



## Review Article

# A prospective for the role of two-pore channels in breast cancer cells

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## Abstract

Breast cancer is one of the most common cancers and the second most frequent cause of cancer death among women worldwide. Recently, potential links between cancer and the calcium mobilizing messenger Nicotinic Acid Adenine Dinucleotide Phosphate (NAADP) and its intracellular target Two-Pore Channels (TPCs) have been identified. However, their role in breast cancer cells has not been extensively evaluated, and the understanding of underlying pathways is still lacking. Here we proposed a hypothesis that TPC function is crucial for the cell biology and tumorigenesis of the breast cancer cell. Further study can be done in this field, such as screening the expression of TPC1 and TPC2 in different breast cancers, then testing whether altered expression of TPC1 and TPC2 affects breast cancer and breast cancer stem cells. It is also valuable to search for the potential targets of TPCs and NAADP calcium signaling in breast cancer cells. This mini-review aims to propose potential research methods and experimental design for future study and will contribute to the raising and development of the research in this field.

## Background

Breast cancer is one of the most common cancers and the second most frequent cause of cancer death among women worldwide [1]. In 2019, more than 10% of all types of cancers are breast cancer, resulting in more than 6% of death in the world [2]. The incidence of breast cancer is predicted to increase by 50% within 25 years [3]. Thus, the study in breast cancer is urging and of utmost importance.

Ion channels are thought to be associated with breast cancer [4]. Recently, potential links between Two-Pore Channels (TPCs) and cancer have been identified. TPCs are eukaryotic intracellular voltage-gated and ligand-gated cation-selective ion channels with one important modulator of such channels being NAADP. There are two known paralogs in the human genome, including TPC1 and TPC2. In humans, TPC1 is sodium selective and TPC2 conducts sodium ions, calcium ions and possibly hydrogen ions [5]. However, their role in breast cancer cells has not been extensively evaluated, and the understanding of underlying pathways is still lacking.

Ong Nam Phuong Nguyen, et al. [6], found that TPC gene knockout or TPC pharmacological inhibitors can decrease

the metastasis of 4T1 mouse breast cancer cell lines in an in vivo study, but a whole animal study assessing human cancer cell lines is also needed. Wei Sun, et al. [7], also found that TPCs are overexpressed in 4T1 cells and play a role in cancer cell migration. The expression of TPC1 and TPC2 in some human breast cancer cell lines has been determined by Aisyah H Jahidin, et al. [8] But they did not link the difference of expression level to the subtypes of breast cancer cells. They also found that TPC1/TPC2 knocking out did not affect the cell proliferation in MDA-MB-468 breast cancer cell line, but they failed to reveal the effects of TPCs on other breast cancer cell lines. So far, the role of TPCs in breast cancer cell biology has not been systematically evaluated, hence the study of the role of TPCs in proliferation and migration in breast cancer cells and the exploration of the mechanisms underlying these effects is needed.

Currently, cancer cells are thought to originate from breast Cancer Stem Cells (CSCs) or “stem-like cells”. A recent study showed that CSCs are essential for metastatic tumor growth [9,10]. As the key components of NAADP-regulated calcium channels, TPCs contribute to changes in intracellular Ca<sup>2+</sup> which regulate a diverse range of cellular processes including proliferation, differentiation, metabolism, gene transcription



and apoptosis [11,12] and TPCs are shown to play a crucial role in cancer cell migration [13-15], which implies that there might be a link between CSCs and TPCs. My hypothesis is that TPC function is crucial for cell biology and tumorigenesis in breast cancer cells.

### Potential available methods and strategies

To explore the role of TPC1 and TPC2 in cancer cell biology and tumorigenesis in breast cancer. Here this review lists several prospective study designs.

(1). Although TPCs are expressed in breast cancer cells, their expression profile and function in subtypes of breast cancer cells [16] has not been extensively evaluated. Therefore, we can screen the expression of TPC1 and TPC2 in different subtypes of breast cancers and determine their function in the calcium signaling pathway. We expect to find greater expression of TPCs and altered TPC function in certain types of breast cancer cell lines.

RT-qPCR and Western blot methods will be used to determine the mRNA and protein expression of TPC1 and TPC2 in different subtypes of breast cancer cell lines, MCF-7 (Luminal A), BT474 (Luminal B), MDA-MB-468 (basal-like), MDA-MB-231 (claudin-low), MDA-MB-453 (HER2-positive), etc. Whole-endolysosomal patch-clamp recordings can be used to test the function of the TPCs in breast cancer cell lines and tetrandrine will be used as a negative control. Intracellular NAADP induced calcium signals can be assessed by calcium imaging.

