GPER Signaling as a Biomarker for Endocrine Therapy Sensitivity in PIK3CA-Mutant HR+ Advanced Breast Cancer

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Abstract

The INAVO 120 clinical trial confirmed the efficacy of first-line palbociclib combined with inavolisib in hormone receptor-positive (HR+) advanced breast cancer patients harboring PIK3CA mutations who developed resistance to adjuvant endocrine therapy. However, the comparative effectiveness of alternative CDK4/6 inhibitors (e.g., abemaciclib, ribociclib) in the first line setting, or the potential of crossline therapy (using abemaciclib or ribociclib combined with inavolisib) following palbociclib resistance in PIK3CA-mutant patients remains unclear. Our prior research identified the G protein-coupled estrogen receptor (GPER) as a pivotal initiator of endocrine resistance in HR+ advanced breast cancer, further driving activation of the PI3K/AKT/mTOR (PAM) signaling pathway. We infer that GPER signaling modulates endocrine therapy sensitivity in PIK3CA-mutant HR+ advanced breast cancer and could serve as a novel biomarker to guide personalized clinical treatment decisions. To demonstrate this hypothesis, we identified MCF-7 (E545K-mutant) and T47D (H1047R-mutant) breast cancer cell lines harboring PIK3CA mutations, and developed tamoxifen-resistant (MCF-7/TAM-R, T47D/TAM-R) and palbociclib-resistant (MCF-7/Palb-R, T47D/Palb-R) models to recapitulate first- and second-line endocrine resistance in clinic. Interestingly, GPER expression was significantly upregulated in all above resistant cell lines and promoting ABCG2 efflux pump expression via the GPER/PI3K/AKT pathway, as validated by quantitative PCR and Western blot. In GPER^{high} tamoxifen-resistant cells, combinations of inavolisib with abemaciclib or ribociclib exhibited superior antiproliferative effects compared to palbociclib-based regimens, which assessed by MTT assays, flow cytometry for apoptosis, and cell cycle analysis. Knockdown of GPER/ABCG2 axis using siRNA normalized the efficacy of palbociclib, abemaciclib, and ribociclib in combination with inavolisib, underscoring GPER's critical role in diverse CDK4/6 inhibitors sensitivity. Notably, in palbociclib-resistant cells, combinations of inavolisib with abemaciclib or ribociclib significantly outperformed inavolisib monotherapy. Furthermore, GPER/ABCG2 knockdown not only partially reversed palbociclib resistance, but also enhanced sensitivity to abemaciclib- and ribociclib-based regimens, demonstrating reduced tumor growth and improved therapeutic response upon GPER signaling inhibition. Therefore, assessing GPER signaling activation in tumor biopsies may enable precise identification of PIK3CA-mutant HR+ advanced breast cancer patients likely to benefit from specific endocrine and targeted therapy combinations. Integrating PI3K, CDK4/6, and GPER-targeted therapies represents a promising strategy to overcome endocrine resistance in the future, offering a path toward personalized treatment paradigms with improved clinical outcomes.

Introduction

Clinical Challenge: While the INAVO 120 trial established the efficacy of palbociclib plus inavolisib in PIK3CA-mutant and endocrine-resistant HR+ advanced breast cancer [1], the optimal sequencing of CDK4/6 inhibitors (CDK4/6i) and the mechanisms underlying cross-resistance remain unclear.
GPER in Endocrine Resistance: Our previous work identified the G-protein coupled estrogen receptor (GPER) as a pivotal driver of endocrine resistance [2-5], promoting the PI3K/AKT/mTOR pathway and chemoresistance via upregulation of the ABCG2 efflux pump [6].
Hypothesis: We hypothesize that GPER signaling modulates sensitivity to diverse CDK4/6i in PIK3CA-mutant models and can serve as a biomarker to guide personalized therapy sequencing.

Methods and Materials

Cell Models: HR+ breast cancer cell lines with endogenous PIK3CA mutations: MCF-7 (E545K) and T-47D (H1047R). Mutational status was confirmed by DNA sequencing.
Tamoxifen-Resistant (TAM-R) Models: Generated by continuous exposure to 1μM Tamoxifen as previously described [6].
Palbociclib-Resistant (Palb-R) Models: Generated by long-term culture with increasing doses of Palbociclib (100nM to 1μM) over 3 months, adapted from Talia et al[7].
Molecular Techniques: qRT-PCR and Western Blot for gene/protein expression, siRNA-mediated knockdown of GPER and ABCG2.
Functional Assays: MTT assay for cell viability, flow cytometry for cell cycle analysis and apoptosis.

