Apolipoprotein J Reshapes Mitochondrial Dynamic in Hormone Receptor-Positive but not in HER2-Positive Breast Cancer

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Abstract

Human epidermal growth factor receptor 2 (HER2)-positive breast cancer accounts for 20% of all breast cancer cases and is mainly treated with trastuzumab (Herceptin). However, most patients develop resistance within one year. Apolipoprotein J (ApoJ), also known as clusterin (CLU), is an extracellular chaperone involved in anti-apoptotic signaling and chemoresistance. Our previous work demonstrated that ApoJ reshapes mitochondrial dynamics and promotes malignancy in hormone receptor–positive MCF-7 breast cancer cells. Take one step forward, the present study extended the investigation to HER2-positive HCC1954 breast cancer cells. We found that trastuzumab reduced phosphorylation of the mitochondrial fission marker DRP1 and promoted mitochondrial elongation, without altering ApoJ expression. Notably, HCC1954 cells expressed a higher abundance of the ApoJ receptor LRP2 than MCF-7 cells, yet failed to uptake circulating ApoJ. Consequently, supplementation with exogenous ApoJ had no effect on mitochondrial dynamics in HCC1954 cells, indicating that ApoJ is not a key regulator in this subtype. Together, these findings reveal a unique pathological role of ApoJ in hormone receptor–positive breast cancer and underscore the importance of subtype-specific strategies for targeting mitochondrial function in breast cancer therapy.

Result

ApoJ disrupts mitochondrial dynamics in hormone receptor–positive breast cancer cells, and alters cellular energy homeostasis

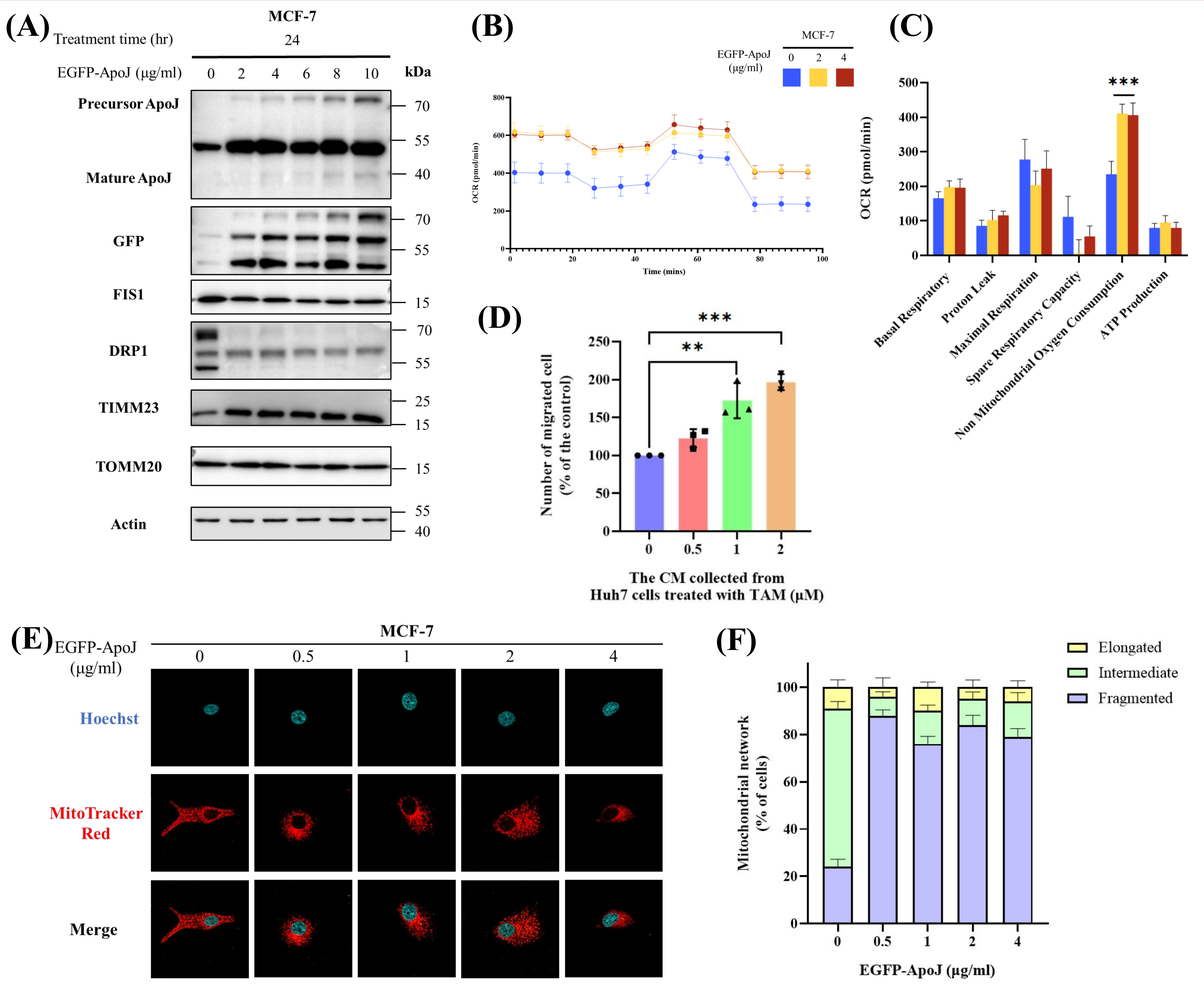
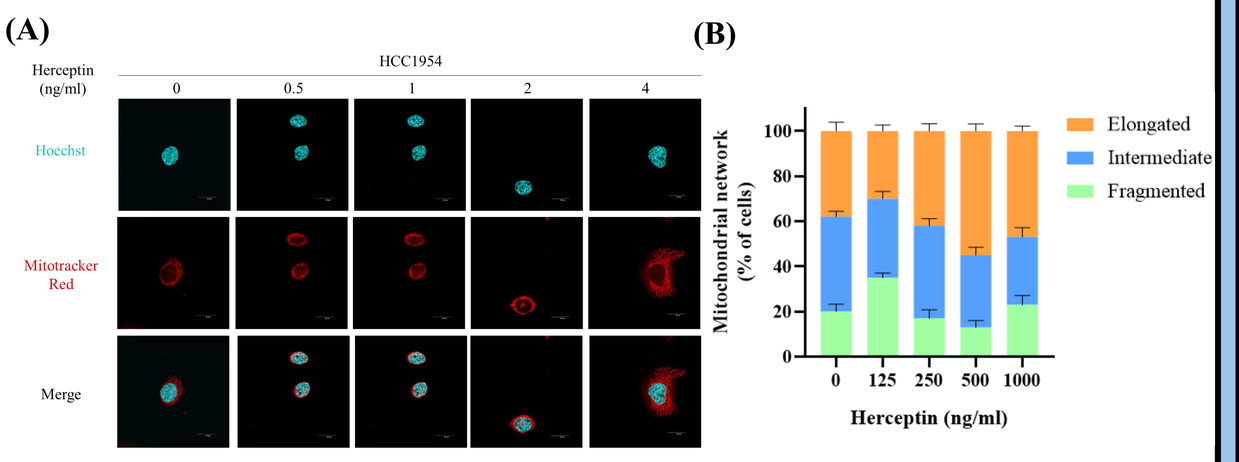


