the Homo sapiens hg38 reference genome assembly (hg38.fa) using tophat2 (version 2.0.13), generating alignment files in bam format. DEG were identified for the pairwise comparison 231/pS vs 231/pR using the tuxedo suite programs including cufflinks-cuffdiff2 (VERSION2.2.1). A \( q \)-value cutoff \( \leq 0.05 \) with |log2FC| \( \geq 1 \) and gene expression > 1 in at least one replicate was used to determine differential expression. RNAseq data available (GEO accession number GSE130437). Gene Ontology Biological Processes (GO:BP) and KEGG Pathways analysis was performed using CategoryCompare [23].

In silico ingenuity network analysis

Pathway and biological processes analysis of all differentially expressed genes was performed using Ingenuity Pathway Analysis (Qiagen).

Western blot analysis

Cells were treated with either vehicle control (H\( \text{2} \text{O} \)) or 500 nM palbociclib (PD-0332991, Selleckchem) for 24 h prior to protein isolation. Whole cell lysates were harvested using RIPA buffer (ThermoFisher) supplemented with protease and phosphatase inhibitors. Protein concentration was determined using the BCA protein assay kit (ThermoFisher) following the manufacturer’s instructions. Proteins were separated on 4–20% Mini PROTEAN TGX gels under reducing conditions and transferred to Immun-Blot PVDF membranes (Bio-Rad). The membranes were blocked with 5% BSA or 5% nonfat milk in TBS-T (0.1% Tween20) and immunoblotted with the indicated antibodies. Antibody against DGKG gamma (PA5-97658) was purchased from Invitrogen. SREBP1 (sc-365513) and MVK (sc-390669) antibodies were purchased from Santa Cruz Biotechnology. Antibody against FASN (no. 3180) was purchased from Cell Signaling. Beta-actin (A2228) antibody was purchased as secondary antibodies. Amersham ECL Prime Western blotting detection reagent (GE Healthcare) was used to detect immunoreactive bands. The bands were visualized on autoradiography film BX (MidSci). Quantitative densitometry was performed with UN-SCAN-IT (Silk scientific, version 6.3), signal density was normalized to the corresponding beta-actin loading control.

Results

Transcriptomic profiling of ER– palbociclib-resistant cells

The development and characterization of palbociclib-resistant, 231/pR cells, and its isogenic parental cell line (231/pS) was previously described by our group [24]. We performed gene expression profiling in 231/pS and 231/pR cells with the goal of characterizing transcriptional alterations upon the development of resistance to palbociclib. Differential gene expression was defined as \( \text{log}_{2} \text{FC} \geq 1 \) with a \( q \)-value cutoff \( \leq 0.05 \) between the resistant and its isogenic sensitive cell line. Hierarchical clustering based on differentially expressed RNA transcripts revealed a distinct transcriptomic profile in 231/pR cells compared to 231/pS (Fig. 1), with 2247 upregulated genes and 1427 downregulated transcripts identified in 231/pR cells. The top 20 upregulated and downregulated genes in 231/pR cells compared to 231/pS cells are shown in Table 1.

KEGG annotation of DEG and enriched biological processes analysis

To identify the underlying molecular mechanisms driving resistance to palbociclib, we performed KEGG pathway analysis of all DEGs identified using CategoryCompare [23]. Table 2 lists the enriched KEGG pathways identified in 231/pR vs. 231/pS cells (false discovery rate (FDR) \( \leq 0.05 \) and \( p \)-value \( \leq 0.001 \)). The KEGG terms associated with resistance to palbociclib included ‘ECM–receptor interaction’ and ‘Focal adhesion’. Next, we sought to identify biological processes that correlated with palbociclib-resistance by performing analysis of GO:BP
(Fig. 2). We observed distinct groups of nodes including ‘type I interferon signaling’, ‘extracellular matrix assembly and organization’, ‘angiogenesis’, ‘regulation of endothelial cells’, ‘regulation of ERK signaling’ and ‘regulation of Rho signaling’ revealing multiple functional “themes” associated with resistance to palbociclib.

**Canonical pathway analysis of DEG**

To uncover pathways significantly associated with resistance to palbociclib, all altered transcripts were mapped to known pathways using Ingenuity Pathway Analysis (IPA). The canonical pathway ‘Aryl hydrocarbon receptor’ emerged as the most significantly associated with resistance to palbociclib. Additionally, we observed significant enrichment of several canonical pathways including three pathways involved in immune responses (‘Antigen presentation pathway’, ‘IL-4 signaling’ and ‘Interferon signaling’), three pathways involved in signal transduction (‘PI3K/AKT signaling’, ‘PTEN signaling’ and ‘mTOR signaling’), among others (Fig. 3).