Results

1. Validation of PIK3CA Mutational Status and Acquisition of EMT Phenotype in Endocrine Resistant Breast Cancer Cells
DNA sequencing confirmed the presence of canonical PIK3CA driver mutations (MCF-7: E545K; T-47D: H1047R) in our model systems, establishing their relevance to the clinical population of interest (Figure 1A). Both TAM-R and Palb-R cell lines exhibited a pronounced morphological shift from a cobblestone-like, epithelial appearance to an elongated, spindle-shaped, fibroblast-like morphology, indicative of an epithelial-mesenchymal transition (EMT, Figure 1B).
2. GPER/ABCG2 Axis is Upregulated in Endocrine Resistant Cell Lines
GPER and ABCG2 expression were significantly elevated in both TAM-R and Palb-R cells compared to parental controls (Figure 2). As our previous work indicated, ABCG2 showed enhanced plasma membrane localization in endocrine resistant cells, a pattern dependent on GPER/PI3K/AKT signaling, facilitating drug efflux [6].
3. GPER Expression Dictates Differential Efficacy of CDK4/6 Inhibitors
In TAM-R (first-line resistance) models, the combination of inavolisib with abemaciclib or ribociclib demonstrated superior anti-proliferative effects and induction of apoptosis compared to palbociclib-based regimens (Figure 3A). Interestingly, in Palb-R (second-line resistance) models, the same combinations (abemaciclib/ribociclib with inavolisib) significantly outperformed inavolisib monotherapy, indicating potential for cross-line efficacy (Figure 3B).
4. Targeting GPER/ABCG2 Re-sensitizes Resistant Cells to CDK4/6 Inhibitors
Knockdown of GPER or ABCG2 via siRNA normalized the efficacy of all three CDK4/6i (palbociclib, abemaciclib, ribociclib) when combined with inavolisib in TAM-R cells (Fig. 3A, First-line). However, GPER/ABCG2 knockdown not only partially reversed palbociclib resistance but also significantly enhanced sensitivity to abemaciclib- and ribociclib-based regimens (Fig. 3B, Second-line).

Figure 1. Validation of PIK3CA Mutational Status and Acquisition of EMT Phenotype in Endocrine Resistant Breast Cancer Cells.

A

Identification of PIK3CA status in diverse breast cancer cell lines			
Cell lines	Phenotype	PIK3CA status (site)	Function
ATCC-HTB-168	TP53	Wild type	Negative control
ATCC-HTB-133	ER/PR+	Mutation (E545K)	Positive control
MCF-7	Luminal	Mutation (E545K)	Selected
T-47D	Luminal	Mutation (H1047R)	Selected
ZR75-1	Luminal	Wild type	Negative control

B

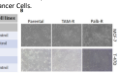


Figure 2. GPER/ABCG2 Axis is Upregulated in Endocrine Resistant Cell Lines

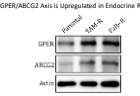
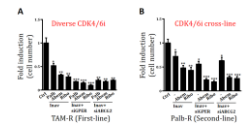


Figure 3. GPER/ABCG2 Signaling as a Biomarker for Endocrine Therapy Sensitivity in PIK3CA-Mutant HR+ Advanced Breast Cancer



Discussion

Mechanistic Insight: Our data establish a model where GPER, upregulated in drug resistant states, activates the PI3K/AKT pathway to enhance ABCG2 expression and membrane localization [6]. This promotes efflux of therapeutic agents, contributing to a endocrine-resistant phenotype. The concurrent induction of EMT phenotype further underscores the aggressive and potential therapy-resistant nature of these cells.
Biomarker Potential: Tumor GPER expression could serve as a functional biomarker to identify patients who are less likely to respond to palbociclib and may benefit from alternative CDK4/6i (abemaciclib or ribociclib) in combination with a PI3Kα inhibitor like inavolisib, the possible reason is that palbociclib may be the drug efflux substrate of ABCG2, but Ribociclib as a potent inhibitor of ABCG2 [8].
Overcoming Resistance: The GPER/ABCG2 axis is a actionable target. Its inhibition can resensitize tumors to multiple CDK4/6i, offering a strategy to overcome or prevent endocrine resistance.
Novel Therapeutic Combinations: We will explore the efficacy of next-generation GPER antagonists, currently in preclinical development, in combination with CDK4/6 and PI3Kα inhibitors, aiming to build a potent triple-combination strategy. Future work will also focus on elucidating the upstream regulators of GPER overexpression in resistant cells and its interplay with other resistance mechanisms, such as ESR1 mutations.
Informed Sequencing: These findings provide a rationale for a more personalized approach to CDK4/6i sequencing in PIK3CA-mutant HR+ advanced breast cancer based on the molecular profile of the tumor, particularly GPER status.

Conclusions

We established clinically relevant PIK3CA-mutant HR+ BC models of sequential therapy resistance, which exhibit hallmark EMT features. GPER is a central mediator of resistance to both endocrine therapy and CDK4/6 inhibitors in these models. Functional assessment of GPER/ABCG2 signaling could enable precise patient stratification, guiding the choice of CDK4/6i and combination therapies to overcome endocrine resistance.

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