Cancer Letters 374 (2016) 117–126

Contents lists available at ScienceDirect

Cancer Letters

journal homepage: www.elsevier.com/locate/canlet

Mini-review

Connexin 43, breast cancer tumor suppressor: Missed connections?

Christina L. Grek [a], J. Matthew Rhett [b], Jaclynn S. Bruce [c], Gautam S. Ghatnekar [a], Elizabeth S. Yeh [c,d,*]

[a] FirstString Research, Inc., 300 W. Coleman Blvd., Suite 203, Mount Pleasant, SC, USA
[b] Department of Surgery, Division of General Surgery, Medical University of South Carolina, Charleston, SC, USA
[c] Department of Cell and Molecular Pharmacology and Experimental Therapeutics, Medical University of South Carolina, 173 Ashley Ave, BSB358, MSC509, Charleston, SC 29425, USA
[d] Hollings Cancer Center, Medical University of South Carolina, Charleston, SC 29412, USA

ARTICLE INFO

Article history:
Received 5 October 2015
Received in revised form 28 January 2016
Accepted 3 February 2016

Keywords:
Breast cancer
Connexin 43
Gap junctions
Metastasis

ABSTRACT

Connexins are a family of transmembrane proteins that are characterized by their capacity to form intercellular channels called gap junctions that directly link the cytoplasm of adjacent cells. The formation of gap junctions by connexin proteins facilitates intercellular communication between neighboring cells by allowing for the transfer of ions and small signaling molecules. Communication through gap junctions is key to cellular equilibrium, where connexins, and the gap junction intercellular communication that connexins propagate, have roles in cellular processes such as cell growth, differentiation, and tissue homeostasis. Due to their importance in maintaining cellular functions, the disruption of connexin expression and function underlies the etiology and progression of numerous pathologies, including cancer. Over the past half a century, the role of connexins and gap junction intercellular communication have been highlighted as critical areas of research in cellular malignancies, and much research effort has been geared toward understanding their dysfunction in human cancers. Although ample evidence supports the role of connexins in a variety of human cancers, detailed examination in specific cancers, such as breast cancer, is still lacking. This review highlights the most abundant gap junction connexin isoform in higher vertebrate organisms, Connexin 43, and its role in breast cancer.

Introduction

The complexity of multicellular organisms necessitates an efficient, coordinated route of cell-to-cell communication. Connexins make up a family of transmembrane proteins that are the primary component in intercellular gap junction pores and have roles in propagating intercellular communication, a property called gap junction intercellular communication (GJIC) [1–3]. Each gap junction is composed of a hexagonally arrayed lattice of intercellular aqueous channels spanning the two membrane bilayers of opposing cells. Each of these channels is made up of 2 hexameric connexin oligomers termed connexons or hemichannels, each one contributed by the two contacting cells. In addition to forming gap junctions, connexin hemichannels have their own physiological roles in regulating cell-to-extracellular communication and purinergic signaling [4].

There are as many as 21 connexin family members in mammals which can combine to form heteromeric and/or heterotypic channels. Studies examining connexin mutations show that the disruption of connexin protein conformation, turnover, and channel function leads to disease phenotypes and reveals the importance of connexins and gap junctions in maintaining tissue homeostasis [5]. A critical role for connexins, gap junctions, and GJIC in tumorigenesis and metastasis was documented as early as 1966 in studies that revealed impaired intercellular electrical coupling in chemically-induced and xenografted rat hepatocarcinomas [6,7]. Expression and functional studies have identified connexins as potential tumor suppressors, where restoration of depressed connexin expression and thus restoration of GJIC inhibit tumor cell growth [8,9]. However, this story has been made more complex by recent studies that report the aberrant increased expression of connexins in a variety of carcinomas and sarcomas as well as the more recently discovered transcriptional functions of connexins that are independent of their gap junction function [10,11].

Connexin 43 (Cx43) is one of the most highly expressed and widely studied connexins and has been found to be aberrantly expressed in several tumor types including liver, prostate, and breast.

