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Review

ENaC/DEG in Tumor Development and Progression

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Abstract

The epithelial Na^+ channel/degenerin (ENaC/DEG) superfamily, including the acid-sensing ion channels (ASICs), is characterized by a high degree of similarity in structure but highly diverse in physiological functions. These ion channels have been shown to be important in several physiological functions of normal epithelial cells, including salt homeostasis, fluid transportation and cell mobility. There is increasing evidence suggesting that ENaC/DEG channels are critically engaged in cancer cell biology, such as proliferation, migration, invasion and apoptosis, playing a role in tumor development and progression. In this review, we will discuss recent studies showing the role of ENaC and ASIC channels in epithelial cells and its relationship to the oncogenesis.

Key words: ENaC, ASIC, proliferation, migration, apoptosis, cancer cells.

Introduction

Epithelium is the most common component of human tissues, which mainly functions in body protection, regulation of fluid homeostasis and exchange, and sensation of external stimuli (1,2). The homeostatic balance between cell regeneration and apoptosis of epithelial cells is under precise control or regulation. Therefore, unrestricted proliferation or apoptosis of epithelial cells can lead to abnormal status and even diseases. Recent studies have revealed that various epithelial ion channels play an important role in the regulation of fluid/ion homeostasis (3). Therefore, the abnormal function in these ion channels may lead to certain congenital diseases (4-10) and human cancers (11-13).

The genes encoding the epithelial Na^+ channel/degenerin (ENaC/DEG) were firstly discovered in the early 1990s. The ENaC/DEG family includes structurally related acid-sensing ion channels (ASIC). It is well known that one of the major physiological functions of the tight and polarized epithelial monolayer is to actively adjust the turnover and resolution of luminal fluid and the machinery for transepithelial salt and fluid transport is distributed in a polarity-dependent manner. Members of the ENaC/DEG family are widely expressed in epithelia including kidney (14-16), lung (17) and colon (18,19). Several studies have shown that the ENaC/DEG channels are responsible for Na^+ uptake and fluid transportation (20) and are necessary to maintain the Na^+ balance, fluid balance and homeostasis for epithelial cells. It has been also reported that the members of the ENaC/DEG family sense the mechanical stimuli and respond with membrane depolarization (21).

The origin of almost 90% of all human cancers is from abnormal epithelial cell functions. Studies have accumulated evidence to support a role for the ENaC/DEG channels in driving malignant cancer cell behaviors and brain tumors (22, 23). In this review, we will discuss the expression of the ENaC and ASIC

channels in tumor cells and their role in tumor development and progression, and highlights them as potential new targets for cancer therapy in the future.

Role of ENaC/DEG in tumor development and progression

Four ENaC subunits, namely α, β, γ and δ, are encoded by the Scnn1 genes (24). The Scnn1 genes have been implicated in oncogenesis (25). ENaC subunits form heterotrimeric complexes that function as constitutively active Na^+ channels, with different subunit composition resulting in channels with distinct kinetics. The ENaC channels are widely reported to mediate the transport of Na^+ from the lumen into the epithelial cells, through which the cell may migrate. As discussed below, there is increasing evidence to suggest a significant role for the ENaC channels in tumor cells, particularly proliferation, migration and apoptosis.

1. α-ENaC

The α-ENaC has been proposed as an important mediator in the processes of cancer cell proliferation. For example, Maryna et al (26) showed an appreciable and reversible increase in the Na^+ conductance in human liver hepatocellular carcinoma HepG2 cells under different degree of hypertonic stress, which could be inhibited by flufenamate and amiloride. These two drugs decreased HepG2 cell proliferation. Similar to treatment with flufenamate and amiloride, knockdown of α-ENaC using small interfering RNA (siRNA) strongly reduced hypertonic stress-induced Na^+ currents. FACS analysis showed that silencing of α-ENaC inhibited cell proliferation, due to an increase in the G2/M phase and a decrease in the G1 phase of the cell cycle and furthermore, increased cell apoptosis. Silencing of α-ENaC led to strong reduction in the cell volume as shown using scanning acoustic microscopy.

In addition to a role in cancer cell proliferation and apoptosis, α-ENaC has been reported to be involved in promoting cancer cell migration (27-29). The α-ENaC protein expression determined by immunocytochemistry was noticeably elevated at the leading edge of wound that was introduced by scratching to choriocarcinoma BeWo cell monolayer cultured with aldosterone (27). Moreover, in wound healing assays, treatment with amiloride alone had no effect on cell migration but completely prevented aldosterone-induced increase in cell migration. Aldosterone-induced increase in cell migration was also abolished in cells treated with antisense oligonucleotides directed against α-ENaC, but not in cells treated with sense oligonucleotides. Taken together, these results provide evidence to suggest that up-regulation of α-ENaC expression by aldosterone or cancer lesion plays an important role in cancer cell migration and post-injury recovery (27). Mirshahi et al showed that cortical actin structures evinced the major factor affecting the activity of ENaC-like channels that are insensitive to amiloride (30).

