Mibefradil inhibits the proliferation of triple-negative breast cancer by targeting AURKA to regulate apoptosis signal pathway

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Research Article

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Abstract

Background: Triple negative breast cancer (TNBC) is a tumor characterized by high recurrence and mortality, but without effective targeted therapy. It is urgent to explore new treatment strategy to improve the efficacy of TNBC therapy.

Methods: Transcriptomic profiling datasets of TNBC were used for screening TNBC specific gene sets. Drug prediction was performed in Connectivity map (CMap) database. Molecular docking method was used for analyzing drug targets. In vitro and in vivo models of TNBC were constructed to examine the drug efficacy.

Results: We screened out Mibefradil, a T-type Ca^{2+} channel blocker, might be a potential therapeutic drug for TNBC by transcriptomics and bioinformatics analysis, and verified that Mibefradil could inhibit the proliferation of TNBC cells by inducing apoptosis and cell cycle arrest. Furthermore, by network pharmacology and molecular docking analysis, AURKA was predicted as the most possible drug target of Mibefradil. Finally, it was proved that Mibefradil treatment could induce apoptosis by decreasing protein expression and phosphorylation level of AURKA in vitro and in vivo.

Conclusions: Mibefradil played anti-cancer role in TNBC cells by targeting to AURKA to induce cell cycle and apoptosis. Our results repurposed Mibefradil as a potential targeted drug of TNBC and provided a fundamental research for a novel strategy TNBC treatment.

Full Text

Due to technical limitations, full-text HTML conversion of this manuscript could not be completed. However, the manuscript can be downloaded and accessed as a PDF.

Tables

Due to technical limitations, table 1 is only available as a download in the Supplemental Files section.

Figures

Figure 1

Screening potential drugs based on TNBC specific expression genes. (A) The flowchart showed the identification procedure of TNBC characteristic expression genes. (B) Heatmaps of DEGs between TNBC and nTNBC in TCGA, METABRIC and GSE76275 datasets, respectively. (C-D) Venn diagrams showed the intersections of upregulated (C) and downregulated (D) genes among three datasets. (E) Heatmap of DEGs between TNBC and normal breast samples in TCGA transcriptome dataset. (F) Schematic diagram

of the drug screening procedure, in which Venn diagrams showed the intersections of DEGs of TNBC/nTNBC and TNBC/normal breast samples (as TNBC specific expression genes). TNBC specific expression genes were then uploaded to CMap database for drug screening.

Figure 2

Mibefradil had an anti-tumor effect on TNBC cells in vitro. (A) Chemical structure of Mibefradil. (B) The IC₅₀ of MDA-MB-468 and MDA-MB-531 cells treated by Mibefradil for 48 h. (C) Cell viability of MDA-MB-

468 and MDA- MB- cells treated with indicated doses of Mibefradil for 48 h. (D-E) Representative flow cytometry analysis and quantification of apoptosis cells of TNBC cells treated with indicated doses of Mibefradil for 48 h. Data are presented as mean $\pm$ SD. n=3 biological replicates. * P< 0.05, *** P<0.001.

Figure 3

Mibefradil promoted cell cycle arrest in TNBC cells. (A-B) Plots and histogram graphs respectively showed the results of KEGG (A) and GO (B) enrichment analysis of 167 DEGs characteristically upregulated in TNBC. (C) Effect of the Mibefradil on cell cycle of MDA-MB-468 and MDA-MB-231 cells. Data are presented as mean $\pm$ SD. n=3 biological replicates. * P< 0.05, ** P<0.01.

Figure 4

AURKA was predicted to be the therapy target for Mibefradil. (A) The compound target network of Mibefradil. Red rhombus represented Mibefradil. Yellow ellipse connected with Mibefradil by the red line are the potential targets. Blue line represented the protein interaction between the targets. (B) PPI network analysis of 167 characteristically upregulated genes in TNBC. Network contains 104 nodes and 1122 edges. (C) Venn diagram showed the intersection between Mibefradil's potential targets and characteristically upregulated genes in TNBC. (D) Molecular docking analyses for AURKA and Mibefradil. (E) The expression of AURKA and p-AURKA in MDA-MB-468 and MDA-MB-231 cells treated with indicated doses of Mibefradil for 48 h.

Figure 5

Mibefradil increased TNBC apoptosis by targeting AURKA. (A) TNBC cells were treated with indicated doses of Mibefradil for 48 h. The expression of cleaved-PARP, BCL2, CyclinD1 and CyclinB1 was detected by Western Blotting analysis. (B) Knockdown efficacy of AURKA in TNBC cells was examined by Western Blotting analysis. (C) Overexpression efficacy of AURKA in TNBC cells was examined by Western Blotting analysis. (D) The effect of Mibefradil on apoptosis of AURKA knockdown TNBC cells. (E) The effect of

Mibefradil on apoptosis in AURKA overexpression TNBC cells. (F) The effect of Mibefradil on the expression of apoptosis- and cell cycle-related proteins in AURKA knockdown TNBC cells. (G) The effect of Mibefradil on the expression of apoptosis- and cell cycle-related proteins in AURKA overexpression TNBC cells. Data were presented as mean $\pm$ SD. n=3 biological replicates. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Figure 6

Mibefradil suppressed tumor progression in xenograft mouse model. (A) Representative images of MDA-MB-468 subcutaneous tumors treated with H2O or Mibefradil. (B) Quantify the weight of the tumor mass. (C) Changes in tumor volume during treatment were recorded every 2 days. (D) Representative IHC staining of Ki67, TUNEL, AURKA, and p-AURKA in subcutaneous tumors. (E) Body weight of the mice administrated with H2O or Mibefradil. (F) Representative HE staining of heart, liver and kidney of the mice administrated with H2O or Mibefradil.

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