Abbreviations: Cx43, connexin 43; Cx, connexin; GJIC, gap junction intercellular communication; WT, wildtype; ErbB2, epidermal growth factor receptor 2; ZO-1, zona occludens-1; ZO-2, zona occludens-2; TCDD, 2,3,7,8-tetrachlorodibenzodioxin; PQ1, substituted quinolones; Akt, protein kinase B; MAPK, mitogen activated protein kinase; αCT1, α-connexin carboxyl-terminal.

* Corresponding author. Tel.: 843-876-2307; fax: 843-792-2475.
E-mail address: yeh@musc.edu (E.S. Yeh).

http://dx.doi.org/10.1016/j.canlet.2016.02.008
0304-3835/

While recent studies support the therapeutic potential of targeting Cx43 as a treatment in breast cancer [12–15], the complexities that are associated with the disseminating roles of Cx43 in the processes of tumorigenesis, tumor cell migration and metastasis in breast cancer remain unresolved. Evidence suggests that Cx43-directed GJIC is critical for normal cell function and loss of this feature promotes malignant transformation of breast/mammary epithelial cells [16–20]. These studies have yielded interesting but early stage information, suggesting that Cx43 has a role in breast cancer cell proliferation, differentiation, and migration. The exact nature of this role is complicated by studies examining human breast cancer tissue that suggest that levels of Cx43 change with cancer stage [16–19]. These studies have also revealed that, regardless of expression level, atypical (e.g., cytoplasmic) expression of Cx43 and impaired GJIC could play a part in determining disease severity and act as an early sign of malignancy, suggesting that preserving Cx43 gap junctions could be an important distinction between normal and malignant breast epithelial cells [16,19,20]. Consequently, maintaining Cx43 gap junctional activity could be a mechanism of Cx43-dependent tumor suppression. A schematic of this concept is shown in Fig. 1A, where it is represented that loss of GJIC maintained by Cx43 in normal mammary gland results in breast cancer malignancy. Furthermore, it is a common feature for breast cancer cells to exhibit Cx43 localized away from the plasma membrane where gap junctions would normally form. This mis-localization is exemplified in Fig. 1B, which shows examples of Cx43 immunofluorescence staining in MCF7 and BT474 breast cancer cell lines where Cx43 is localized away from the plasma membrane, likely in endocytic vesicles in the cytoplasm. Here, we review the current literature on Cx43 and breast cancer in order to highlight a possible tumor suppressor role for Cx43 in breast cancer, what circumstances have been observed where Cx43 acts as a tumor suppressor or does not, and the potential for therapeutic targeting of this protein for treatment of primary and metastatic disease (summarized in Table 1).

Materials and methods

Immunofluorescence

The anti-Cx43 antibody used for immunofluorescence detection of Cx43 in Fig. 1B was purchased from Sigma-Aldrich (C-terminal directed antibody). Wheat Germ Agglutinin (WGA) stain was purchased from Life Technologies (Thermo-Fisher). Images were obtained using a Leica L5 scanning confocal microscope.

