Cells 2025 Conference
The 3rd International Online Conference on Cells
25–27 March 2025 | Online
MDPI

UNIVERSITÀ DELLA CALABRIA

The silencing of the G protein-coupled estrogen receptor (GPER) drives apoptotic death in triple-negative breast cancer cells

Marianna Talia, Francesca Cirillo, Domenica Scordamaglia, Salvatore De Rosis, Azzurra Zicarelli, Marika Di Dio, Rosamaria Lappano and Marcello Maggiolini

Department of Pharmacy, Health and Nutritional Sciences, University of Calabria, 87036 Rende, Italy

Introduction & Aim. The G protein-coupled estrogen receptor (GPER) is able to mediate estrogen signaling in diverse normal and malignant cell contexts, including breast cancer (BC)[1,2]. Of note, a role for GPER in promoting pro-tumorigenic traits in triple-negative breast cancer (TNBC) has been suggested[3,4]. Here, we sought to provide novel insights into the transcriptional-guided biological behavior of TNBC cells lacking GPER expression.

Methods. GPER knock-out (KO) MDA-MB-231 TNBC cells were obtained by using the CRISPR/Cas9 genome editing technology. RNA-sequencing (RNA-seq) and Gene Ontology (GO) enrichment analyses served to assess the differentially expressed genes (DEGs) of GPER KO respect to wild type (WT) cells and their biological roles. Chromatin immunoprecipitation assays, real-time PCR, immunoblots, immunofluorescence, ELISA and flow cytometric experiments as well as RNA interference techniques allowed us to uncover the molecular routes implicated in the biological features of TNBC cells silenced for GPER expression. Survival analyses were performed on TNBC patients of the METABRIC dataset.

