Potassium channels and proliferation and migration of breast cancer cells

ZHANG Lei1, ZOU Wei1,*, ZHOU Shi-Sheng2, CHEN Dong-Dong1

1College of Life Science, Liaoning Normal University, Dalian 116029, China; 2Medical College of Dalian University, Dalian 116600, China

Abstract: Potassium channels (K+ channels), a family of special ion channel proteins, are involved in various physiological functions. Recent data show that the abnormalities of K+ channels are not only responsible for some neurological and cardiovascular diseases but also for channelopathies. Furthermore, many groups reported that the abnormalities of K+ channels had shown their oncogenic potential in breast introduction and other malignant tumors, promoting proliferation, invasion and metastasis. The aim of this review is to give an updated introduction of research progress in K+ channels associated with breast cancer.

Key words: potassium channels; breast cancer; proliferation; metastasis

1 Introduction

Potassium channels (K+ channels) are the most diverse and ubiquitous class of ion channels which are involved in many physiological functions such as solute transport, volume control, enzyme activity, secretion, excitation-contraction coupling and intercellular communication. K+ channels had also been recognized for their oncogenic potential in some malignant tumors, promoting proliferation, invasion, metastasis, etc.[1-3]. Recent reports indicated that K+ channels played a key role in breast cancer cells proliferation, cell cycle progress, apoptosis and metastasis[4]. This brief review gives an introduction of research progress in K+ channels associated with breast cancer.

2 Classification of K+ channels

K+ channels are membrane-spanning proteins that selectively conduct K ions across the cell membrane along its electrochemical gradient. They are usually classified into three groups based upon the primary amino acid sequence of pore-forming subunits: voltage-gated K+ channels con-
taining six transmembrane regions with a single pore, inward rectifier K⁺ channels containing two transmembrane regions with a single pore, and two-pore K⁺ channels containing four transmembrane domains with two pore regions⁵. In recent years, many K⁺ channels have been found in specific cancer cells. They have been classified into several sub-families, which include: voltage-gated K⁺ channels (Kv), inward rectifier K⁺ channels (Kir), Ca²⁺ activated K⁺ channels (KCa), ether-α-go-go K⁺ channels (EAG), ATP sensitive K⁺ channels (KATP)⁶. Table 1 showed the different types of K⁺ channels found in normal and cancer tissues of breast.

### 3 K⁺ channels in the occurrence of breast cancer cells

The reasons for the occurrence of breast cancer cells are multi-factorial. A single factor or a combination of factors can cause gene mutation and/or the overexpression of an oncogene. The studies have confirmed that the irregular expression of BRCA1/2, p53, and HER2(neu) are involved in the occurrence of breast cancer. Furthermore, many genes, such as MUC1, NM23, p53, are considered to be the markers of mammary gland metastasis. Previous studies have shown that some K⁺ channels exhibited oncogenic potential in the progression of breast cancer⁴⁶. Recently two-pore (2P) domain K⁺ (TWIK) channels were suggested to have a critical role in both cell apoptosis and tumorigenesis⁷⁻⁸. TWIK-related acid-sensitive K⁺ channel 3 (TASK3), a 2P channel, is the only one overexpressed in a series of breast cancer samples. The overexpression promotes the formation of tumors, which suggests that TASK-3 is an important factor in the carcinogenesis of breast cancer. In fact, It was found that TASK3 was amplified from 3-fold to 10-fold in 10% of breast tumors and overexpressed from 5-fold to over 100-fold in 44% of breast tumors⁹. Additionally, Pei et al.¹⁰ reported a dominant-negative mutation of TASK3, TASK3G95E, could abolish its K⁺ channel activity and also abrogate the oncogenic function, but had no effect on normal cell growth. Co-expression of the wild-type and the mutant TASK3 resulted in the inhibition of K⁺ current of wild-type TASK3 and tumor occurrence in nude mice. In addition, human EAG mRNA was detected in several cancer cell lines and its expression was