**Metabolic pathways analysis of DEGs**

We next performed metabolic pathway analysis of all DEGs using IPA with the goals of determining the underlying metabolic differences between 231/pR and 231/pS cells (Fig. 4). We observed a significant enrichment of metabolic pathways involved in cholesterol biosynthesis (‘Cholesterol biosynthesis I’, ‘Cholesterol Biosynthesis II’ and ‘Superpathway of cholesterol biosynthesis’) and fatty acid biosynthesis (‘Fatty acid biosynthesis’, ‘Palmitate biosynthesis I’ and ‘Triacylglycerol biosynthesis’). Among other pathways, we found ‘NAD biosynthesis (from Tryptophan)’ and ‘Pyrimidine deoxyribonucleotides de novo biosynthesis I’ to be enriched in our dataset. These results indicate that deregulated metabolism, particularly cholesterol and fatty acid metabolism, may play an essential role in mediating resistance to palbociclib.

**Validation of expression of DEGs identified by RNA-seq**

To validate our RNA-seq data, we selected a number of DEGs with potential biological relevance (as identified by our IPA analysis) to determine their expression at the protein level in 231/pS and 231/pR cells exposed to either vehicle control or palbociclib. Given that DGKGγ was identified as one of the top 20 up-regulated genes in our dataset (Table 1) we next examined DGKGγ protein expression in these cells. Diacylglycerol kinase gamma (DGKGγ) belongs to a family of DGKGs that convert diacylglycerol to phosphatidic acid thus, regulating the levels of two lipids with key roles in cell signaling and metabolism. We observed a marked increase in DGKGγ protein expression in 231/pR cells compared to 231/pS. Treatment with palbociclib had no effect on DGKGγ protein expression.
expression compared to the corresponding vehicle control (Fig. 5a). Our metabolic pathway analysis identified an enrichment in fatty acid and cholesterol biosynthesis in 231/pR vs. 231/pS cells. Lipid metabolism is tightly regulated by sterol regulatory element-binding factors (SREBPs), which activate the transcription of genes encoding for key enzymes involved in fatty acid and cholesterol biosynthesis [25, 26]. Protein expression analysis of SREBP1 showed a modest increase in SREBP1 in 231/pR control-treated cells relative to 231/pS (Fig. 5a).

Table 1 Top 20 up-regulated and down-regulated genes between 231/pS and 231/pR ranked by p-value ($p_{val} \leq 0.05$; $q_{val} \leq 0.05$; $|\log_{2}FC| \geq 1$)

| Ensembl ID | Gene symbol | Description | Log2FC | p-value  | Q-value  |
|------------|-------------|-------------|--------|----------|----------|
| ENSG00000058866 | DGKG | Diacylglycerol kinase, gamma 90 kDa | 19.8941 | 5.00E−05 | 0.000208859 |
| ENSG00000079393 | DUSP13 | Dual specificity phosphatase 13 | 19.8941 | 5.00E−05 | 0.000208859 |
| ENSG00000099260 | PALM | Palmitoliphin | 19.8941 | 5.00E−05 | 0.000208859 |
| ENSG00000105472 | CLEC11A | C-type lectin domain family 11, member A | 19.8941 | 5.00E−05 | 0.000208859 |
| ENSG00000108691 | CCL2 | Chemokine (C–C motif) ligand 2 | 19.8941 | 5.00E−05 | 0.000208859 |
| ENSG00000115263 | GCG | Glucagon | 19.8941 | 5.00E−05 | 0.000208859 |
| ENSG00000124731 | TREM1 | Triggering receptor expressed on myeloid cells 1 | 19.8941 | 5.00E−05 | 0.000208859 |
| ENSG00000131303 | SREBP1 | Sterol regulatory element-binding protein 1 | 19.8941 | 5.00E−05 | 0.000208859 |
| ENSG00000133665 | DYDC2 | DPY30 domain containing 2 | 19.8941 | 5.00E−05 | 0.000208859 |
| ENSG00000137491 | IDO1 | Indoleamine 2,3-dioxygenase 1 | 19.8941 | 5.00E−05 | 0.000208859 |
| ENSG00000132185 | FCRLA | Fc receptor-like A | 19.8941 | 5.00E−05 | 0.000208859 |
| ENSG00000131203 | IDO1 | Indoleamine 2,3-dioxygenase 1 | 19.8941 | 5.00E−05 | 0.000208859 |
| ENSG00000132185 | FCRLA | Fc receptor-like A | 19.8941 | 5.00E−05 | 0.000208859 |
| ENSG00000133665 | DYDC2 | DPY30 domain containing 2 | 19.8941 | 5.00E−05 | 0.000208859 |
| ENSG00000137491 | IDO1 | Indoleamine 2,3-dioxygenase 1 | 19.8941 | 5.00E−05 | 0.000208859 |
| ENSG00000132185 | FCRLA | Fc receptor-like A | 19.8941 | 5.00E−05 | 0.000208859 |
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| ENSG00000133665 | DYDC2 | DPY30 domain containing 2 | 19.8941 | 5.00E−05 | 0.000208859 |
Notably, while palbociclib treatment in 231/pS cells led to a marked decrease in SREBP1 expression, 231/pR cells failed to decrease SREBP1 expression in response to palbociclib exposure. Next, we examined the expression of fatty acid synthase (FASN), a key enzyme regulating fatty acid synthesis and a direct target of SREBP1. We found that FASN protein levels were significantly increased in 231/pR cells compared to 231/pS cells in the presence and