Results

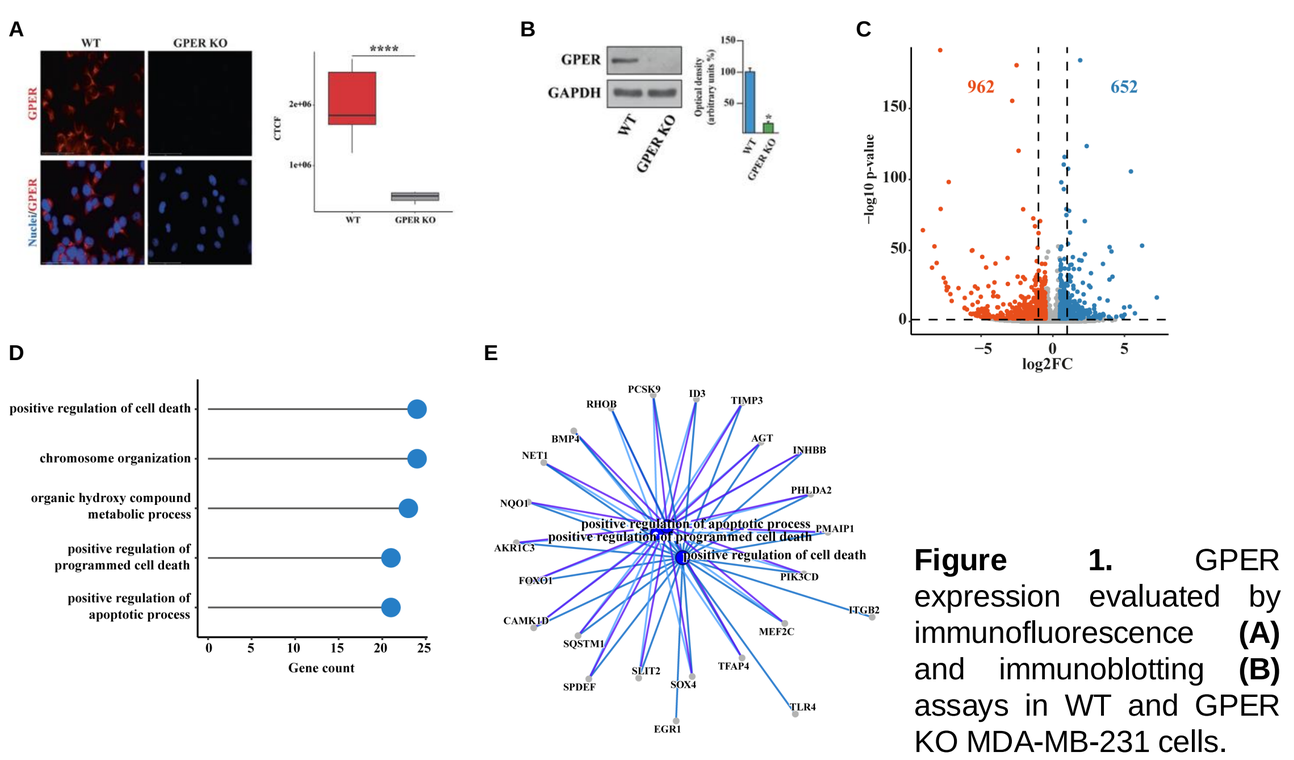

Figure 1. GPER expression evaluated by immunofluorescence **(A)** and immunoblotting **(B)** assays in WT and GPER KO MDA-MB-231 cells. **(C)** Volcano plot showing the differentially expressed genes (DEGs) in GPER KO respect to WT MDA-MB-231 cells, as found by RNA-sequencing. The downregulated genes (log2FC ≤−0.5 and p ≤ 0.01) are shown in orange (n. 962), the upregulated genes (log2FC ≥ 0.5 and p ≤ 0.01) are shown in blue (n. 652), non-significant genes are shown in gray (p > 0.01). **(D)** Gene ontology (GO) analysis of the upregulated genes in GPER KO respect to WT MDA-MB-231 cells, bubble chart shows the top 5 GO terms for biological process (BP) ranked by gene number (high to low). **(E)** Interrelation network of the genes belonging to the "positive regulation of cell death", "positive regulation of programmed cell death" and "positive regulation of apoptotic process" BPs. ****p < 0.0001.