### Table 1. Classification of K⁺ channels in human breast tissues

<table>
<thead>
<tr>
<th>Type</th>
<th>Nomenclature</th>
<th>References</th>
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<tbody>
<tr>
<td>Voltage-gated K⁺ channel</td>
<td>Kv1.1</td>
<td>Ouadid-Ahidouch H et al., 2000¹³</td>
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<td></td>
<td>Kv1.3</td>
<td>Abdul M et al., 2003¹⁴</td>
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<td>Ca²⁺-activated K⁺ channel</td>
<td>BK&lt;sub&gt;Ca&lt;/sub&gt;</td>
<td>Ouadid-Ahidouch H et al., 2004¹⁵</td>
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<tr>
<td></td>
<td>SK&lt;sub&gt;Ca&lt;/sub&gt;</td>
<td>Roger S et al., 2004¹⁶</td>
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<td></td>
<td>hEAG</td>
<td>Coiret G et al., 2007²⁷</td>
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<td></td>
<td>hIK1-like K⁺ channel</td>
<td>Ouadid-Ahidouch H et al., 2004¹⁵</td>
</tr>
<tr>
<td>Voltage and Ca²⁺-sensitive K⁺ channel</td>
<td>Maxi-K channel</td>
<td>Coiret G et al., 2005²⁶</td>
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<td>Inward rectifier K⁺ channel</td>
<td>GIRK1</td>
<td>Stringer BK et al., 2001²⁹</td>
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<td></td>
<td>GIRK2</td>
<td>Plummer HK 3rd et al., 2004³⁰</td>
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<td></td>
<td>GIRK4</td>
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<td>Two-pore K⁺ channel</td>
<td>TASK-3 K⁺ channel</td>
<td>Patel AJ et al., 2004⁷</td>
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<td></td>
<td>Mu D et al., 2003³⁸</td>
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<td>Pei L et al., 2003¹⁰</td>
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<tr>
<td>ATP sensitive K⁺ channel</td>
<td>ATP sensitive K⁺ channel</td>
<td>Woodfork KA et al., 1995³⁸</td>
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<td></td>
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<td>Wonderlin WF et al., 1995³⁹</td>
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<td>Klimatcheva E et al., 1999³⁰</td>
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higher than that in normal cells. Furthermore, when transfected cells (hEAG overexpression) were injected, the tumors in immune-depressed mice showed faster growth than that in controls\cite{11,12}. These data demonstrate the oncogenic potential of TASK3 and EAG.

4 K⁺ channels and the proliferation of breast cancer cells

The proliferation of mammary carcinoma cells is regulated by many factors. There are many signaling molecules involved in modulate initiation, transcription and translation etc., and in each phase of cell cycles. Recent studies have found that the proliferation of breast cancer cells can be inhibited by K⁺ channels blockers, although the precise mechanisms are still unknown.

Ouadid-Ahidouch et al.\cite{13} perfused MCF-7 cells with α-dendrotoxin (α-DTX), a blocker of Kv1.1, resulting in the decrease of cell proliferation in a dose-dependent manner. Abdul et al.\cite{14} reported that K⁺ channel-blockers, dequalinium and amiodarone, inhibited MCF-7 proliferation while K⁺ channel-opener, Minoxidil, promoted the growth of MCF-7 human breast cancer cells. In addition, they also investigated the expression of Kv1.3 voltage-gated K⁺ channels in 60 human breast cancer specimens by immunohistochemistry. The results showed that 30% of the specimens expressed a high level of Kv1.3 in the epithelial compartment, 58% moderate and 12% low. These results indicate that Kv channels, active or inactive, are involved in breast cancer cell proliferation.