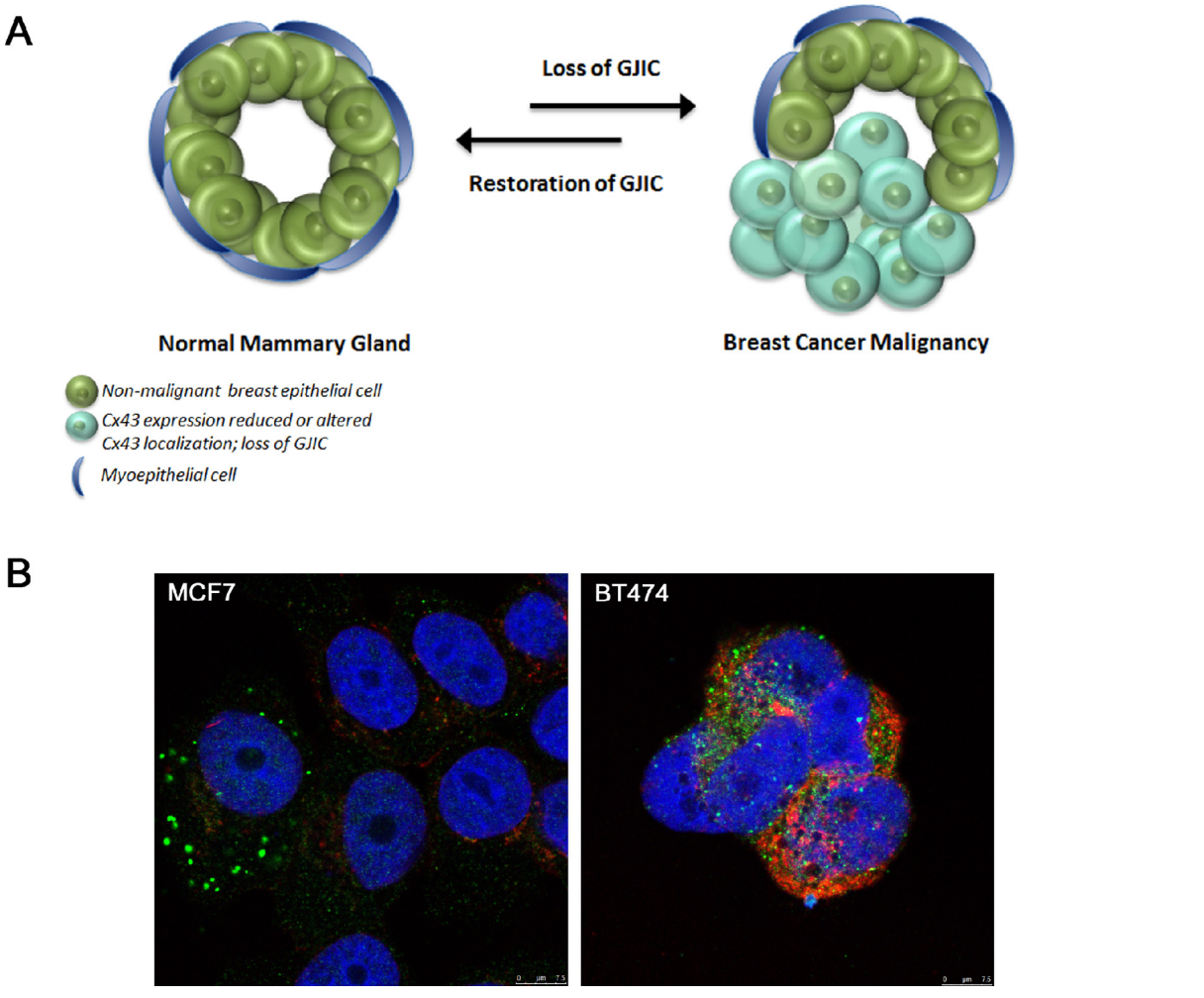

Fig. 1. Gap junctional intercellular communication and breast cancer malignancy. (A) Normal mammary gland structure shown on the left is maintained by mammary epithelial and myoepithelial cells that have functional Cx43 gap junctions. Loss of the GJIC mediated by Cx43 is thought to promote the development of breast cancer malignancies that contain cells that no longer have functional Cx43 gap junctions either due to loss of Cx43 expression or mis-localization of Cx43 away from the plasma membrane. Restoration of Cx43 activity is proposed to reverse the malignant phenotype driven by loss of Cx43 GJIC. (B) Cx43 immunofluorescent staining in MCF7 and BT474 cells demonstrates localization of Cx43 to cytoplasmic areas not associated with gap junction or hemichannel formation at the plasma membrane. Cx43 staining in green. Wheat germ agglutinin staining to show the plasma membrane in red. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)