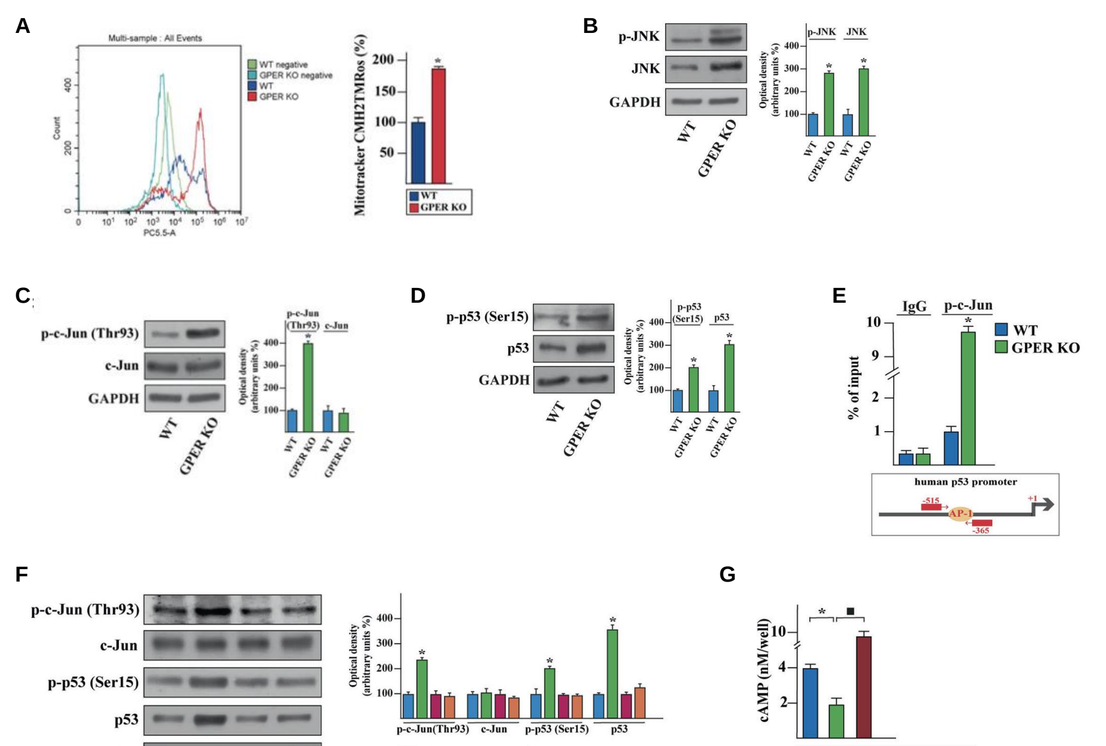

Figure 2. (A) Mitochondrial membrane potential in WT and GPER KO MDA-MB-231 cells exposed to MitoTracker Orange CMH2TMRos probe. Protein levels of p-JNK and JNK **(B)**, p-c-Jun (Thr93) and c-Jun **(C)**, p-p53 (Ser15) and p53 **(D)** in WT and GPER KO MDA-MB-231 cells. **(E)** Recruitment of p-c-Jun to the AP-1 site located within the p53 promoter sequence in WT and GPER KO MDA-MB-231 cells. (*) indicates p < 0.05. **(F)** Immunoblotting showing the protein levels of p-c-Jun (Thr93), c-Jun, p-p53 (Ser15) and p53 in WT and GPER KO MDA-MB-231 cells exposed for 12 h to vehicle or 400 nM JNK inhibitor SP600125. **(G)** Measurement of cAMP in WT and GPER KO MDA-MB-231 cells exposed for 12 h to vehicle or 10 μM cAMP specific activator forskolin. **(H)** Protein levels of p-JNK, JNK, p-c-Jun (Thr93), c-Jun, p-p53 (Ser15) and p53 in WT and GPER KO MDA-MB-231 cells exposed for 12 h to vehicle or 10 μM forskolin. (*), (■) indicate p < 0.05.

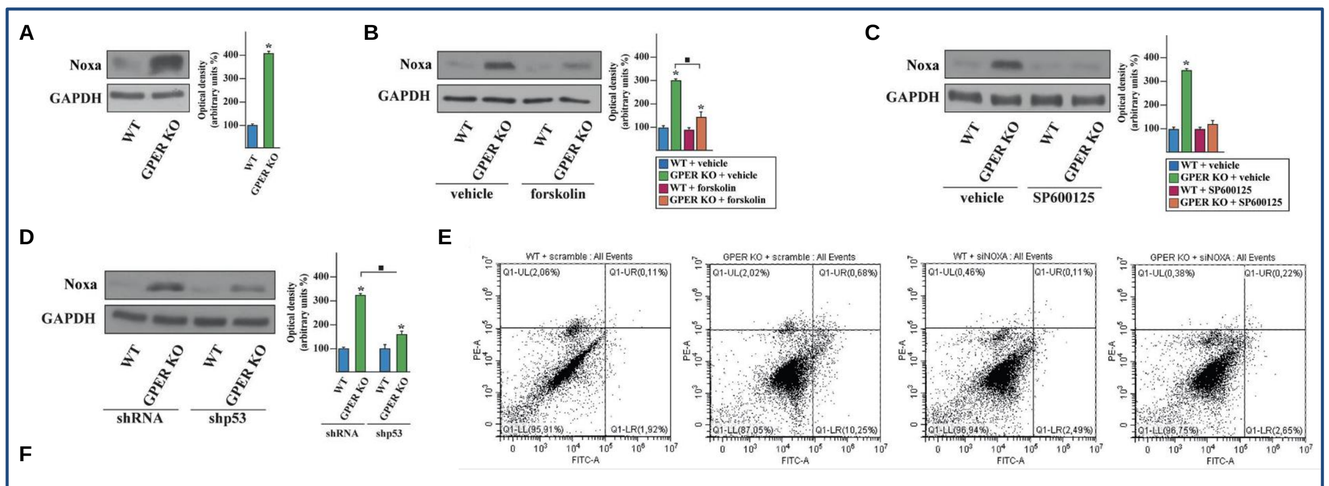

Figure 3. (A) Immunoblotting depicting the protein levels of Noxa in WT and GPER KO MDA-MB-231 breast cancer cells. Noxa levels in WT and GPER KO MDA-MB-231 cells exposed for 12 h to vehicle or 10 μM cAMP activator forskolin **(B)** or 400 nM JNK inhibitor SP600125 **(C). (D)** Protein levels of Noxa in WT and GPER KO MDA-MB-231 cells transfected for 36 h with shRNA or shp53. **(E)** Assessment of apoptosis by cytometric analyses in WT and GPER KO MDA-MB-231 cells transfected for 36 h with scramble or siNoxa. Cells were stained with Annexin V-FITC (Alexa Fluor 488) conjugate to identify apoptotic cells and with propidium iodide (PI) to identify dead cells. The four quadrants represent living cells (lower left, AnnexinV-/PI-), early apoptotic (lower right, Annexin V+/PI-), late apoptosis (upper right, Annexin V+/PI+) or necrotic (upper left, Annexin V-/PI+) stages. (*), (■) indicate p<0.05. **(F)** Survival analysis of Noxa mRNA expression and OS in TNBC patients.

Conclusions. Our findings unveil a role for GPER in sustaining anti-apoptotic signals in TNBC cells, thus suggesting this receptor a potential valuable therapeutic target to prevent the progression of the breast malignancy.

References. 1) Barton M et al., J Steroid Biochem Mol Biol. 2018. 2) Hall KA et al., Cells. 2023. 3) Lappano R et al., J Exp Clin Cancer Res. 2020. 4) Cirillo F e al., J Transl Med. 2024.