

Progress in Cellular Physiological Researches on Esophageal Cancer

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INTRODUCTION

Esophageal cancer is an aggressive type of neoplasm constituting a major cause of cancer-related deaths in worldwide^[1]. Recently, the outcome of esophageal squamous cell carcinoma (ESCC) is improved with advances in surgical techniques, adjuvant therapy, chemoradiotherapy and perioperative managements^[2]. However, patients with advanced disease still frequently develop recurrence, and their prognosis remains poor^[3]. To improve the treatment of recurrent or metastatic esophageal cancer, it is important to understand the molecular mechanisms regulating the tumorigenesis and the progression of the disease.

Over the past few decades, many reports have revealed that ion and water transporters play crucial roles in fundamental cellular functions. Alteration in function of these transporters has been reported in different human pathologies. Recently, the roles of ion and water transporters have been studied in cancer cells^[4-6], and various types of transporters have been found in cancers of gastrointestinal tract.

The objective of this article is to systematically review the current knowledge on expression and function of ion and water channels/transporters in esophageal cancer cells and tissues. The ultimate aim is to evaluate the potential of cellular physiological approach, such as regulation of ion transporters, water channels, intracellular pH and osmolality, in clinical management of esophageal cancer.

ION CHANNELS AND TRANSPORTERS IN ESOPHAGEAL CANCER

Recent studies have demonstrated that several subtypes of K⁺ channels are expressed in human esophageal cancer cells, and are

ABSTRACT

Recently, the roles of ion and water transporters have been studied in cancer cells, and various types of transporters have been found in esophageal cancer. This article aims to systematically review the current knowledge on expression and functioning of cellular physiological factors in esophageal cancer. Various types of ion transporters, such as voltage-gated K⁺ channels, Cl⁻ transporters, Ca²⁺ channels and transient receptor potential channels have been found to express in esophageal cancer cells and tissues, and to control cancer progression. With regard to water channels, aquaporin 3 and 5 play an important role in the progression of esophageal cancer. Regulators of intracellular pH, such as anion exchanger, sodium-hydrogen exchanger, vacuolar H⁺-ATPases and carbonic anhydrases also involved in cellular behaviours of esophageal cancer. Their pharmacological inhibition and gene silencing affected tumorigenesis, suggesting their potentiality as therapeutic targets for esophageal cancer. We demonstrated the cytoskeletal effects of hypotonic stress, and indicated that regulation of ion transport enhanced these effects in esophageal cancer cells. A deeper understanding of molecular mechanisms may lead to the discovery of these cellular physiological strategies as a novel therapeutic approach for esophageal cancer.

associated with prognosis. Altered expression of several voltage-gated K^+ channels (K_v) has been observed in esophageal cancer. Eag1 ($K_v10.1$) is the prototypic member of the ether a' go-go family of K_v . Ding *et al* have demonstrated that Eag1 is aberrantly expressed in ESCC tissues and correlates with poor prognosis after surgery^[7]. Human ether-a-go-go-related gene (HERG) encodes one of the components of delayed rectifier K^+ currents. Overexpression of hERG1 in resected ESCC has been observed, and correlated with poor prognosis after surgery^[8]. Further, Lastraioli *et al* have shown that the surveillance of patients with Barrett's esophagus indicated that 89% of those who later developed adenocarcinomas displayed hERG1 expression^[9]. Their results suggest that hERG1 expresses from an early stage of the progression of esophageal cancer through a dysplasia.