Recent researches on K⁺ channels and their relationship with the tumors have mostly focused on the KᵥCa channels. Ouadid-Ahidouch et al.\cite{13} reported the activity of large-conductance Ca²⁺ activated K⁺ (BKᵥCa) channels was involved in the proliferation of breast cancer cells. They found that the currents of BKᵥCa induced by increasing the intracellular Ca²⁺ concentration ([Ca²⁺]i) were inhibited by iberiotoxin and charybdoxin, which resulted in a slight depolarization in cells arrested in the early G1, late G1, and S phases and accumulated cells in the S phase; however, maybe due to their trivial role in the membrane potential, it failed to induce cell proliferation. Roger et al.\cite{15} reached the same conclusion by using iberiotoxin (a specific blocker of BKᵥCa) and NS1619 (an activator of BKᵥCa). Those regents did not interfere with either the cell proliferation or the invasive properties of the cells under normal culture conditions. However, after [Ca²⁺]i increased, iberiotoxin exhibited a marked decreasing effect on cell proliferation. This indicated that iberiotoxin-sensitive current was involved in cell proliferation when [Ca²⁺]i increased, while iberiotoxin had no effect on cell proliferation in basal conditions.

Membrane potential hyperpolarization is a key requirement for cells to cross the transitory phase G0/G1 or G1/S. Ouadid-Ahidouch et al.\cite{17} reported that membrane potential hyperpolarization could be achieved by the activation of human EAG (hEAG) K⁺ channel in the early G1 phase. However, there were more than one type of K⁺ channels involved in cell cycle progression. Their results showed that hIK1 current-density increased at the end of the G1 or S phase compared with that in the early G1 phase. Further, they found blocking hIK1 channels with clotrimazole (a specific blocker) induced membrane potential depolarization at the end of G1 and S phases but not in cells arrested early in the G1 phase. Moreover, blocking hEAG with astemizole induced the inhibition of cell proliferation to a greater degree than blocking hIK1 with clotrimazole. It indicated that the activation of hEAG K⁺ channels was essential during the progression of MCF-7 cells through the early G1 phase, while the hIK1-activity level became the primary factor when it came to G1 and checkpoint G1/S transition.

Otherwise, as stated previously, whole-cell recordings identify that the ion current(s) required for progression through G1 phase of the cell cycle in MCF-7 human breast cancer cells are K⁺ currents. Moreover, MCF-7 cells could be arrested in the G0/G1 phase by the agents known to block the activity of K₅ATP channels. This indicates that the K₅ATP channels play roles in cell proliferation in these human mammary carcinoma cells\cite{18,20}.

As described above, K⁺ channels play an important role in proliferation of tumor cells, but the underlying mechanism is still unclear. At present, three hypotheses have been proposed, although none was fully confirmed yet. With the development of these studies, the relationship between K⁺ channels and the proliferation of the tumor cells became clearer\cite{21-25}. Figure 1 showed the hypothetical relations between K⁺ channels and cell proliferation. Firstly, mitosis signal transduction pathway is involved in the regulation of K⁺ channel activation. This hypothesis has been recently partially confirmed\cite{20}; Secondly, the changes in ion concentration, intercellular or extracellular, maybe the first sign of induced proliferation or migration\cite{21,22}; And thirdly, ion channels play an important role in the cell volume regulation\cite{23,24}. For example, K⁺ channels could control the activity of cyclin through regulating cell volume.

As we know, 17-β-estradiol (E₂), a steroid hormone, is a key regulator in the normal mammary gland growth, and also is a leading cause of sporadic female breast cancer.
Recently, scientists found that E2 was involved in the mechanism of K+ channel promoting breast cancer cell proliferation. E2 could induce a rapid and irreversible augmentation of the K+ current for all membrane potentials superior to -25 mV in MCF-7 cells. Its amplifying effect was suppressed by the inhibitors of the maxi-K channel and anti-estrogen[26]. Tamoxifen is a selective estrogen receptor modulator (SERM) used for breast cancer treatment. The perfusion of 10 nmol/L tamoxifen significantly increased the magnitude of BK current. Even in the presence of the selective estrogen receptor antagonist, faslodex, the BK current was still recorded. Given these above results, it can be deduced that BK channel is the target of the tamoxifen (not through the endoplasmic reticulum pathway) and is involved in cell proliferation[27].

