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Mechanisms of Acquired Resistance to PI3K Inhibitors in Breast Cancer: The Central Role of Autophagy

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Introduction

Hyperactivation of the Phosphatidylinositol 3-Kinase (PI3K)/AKT/mTOR signaling axis represents one of the most frequently observed and clinically relevant oncogenic alteration in breast cancer (1). This aberrant activation is particularly prevalent in Estrogen Receptor-positive (ER-positive) luminal tumors, affecting nearly 40% of cases and driving proliferative and survival signals. The most common molecular drivers include activating somatic gain-of-function mutations in the catalytic subunit PIK3CA and loss or reduced expression of the tumor suppressor gene PTEN (2).

Several selective PI3K inhibitors, such as Alpelisib and Capitasertib, have been developed and gained regulatory approval for the treatment of breast cancer, demonstrating variable clinical benefit in patients harboring PI3K/AKT/PTEN alterations. However, despite their responses, the long-term clinical efficacy of these agents remains largely constrained by the ubiquitous challenge of acquired drug tolerance and the emergence of adaptive resistance mechanisms (3).

In this study, we aimed to elucidate the mechanism underlying resistance to PI3K inhibitors and to identify how tumor cells evade the selective pressure exerted by the drug. To achieve this, we developed an *in vitro* model of acquired resistance to Taselisib, a highly selective inhibitor targeting the mutant forms of PIK3CA (4). Specifically, we generated a resistant subline, designated MCF7 Tas-r, derived from the parental human breast cancer cell line MCF7-Par through a process of stepwise exposure to incrementally increasing concentrations of Taselisib over several months. The subsequent comprehensive analysis of the MCF7 Tas-r cells compared to the parental line provides a unique platform to characterize the phenotypic and molecular determinants of resistance.

Materials and Methods

Taselisib (GDC0032) was supplied by Genentech (Research proposal no. OR-215703). Taselisib-resistant MCF7 cells (MCF7 Tas-r) were developed by long-term exposure of parental cells to increasing drug concentrations. Cell viability was assessed by SRB assay. Phosphoproteomic profiling was performed using LC–MS/MS followed by bioinformatic and pathway enrichment analyses. Differentially phosphorylated proteins were validated by Western blot. Functional and network analyses were conducted using DAVID and IPA software, and statistical evaluations were performed with Progenesis QI, Perseus, and GraphPad Prism.

Results

Autophagy emerged as an upregulated signaling pathway in a cellular model of resistance to the PI3K inhibitor

Table 1

Cells	Drug	IC^{50}	RI
MCF7	Taselisib	68.3 nM±2.7	2.4
MCF7 Tas-r	Taselisib	163.9 nM±8.5	
MCF7	Ipertaselisib	13.3 µM±0.5	1.3
MCF7 Tas-r	Ipertaselisib	17.5 µM±0.5	

Table1. Taselisib and Ipatasertib IC_{50} [72h] in MCF7 and MCF7 Tas-r cells.

Fig. 1

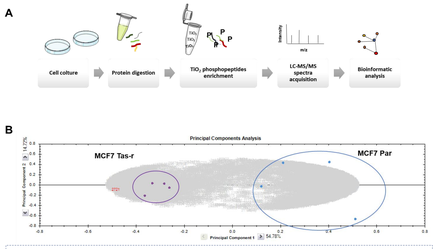


Fig.1. Phosphoproteomic profiling of MCF7 Par vs MCF7 Tas-r. A, Workflow: protein digestion, phosphopeptide enrichment, LC-MS/MS and bioinformatic analysis. B, Principal Component Analysis (PCA) shows high sample reproducibility and clear separation between MCF7 Par and MCF7 Tas-r cells. LC-MS/MS analysis identified 253 differentially expressed phosphoproteins between the two cell lines.

Table 2

Phosphoproteins Enriched by KEGG	Description	Function (www.uniprot.org)	Fold change MCF7 Tas-r/MCF7 Par
CL16A_HUMAN	C-type lectin domain family 16 member A (CLEC16A)	Regulator of mitophagy through the upstream regulation of the RNF41/NRDP1-PRKN pathway	-8.86
VPS18_HUMAN	VPS18, CORVET/HOPS core subunit (VPS18)	Plays a role in vesicle-mediated protein trafficking to lysosomal compartments including the endocytic membrane transport and autophagic pathways	-12.86
XBP1_HUMAN	X-box binding protein 1 (XBP1)	Functions as a transcription factor during endoplasmic reticulum (ER) stress by regulating the unfolded protein response (UPR)	+1.78
ATG4B_HUMAN	Autophagy related 4B cysteine peptidase (ATG4B)	Cysteine protease that plays a key role in autophagy by mediating both proteolytic activation and delipidation of ATG8 family proteins	+2.17
ATG4D_HUMAN	Autophagy related 4D (ATG4D)	Cysteine protease that plays a key role in autophagy by mediating both proteolytic activation and delipidation of ATG8 family proteins	+4.31
ATG9B_HUMAN	Autophagy related 9B cysteine peptidase (ATG9B)	Phospholipid scramblase involved in autophagy by mediating autophagosomal membrane expansion	+2.39
SQSTM_HUMAN	Sequestosome 1 (SQSTM1)	Autophagy receptor required for selective macroautophagy (aggrephagy)	+2.86
ULK1_HUMAN	Unc-51 like autophagy activating kinase 1 (ULK1)	Serine/threonine-protein kinase involved in autophagy in response to starvation	+3.07

Table 2. Identified differentially expressed phosphoproteins involved in the 'Regulation of autophagy' according to the KEGG pathway.