Figure 1. The circulating ApoJ was taken up by hormone receptor-positive breast cancer cells and altering both mitochondria and energy homeostasis. (A) The MCF-7 cells were treated with EGFP-ApoJ at indicated dose and the expression levels of protein were evaluated by Western blotting analysis with antibodies against GFP, Apolipoprotein J(ApoJ), Fission 1(FIS1), dynamin-related protein 1 (DRP1), translocase of inner mitochondrial membrane 23(TIMM23), translocase of outer mitochondrial membrane 20 (TOMM20), and actin. (B) The MCF-7 cells were treated with EGFP ApoJ (0, 2, and 4 μg/mL) for 24 hours and mitochondrial function was assessed using the Seahorse XF Cell Mito Stress Test. OCR was measured at baseline and following the sequential injection of ATP synthase inhibitor Oligomycin, mitochondrial oxidative phosphorylation uncoupler FCCP, and complex I/III inhibitor Rotenone. (C) Quantitative analysis of basal respiration, proton leak, maximal respiration, spare respiratory capacity, non respiration, spare respiratory capacity, non mitochondrial oxygen consumption and ATP mitochondrial oxygen consumption and ATP production. Data are presented as mean ± SD. Statistical analysis was performed using one-way ANOVA. *p<0.05, **p<0.01, ***p<0.001. (D) The MCF-7 cells exposed to cultured medium (CM) from TAM-treated Huh7 cells for 48 hours and a transwell migration assay was performed to assess the migratory response of MCF-7 cells. Quantification of migrated cells from three independent experiments is displayed as a percentage ± SD, with values normalized to the control group. Statistical analysis was performed using one-way ANOVA. *p<0.05, **p<0.01, ***p<0.001. (E) The MCF-7 cells at a density of 1×10^4 were treated with EGFP-ApoJ at indicated dose for 24 hours. The mitochondria (red) and nuclei (blue) were visualized by mitotracker red and Hoechst staining, respectively. Scale bar, 5 μm. 100X magnification. (F) Mitochondrial morphology in MCF-7 cells was quantitatively assessed and results were expressed as a percentage of the total cell population. Data are presented as mean ± SD.