2.γ-ENaC

A recent study reports a role for γ-ENaC in mediating hypotonic stress-induced proliferation and apoptosis of inner medulla collecting duct (IMCD) cells (31). As determined by FACS and MTT assays, exposure of IMCD cells to hypotonic stress resulted in significant decrease in cell proliferation and increase in cell apoptosis. Such hypotonic stress-induced effects on cell proliferation and apoptosis were prevented by treatment with 11,12-epoxyeicosatrienoic acids (EET) or adenovirus-mediated overexpression of cytochrome P2C23(CYP), the predominant epoxygenase isoform responsible for EET synthesis in rat kidney. Western blotting analysis demonstrated that the γ-ENaC expression in IMCD cells was significantly up-regulated by hypotonic stress, which was prevented by treatment with EET or overexpression of CYP2C23. These observations have led to the notion that regulation of the γ-ENaC expression represents a molecular mechanism responsible for the anti-apoptotic actions of EETs in IMCD cells under hypotonic stress conditions.

3. δ-ENaC

The δ-ENaC expression was shown in human melanoma G-361 cells using reverse transcription-polymerase chain reaction and cell-based *in situ* hybridization techniques (32). The δ-ENaC expression has also been documented using immunocytochemistry in human melanoma cells (32). This finding led to the proposal that δ-ENaC is a novel therapeutic target for treating malignant melanoma.

Role of ASICs in tumor development and progression

In addition to ENaC channels, ASICs are members of the ENaC/DEG family. Four different genes are identified, encoding six subunit isoforms, termed ASIC1a, ASIC1b, ASIC2a, ASIC2b, ASIC3 and ASIC4. These subunits can assemble functional hetero/homotrimeric channels that are sensitive to inhibition by amiloride (33).

1. ASIC1

Cheng et al (34) found that overexpression of ASIC1a was associated with tumor cell migration and

invasion. Under hypoxic and acidic conditions, an important feature of the tumor microenvironments, the ASIC1a mRNA and protein expression was obviously reduced in human hepatocellular carcinoma SMMC-7721 cells. In contrast, the ASIC1a expression was enhanced in a moderately acidic extracellular environment (e.g., pH 6.5). Therefore, ASIC1a may be overexpressed in hepatocellular carcinoma tissues, which are correlated with the disease development. Silencing of the ASIC1a expression inhibited cancer cell migration and invasion.

The study by Ross al (35) identified a cation conductance in D54-MG1 glioma cells, which was absent in normal human astrocytes. This conductance was sensitive to inhibition by psalmotoxin, an ASIC1-specific peptide inhibitor, as well as amiloride, suggesting that it was mediated by ASCI1a. Regulatory volume recovery from hyperosmotic stimulus-induced cell shrinkage was inhibited by replacement of extracellular Na+ with NMDG+ or treatments of cells with amiloride and psalmotoxin. These results support a role for the ASCI1a channel in restoring cell volume during migration of human glioma cells in the brain.

2. ASIC2

An early electrophysiological study documented amiloride-sensitive constitutive currents in cells from high-grade human malignant glioma, but not in cells from low grade tumors or human normal astrocytes (36). In a subsequent study, the same group revealed that surface expression of ASIC2 protein was detected in normal astrocytes but completely absent in high-grade human malignant glioma, immortalized human glioma cells (37). In further experiments, it was shown that treatments with glycerol, a chemical chaperone, or sodium 4-phenylbutyrate, a transcriptional regulator, stimulated trafficking of the intracellularly localized ASIC2 protein to the plasma membrane, leading to significant increase in ASIC2 protein at the cell surface and suppression of amiloride-sensitive currents. Both treatments reduced glioma cell proliferation and migration. These results led to the hypothesis that promotion of the ASIC2 cell surface expression and down-regulation of the amiloride-sensitive channel activity may reverse high-grade glioma cells to cells with normal astrocyte phenotypes.

Vascular smooth muscle cell (VSMC) migration is important for vascular genesis and vascular remodeling after injury. A separate study showed that ASIC2 also participated in inhibiting VSMC cell migration (38). Similar to what described above in high-grade glioma cells, ASIC2 was largely retained intracellularly. Treatment with glycerol increased ASIC2 cell surface expression and inhibited platelet-derived growth factor-induced cell migration. Moreover, this study showed that siRNA-mediated silencing of heat shock protein 70 (Hsc70) promoted ASIC2 protein cell surface expression and inhibition of cell migration, which was prevented by silencing of ASIC2 expression. These results suggest that Hsc70-dependent regulation of ASIC2 surface expression is an important regulatory mechanism in the processes of vascular genesis and vascular remodeling.

Conclusions and Future Perspectives

It has been 20 years since ENaC was first molecularly cloned from mouse colonic epithelium. As the earlier studies on the ENaC/DEG channels predominantly focused on their physiological functions in liver, kidney and colon epithelial cells, and showed that ENaC channel function was necessary for homeostasis of water and ion. A number of recent studies have attracted attention to the expression and function of these channels in cancer cells. As discussed above, evidence is emerging to support that the ENaC/DEG channels play a role in tumor development and progression, and stimulation or inhibition of particular ion channel expression and activity may be beneficial in certain disease conditions. Evidently, more efforts are required to better inform the relationships between the ENaC/DEG channels and tumor cells and thereby how to treat tumors by targeting these channels.

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Author Contributions

Cui Liu, Li-Li Zhu and Si-guang Xu contributed to manuscript writing, and Xiu-min Li and Hong-Long Ji contributed to manuscript revision.

Competing of Interest

The authors declare that they have no competing interests.

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