Fig. 2

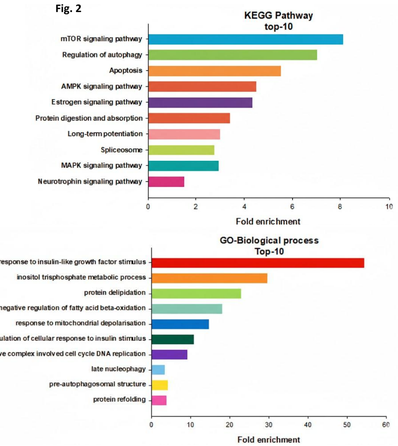


Fig. 2. DAVID-based enrichment of 253 differentially expressed phosphoproteins for Gene Ontology (GO) terms and KEGG pathways.

Fig. 3

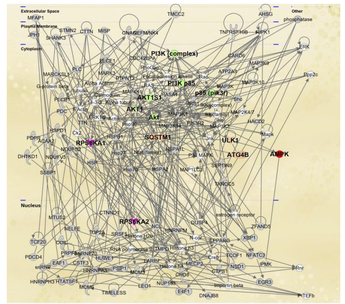


Fig. 3. Ingenuity Pathway Analysis (IPA). Network from 253 differential phosphoproteins highlights PI3K (green), autophagy (orange), AMPK (red), and mTOR (violet) as main pathways.

Inhibition of Autophagy by Chloroquine Induces Reduction of Cell Proliferation

Fig. 4

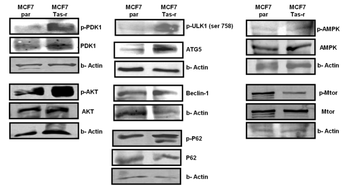


Fig. 4. Western blot analysis. Basal levels of proteins identified as differentially expressed in the phosphoproteomic analysis in MCF7-Par and MCF7 Tas-r cells .

Fig. 5

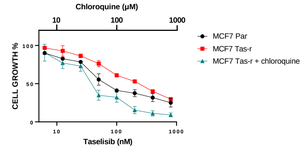


Fig. 5. Antiproliferative effect of combined treatment. Chloroquine (CQ) potentiates taselisib activity in MCF7 Tas-r cells after 72 h exposure.

Fig. 6

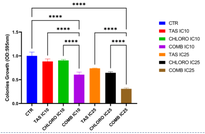


Fig. 6 Clonogenic assay. Taselisib–CQ combination prevents clonogenic growth in MCF7 Tas-r cells at IC1072h and IC2572h doses of parental cells.

Fig. 7

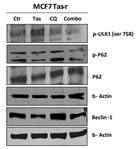
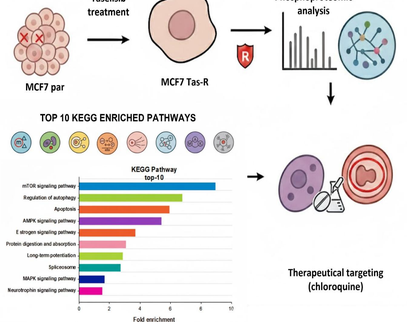


Fig. 7. Western blot analysis. Taselisib and CQ treatment induced reduction of autophagy markers in MCF7 Tas-r cells.

Conclusions

- Stepwise exposure to increasing concentrations of taselisib enabled the generation of a robust cellular model of acquired PI3K inhibitor resistance.
- Phosphoproteomic profiling of MCF7 Tas-r cells identified 253 differentially phosphorylated proteins relative to parental cells, highlighting extensive rewiring of signaling networks associated with resistance.
- Functional enrichment analyses revealed significant dysregulation of autophagy-related pathways, including mTOR and AMPK signaling. Consistently, Western blot validation confirmed increased activation of key autophagy-associated proteins (p-ULK1, SQSTM1/p62, ATG5, Beclin-1) and elevated AMPK phosphorylation, accompanied by reduced mTOR activity in resistant cells.
- Inhibition of autophagy by chloroquine potentiates taselisib activity.
- Overall, these findings indicate that autophagy activation represents a critical adaptive mechanism that enables cancer cells to evade PI3K inhibition. Targeting autophagy may offer a promising therapeutic strategy to overcome or delay acquired resistance to PI3K inhibitors.



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