Herceptin treatment induced alterations in mitochondria homeostasis in HER2-positive breast cancer cells



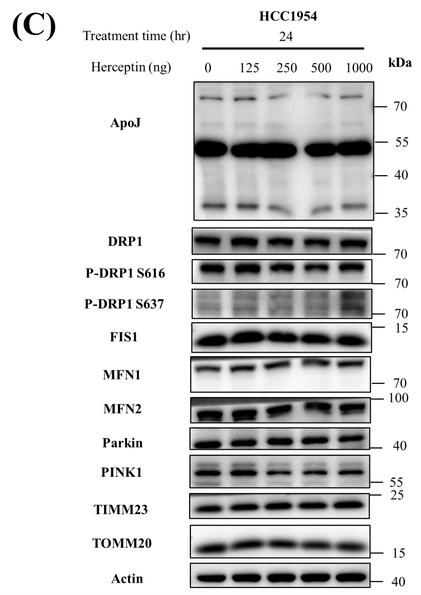


Figure 2. Herceptin treatment can alter mitochondria homeostasis in HER2-positive breast cancer cells. (A) The HCC1954 cells at a density of 1×10^4 were treated with Herceptin at indicated dose for 24 hours. The mitochondria (red) and nuclei (blue) were visualized by mitotracker red and Hoechst staining, respectively. Scale bar, 5 μm. 100X magnification. (B) Mitochondrial morphology in HCC1954 cells was quantitatively assessed and results were expressed as a percentage of the total cell population. Data are presented as mean ± SD. (C) The HCC1954 cells were treated with Herceptin at indicated dose and the expression levels of protein were evaluated by Western blot analysis with antibodies against ApoJ, FIS1, DRP1, TIMM23, TOMM20, Phospho-DRP1 (Ser616), Phospho-DRP1 (Ser637), Mitofusin1(MFN1), Mitofusin2(MFN2), PTEN-induced kinase 1(PINK1), Parkin and actin.

ApoJ has minimal effect on mitochondria imbalance in HER2-positive breast cancer cells