Table 2  Top enriched KEGG terms between 231/pS and 231/pR ranked by p-value ($p_{val} \leq 0.05$; $q_{val} \leq 0.05$; $|\log_2 FC| \geq 1$)

| KEGG ID | Description                              | p-value | FDR  |
|---------|------------------------------------------|---------|------|
| 5150    | Staphylococcus aureus infection           | 3.19E−05| 0    |
| 4512    | ECM–receptor interaction                  | 7.69E−05| 0    |
| 5323    | Rheumatoid arthritis                      | 9.74E−05| 0.006666667|
| 4610    | Complement and coagulation cascades       | 3.30E−04| 0.005|

Fig. 2  Enriched biological processes (BP) analysis of ER− palbociclib-resistant breast cancer cells

Fig. 3  IPA analysis of ER− palbociclib-resistant breast cancer cells. A higher $-\log(B−H p$-value) shown on the left Y axis represents more significant pathways. The ratio (right Y axis) refers to the number of genes from the dataset that map to the pathway divided by the total number of genes that map the canonical pathway from the IPA database. $p_{val} \leq 0.05$; $q_{val} \leq 0.05$; $|\log_2 FC| \geq 1$
absence of palbociclib. Mevalonate kinase (MVK) is an essential early enzyme in the mevalonate pathway which is required for the generation of isoprenoids and cholesterol. We observed a marked increase in MVK in 231/pR cells compared to 231/pS cells in the presence and absence of palbociclib. Given the known heterogeneity of TNBC, we next sought to determine whether the observed changes in gene expression upon the development of resistance were cell-type specific. We used MFM-223 cells, which are sensitive to palbociclib, and generated palbociclib-resistant MFM-223 cells (223/pR) by continuous exposure to palbociclib for 4 months. Protein expression analysis of 223/pR cells showed an increase in DGKGγ and MVK levels compared to 223/pS cells. In contrast, a modest decrease was observed in the expression levels of SREBP1 and FASN in 223/pR cells compared to the parental 223/pS cells. These observations suggest that DGKGγ and MVK may be key mediators of resistance to palbociclib. In line with a previous study, the 223/pR cells acquired increased expression of cyclin E which is a known mechanism of resistance to CDK4/6 inhibition (Fig. 5b) [17]. This increase in cyclin E was also observed in 231/pR cells compared to 231/pS (Fig. 5a).

Taken together, our results validate the expression of various DEGs identified in our RNA-seq analysis. Furthermore, our findings suggest an up-regulation of cholesterol biosynthesis in both TNBC models that may mediate resistance to palbociclib.
Discussion

A significant number of patients with TNBC experience disease progression within 2–5 years from initial diagnosis. To benefit these patients, we need to develop effective targeted therapies against this cancer subtype [27]. Given the success of CDK4/6 inhibitors in advanced ER+ breast cancer, trials are underway to expand the use of this treatment modality against TNBC. Resistance to CDK4/6 inhibition is a major limitation of this treatment regimen thus, a mechanistic understanding of CDK4/6 resistance is needed to design effective combinatorial therapies that block this adaptive response to CDK4/6 inhibition. In this study, we performed transcriptomic analysis to characterize a pair of palbociclib-sensitive and -resistant ER- MDA-MB-231 breast cancer cells. To the best of our knowledge, we are the first group to generate MDA-MB-231 cells with acquired resistance to palbociclib and to identify clinically relevant pathways associated with resistance in these cells.