Breast cancer tissues with complete clinical-pathological data (including cancer subtype) will be collected from patients and made into paraffin-embedded tissue microarrays. mRNA and protein expression of TPC1 and TPC2 in patients' tumors will be determined by in situ RNA hybridization and immunohistochemistry methods.

(2). TPCs play a role in cancer cells, but their role in breast cancer cell biology has not been systematically evaluated, so I will test whether the expression of TPC1 and TPC2 affects breast cancer cells. I expect to find the knocking out of TPCs can decrease breast cancer cell migration and metastasis.

Cell viability, migration, invasion, and adhesion viability in *Tpcn1/2<sup>+/+</sup>*, *Tpcn1/2<sup>-/-</sup>*, *Tpcn1<sup>-/-</sup>*, and *Tpcn2<sup>-/-</sup>* breast cancer cell lines will be determined using MTT, Cell Cytotoxicity Assay Kit, apoptosis ELISA Kit [17], Flow Cytometry, wound healing, transwell, matrices assay, colony-forming assay, trypsin detaching assay, etc. Intracellular NAADP induced calcium signals will be assessed by calcium imaging. The breast cancer subtypes that have more TPC expression will be compared with those that have less expression.

NSG mice and *Tpcn1/2<sup>+/+</sup>*, *Tpcn1/2<sup>-/-</sup>*, *Tpcn1<sup>-/-</sup>*, and *Tpcn2<sup>-/-</sup>* luciferase MDA-MB-231 (human breast cancer cell lines) will be used in this study [18]. Cells will be injected into the breast pads. Bioluminescence signals will be measured by luciferase imaging. The primary tumor will be excised and collected after four weeks and the primary recurrent and metastasis will be evaluated.

(3). As Cancer Stem Cells (CSCs) have been shown to be essential for metastatic tumor growth and TPCs is shown to play a crucial role in cancer cell migration, I expect to find that knocking out of TPCs will decrease the population of CSCs and that the CSCs have higher levels of TPC expression.

Self-renewal ability of *Tpcn1/2<sup>+/+</sup>*, *Tpcn1/2<sup>-/-</sup>*, *Tpcn1<sup>-/-</sup>*, and *Tpcn2<sup>-/-</sup>* breast cancer cell lines will be determined using serial replating assays [19]. The level of BCSCs will be determined by CSC marker [20,21], (CD44<sup>+</sup> CD24<sup>-low</sup> and ALDH<sup>+</sup>) stain followed by flow cytometry and immunohistochemistry methods. The number of breast CSCs and cancer cells in tumor and metastatic tissue in animal models will be determined by cell marker staining using the immunohistochemistry method.

Immuno-magnetic cell sorting of stem cell subpopulation will be used [22]. Breast cancer cell lines will be exposed to FITC-conjugated anti-CD44, anti-ALDH, and anti-LGR5 antibody, and further labeled with dextran-coated magnetic nanoparticles using bispecific tetrameric antibody complexes (TAC). These cells will be subjected to immuno-magnetic cell separation and CD44<sup>+</sup>, ALDH<sup>+</sup>, and LGR5<sup>+</sup> cells will be identified as CSCs. Sphere culture [23,24], will also be used as another method to extract cancer stem-like cells. Cells will be cultured in ultralow attachment plates and the sphere passage cells will be identified as CSCs. The expression of TPCs in CSCs will be tested.

(4). To have a better understanding of TPCs in breast cancer biology, the precise mechanisms of TPCs action, and their links with NAADP calcium signaling, I will search for potential targets of TPCs and NAADP calcium signaling in breast cancer cells. I expect to find some potential targets that can explain the effect of TPCs on breast cancer cells.

Total protein and total RNA of *TPCN1/2<sup>+/+</sup>*, *TPCN1/2<sup>-/-</sup>*, *TPCN1<sup>-/-</sup>*, and *TPCN2<sup>-/-</sup>* breast cancer cells will be extracted, and proteomic analysis and gene array methods will be used to screen altered protein and mRNA expression. QPCR and western blotting methods can be used to confirm the results, the method was described previously [25,26]. The gene knockout method will be used to test the potential targets of TPCs and NAADP induced calcium signals. Sorted CSCs will also be studied to search for potential targets underlying the effect of TPCs on breast CSCs.

### Conclusion

This review hopes to propose a prospective study for the role of two-pore channels in breast cancer cells and will contribute to the raising and development of the research in this field.

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