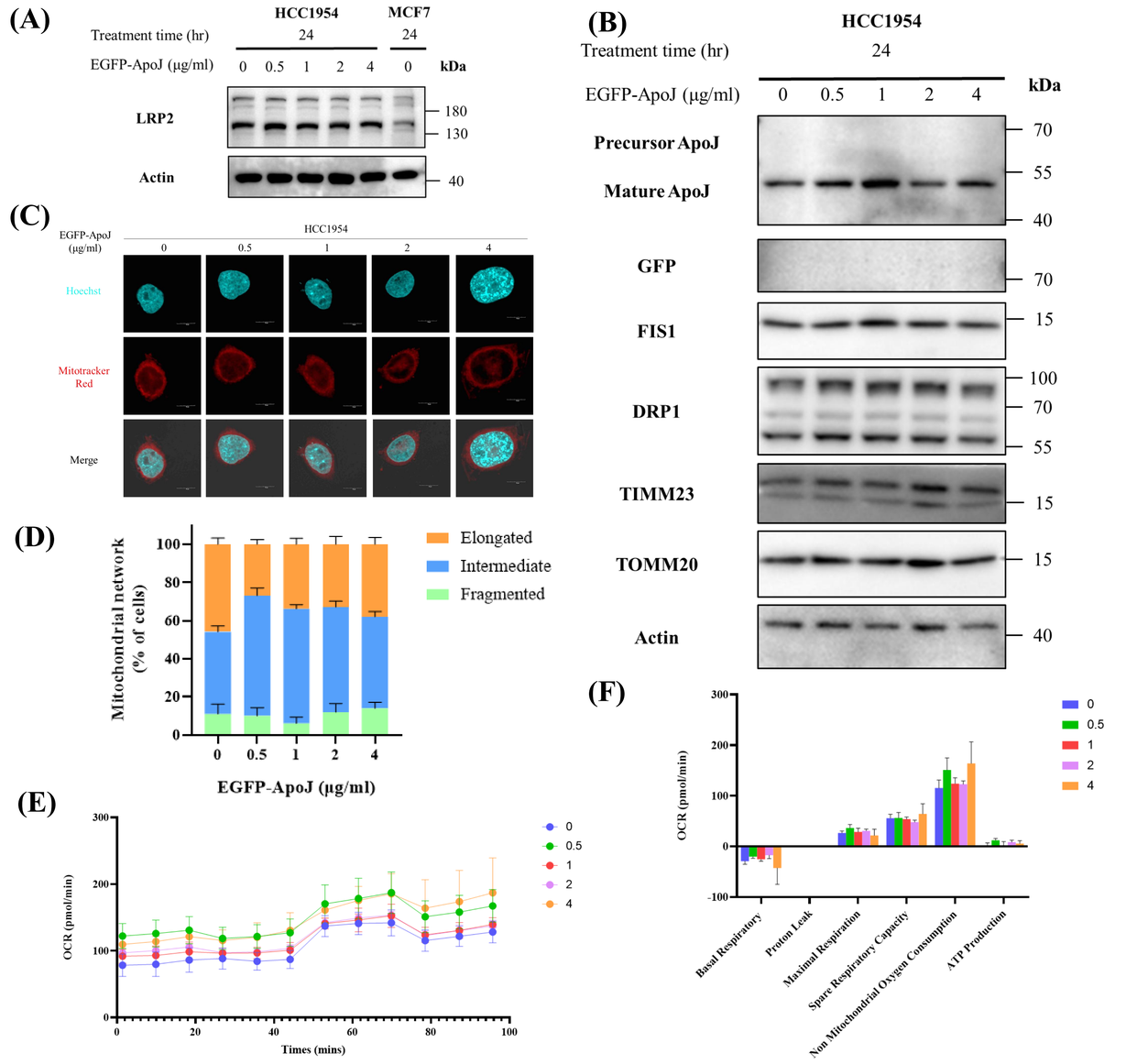
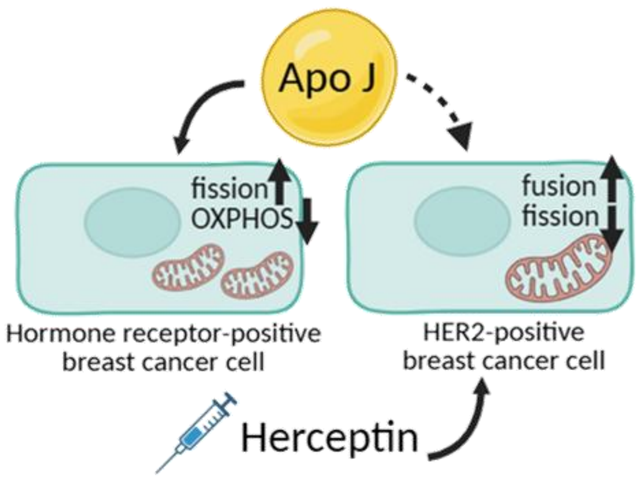


Figure 3. The circulating ApoJ was not taken up by HER2-positive breast cancer cells through LRP2 and thus showing no significant difference in mitochondria and energy homeostasis. (A) The HCC1954 cells were treated with EGFP-ApoJ at indicated dose and the expression levels of protein were evaluated using Western blot analysis with antibodies against Low-density lipoprotein receptor-related protein 2(LRP2) and actin. (B) The HCC1954 cells were treated with EGFP-ApoJ at indicated dose and the expression levels of protein were evaluated by Western blot analysis with antibodies against GFP, ApoJ, FIS1, DRP1, TIMM23, TOMM20, and actin. (C) The HCC1954 cells at a density of 1×10^4 were treated with EGFP-ApoJ at indicated dose for 24 hours. The mitochondria (red) and nuclei (blue) were visualized by mitotracker red and Hoechst staining, respectively. Scale bar, 5 μm. 100X magnification. (D) Mitochondrial morphology in MCF-7 cells was quantitatively assessed and results were expressed as a percentage of the total cell population. Data are presented as mean ± SD. (E) The HCC1954 cells were treated with EGFP ApoJ (0, 0.5,1,2 and 4 μg/mL) for 24 hours and mitochondrial function was assessed using the Seahorse XF Cell Mito Stress Test. OCR was measured at baseline and following the sequential injection of ATP synthase inhibitor Oligomycin, mitochondrial oxidative phosphorylation uncoupler FCCP, and complex I/III inhibitor Rotenone. (F) Quantitative analysis of basal respiration, proton leak, maximal respiration, spare respiratory capacity, non respiration, spare respiratory capacity, non mitochondrial oxygen consumption and ATP mitochondrial oxygen consumption and ATP production. Data are presented as mean ± SD. Statistical analysis was performed using one-way ANOVA. *p<0.05, **p<0.01, ***p<0.001.

Conclusion



ApoJ can be internalized by MCF7 cells, leading to an increase in fragmented mitochondria and a downregulation of oxidative phosphorylation. However, ApoJ is not efficiently uptaken by HCC1954 cells. Moreover, the effects of Herceptin on mitochondrial homeostasis appear to be minimally associated with ApoJ.