Examination of canonical pathways associated with resistance to palbociclib in ER- breast cancer cells revealed a deregulation of PI3K/AKT/mTOR signaling (Fig. 3). These results are in line with previous studies showing that PI3K/AKT/mTOR activation mediates resistance to CDK4/6 inhibition in ER+ breast cancer [28]. Importantly, the PI3K/AKT/mTOR axis play a critical role in regulating cellular metabolism including lipid metabolism [29, 30] and may be responsible for the deregulation of fatty acid and cholesterol observed in palbociclib-resistant TNBC cells. Similarly, we observed an increase in cyclin E in both TNBC cell models of acquired resistance to palbociclib. The role of cyclin E in mediating resistance to CDK4/6 inhibition has been extensively documented in ER+ breast cancer cells. Taken together, our results suggest that reactivation of the PI3K/AKT/mTOR pathway and increased cyclin E are universal mechanisms of resistance to CDK4/6 inhibition independent of ER status.

Metabolic rewiring is consistently observed in cancer cells and is an emergent mechanism of resistance to CDK4/6 inhibition. For instance, a previous study showed that palbociclib-resistant ER+/HER2- breast cancer cells exhibit increased dependency in glucose metabolism [31]. In this context, elucidating the unique metabolic dependencies of sensitive versus therapy-resistant cells remains an important challenge that may reveal novel pharmacological interventions. Our transcriptomic analysis uncovered that fatty acid and cholesterol metabolism are the most common metabolic alterations in 231/pR compared to 231/pS cells, suggesting that lipid reprogramming is associated with resistance to palbociclib. Here, we show that 231/pR cells increase the expression of important drivers of fatty acid and cholesterol metabolisms such as SREBP1, FASN and MVK (Fig. 5a). Notably, MVK expression was also found to be increased in 223/pR cells compared to 223/pS cells, suggesting that MVK may be a key mediator of resistance to palbociclib across multiple TNBC cells. Conversely, we observed a modest decrease in SREBP1 and FASN in 223/pR cells compared to 223/pS. It should be noted that other drivers of fatty acid biosynthesis include key enzymes such as acetyl CoA carboxylase (ACC) and ATP citrate lyase (ACLY) [32, 33], thus additional studies will be required to determine if increased fatty acid metabolism occurs in 223/pR cells via upregulation of these enzymes. Lipid metabolic rewiring is an emerging mechanism of resistance to many targeted therapies in breast cancer [34]. Several groups have demonstrated that lipid reprogramming promotes the growth and survival of cancer cells and often correlates with cancer cell stemness and resistance to therapy [35–40]. Moreover, elevated total cholesterol levels have been shown to correlate with early recurrence in breast cancer [41] while the use of cholesterol-lowering medications associates with improved overall survival in a subset of TNBC patients and decreases the survival of TNBC cell lines [42, 43]. Our studies provide further evidence of the potential role of lipid metabolism deregulation in mediating resistance to CDK4/6 inhibition and provide rationale for the future testing of drugs targeting lipid metabolism to re-sensitize TNBC tumors resistant to CDK4/6 inhibitors.

Our findings indicate that cholesterol metabolism was significantly altered in 231/pR and 223/pR cells compared to their respective sensitive counterparts. Future metabolomic profiling will be needed to confirm our initial findings and provide further evidence to whether cholesterol-lowering drugs such as statins may provide anticancer activity against palbociclib-resistant TNBC. Importantly, while resistance to palbociclib has been associated with changes in glucose and nucleotide metabolism in ER+ breast cancer cells [31, 44], the metabolic changes herein described have not been reported in ER+ breast cancer cells thus far. Given the heterogeneity of TNBC, a limitation of our studies is the use of only two isogenic TNBC cell lines to characterize the mechanisms of resistance to palbociclib. Future studies will be needed to determine whether our findings extend across multiple TNBC subtypes and to functionally validate whether these metabolic alterations may have potential clinical utility. Another limitation of the current analysis is the use of cells that are resistant to a single CDK4/6 inhibitor, palbociclib. Preclinical evidence in some ER+ models suggests that resistance to palbociclib confers cross-resistance to the other CDK4/6 inhibitors and thus, it is likely that a number of mechanisms of resistance described here will be shared with other CDK4/6 inhibitors [45]. Nevertheless, the data presented here will help guide future efforts to study these potentially targetable alterations in response to CDK4/6 inhibition in TNBC.
Collectively, our studies identified clinically relevant canonical and metabolic pathways associated with resistance to palbociclib in ER-MDA-MB-231 cells. Future studies will focus on characterizing the dependency of TNBC resistant to CDK4/6 inhibitors on fatty acid and cholesterol reprogramming and determine the utility of targeting key enzymes such as MVK for the prevention and/or treatment of drug-resistant TNBC patients.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s10549-021-06127-5.

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Compliance with ethical standards
Conflict of interest The authors declare that they have no conflict of interest.

Research involving human participants and/or animals This article does not contain any studies with human participants or animals performed by any of the authors.

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