Previous work has shown that hEAG K+ channels are crucial for breast cancer cell proliferation and cell cycle progression. The question is how the cell signaling pathway regulates the expression of hEAG. Further study by Borowiec et al.[21] showed that insulin-like growth factor-1 (IGF-1) increased mRNA level of hEAG in a time-dependent manner in parallel with an enhancement of cell proliferation. The effects of IGF-1 was blocked by wortmannin (PI3K inhibitor). Wortmannin inhibited Akt phosphorylation, thus reducing hEAG mRNA levels. Either MAPK or PI3K is known to mediate IGF-1 cell proliferation signals through activation of ERK 1/2 and Akt. These results suggest that IGF-1 increases both the activity and the expression of hEAG channels through an Akt-dependent pathway. hEAG channel, necessary for cell proliferation and regulated by IGF-1, may also play an important role in the proliferation of the breast cancer cells.

5 K+ channels and the migration of breast cancer cells

Further studies have shown that a great number of different K+ channels are involved in cell migration. Breast tissue biopsies showed that, SK3 channels were expressed in highly metastasizing mammary cancer cell lines. These channels were not, however, expressed in non-cancerous breast tissue. Regulation of membrane potential and [Ca2+]i showed that, while SK3 channels had no effect on MDA-MD-435s cell proliferation, they did affect its migration. Furthermore, Potier et al.[28] demonstrated that, treated with small interfering RNA (siRNA) against SK3 channels, the cell migration of MDA-MD-435, MCF-7, 184A1 cell lines and transient expression of SK3 were almost completely abolished. The results indicated KCa channels might be a new mediator of breast cancer cell migration and thus became a potential target for anti-carcinogenic agents.

G-protein-coupled inwardly rectifying K+ channel (GIRK)

Fig. 1. Hypothetical relations between K+ channels and cell proliferation.
is one of the Kir sub-families that consist of five sub-types: GIRK 1 to 5. Stringer et al. measured GIRK1 mRNA expression in benign breast tissues, primary invasive breast carcinomas, and metastatic breast carcinomas from auxiliary lymph nodes using quantitative TaqMan reverse transcription-PCR and correlated the results with clinical parameters. It was found that GIRK1 overexpression correlated with lymph node metastasis. The overexpression level was highest in the tumors with more than one positive lymph node. More studies on breast cancer cell lines showed that, the GIRK1 was expressed in most of them, such as MCF-7, MDA-MB-361, MDA-MB 453, and ZR-75-1, while not in MDA-MB-468, MDA-MB-435S and normal breast cell line MCF-10A. As GIRK1 cannot form functional channels alone, it needs to be combined with other GIRK channels. They found that all of six breast cancer cell lines expressed GIRK2 and/or GIRK4, indicating that functional GIRK potassium channels exist in breast cancer cell lines. When the MDA-MB 453 was exposed to a β-adrenergic agonist and antagonist for 24 h, no effect was observed on GIRK1 expression but K+ influx increased. This increase was inhibited by the GIRK channel inhibitor, clozapine. It means that the GIRK channels are involved in the signaling pathway associated with β-adrenergic receptors. Ethanol is an established risk factor for breast cancer and has been found to open GIRK. After the MDA-MB 453 cell line was exposed to ethanol or serum-free media (lack of estrogen in the media), GIRK1 protein expression levels were decreased. All these data indicate that functional GIRK channels exist in breast cancer cells and that they are involved in the cell signaling pathway of breast cancer cell migration.

6 Conclusion

In recent years, studies on K+ channels and breast cancer have progressed significantly. The abnormality of K+ channel is an important feature in breast cancer, and K+ channel activity affects the proliferation and/or migration of tumor cells. However, the mechanism of K+ channels in tumor occurrence and development, and the role of K+ channels in cell signaling transduction of tumor cells are yet to be defined. With further in-depth studies, the K+ channels are expected to become the new target in the prevention and treatment of breast cancer.

* * *

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REFERENCES