There is evidence also for Cl^- transporters involvement in esophageal cancer. We have investigated roles of intracellular chloride concentration ($[Cl^-]_i$) in cell cycle progression of cancer cells, and reported that the $[Cl^-]_i$ regulated by Cl^- channels/transporters would be one of critical messengers^[10-15]. In ESCC, we found that the expression of $Na^+/K^+/2Cl^-$ cotransporter 1 (NKCC1) was related to the degree of histological differentiation^[16]. Furosemide, a blocker of NKCC1, diminished cell growth of ESCC by affecting the G₂/M checkpoint^[16]. NKCC is one of the important transporters controlling the $[Cl^-]_i$ via uptake of Cl^- into the intracellular space and, therefore, furosemide decreases the $[Cl^-]_i$ ^[17]. Furosemide is often used as a diuretic to maintain urine output and improve edema, ascites, or pleural effusion for the treatment of patients with terminal stage esophageal cancers. From this viewpoint, our observation that the blockage of NKCC1 diminished the proliferation of ESCC cells provides strong clinical evidence that furosemide can be used for ESCC patients. We have also investigate the role of K^+-Cl^- cotransporter 3 (KCC3) in the regulation of cellular invasion and the clinicopathological significance of its expression in ESCC^[18]. The survival rate of patients in whom KCC3 was expressed in the invasive front of ESCC was lower than that of the patients without it, and multivariate analysis demonstrated that it was one of the most important independent prognostic factors^[18]. Further, the knocking-down of KCC3 using siRNAs inhibited cell migration and invasion in human ESCC cell lines^[18].

The Ca^{2+} channels, through which the intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) can be regulated, also have critical roles in cancer progression. Lu *et al* showed that T-type Ca^{2+} channels play a critical role in cell proliferation of ESCC via a p53-dependent p21^{CIP1} pathway^[19]. Zhu *et al* have demonstrated that tumors from patients with ESCC were found to display elevated expression of Orai1, a storeoperated Ca^{2+} entry (SOCE) channel, which mediates $[Ca^{2+}]_i$ oscillations, and the high expression of Orai1 was associated with poor prognosis^[20]. Further, inhibition of Orai1 activity suppressed proliferation and migration of ESCC *in vitro* and slowed tumor growth in *in vivo* xenografted mice^[20]. The transient receptor potential (TRP) superfamily consists of a highly diverse group of ion channels that are mostly permeable to monovalent and divalent cations. TRP channels are divided into seven subfamilies, including the canonical (TRPC) and the vanilloid receptor related (TRPV) channels. In esophageal cancer, Shi *et al* have shown that the expression of TRPC6 at protein and mRNA levels was markedly increased in human ESCC specimens, and that the $[Ca^{2+}]_i$ elevation regulated by TRPC6 channels is essential for G₂ phase progression and ESCC development^[21]. High expression of TRPC6 correlated with poor prognosis in patients with ESCC^[22]. Zhou *et al* have demonstrated that overexpression of TRPV2 mRNA was associated with poor prognosis of patients with ESCC and might

serve as a novel prognostic biomarker in early stage^[23].

Because there is significant evidence for involvement of these ion channels and transporters in esophageal cancer cell proliferation and disease progression, their clinical potential would be worth investigating further.

WATER CHANNELS IN ESOPHAGEAL CANCER

Aquaporins (AQPs), transmembrane proteins that facilitate transport of water, are important for cell volume regulation and electrolyte balance under both physiological and pathophysiological conditions. To date, 13 AQP subtypes and their crucial roles have been characterized in humans. In ESCC, Kusayama *et al* found the overexpression of AQP3 in tumor areas of human ESCC and its potentially important role on the cell growth^[24]. We have examined the pathophysiologic role of AQP5 in cell proliferation and survival, and also investigated its expression and effects on the prognosis of ESCC patients^[25]. In ESCC cells, the knockdown of AQP5 using siRNA inhibited cell proliferation and G₁-S phase progression, and induced apoptosis^[25]. The expression pattern of AQP5 and p21 protein was sharply contrasted, but AQP5 and CCND1 protein expression showed a similar pattern in ESCC tissue^[25]. Immunohistochemical staining of ESCC patients showed the AQP5 expression is associated with tumor size, histological type, and tumor recurrence^[25]. Liu *et al* showed that co-expression of AQP3 and AQP5 in ESCC correlates with aggressive tumor progression and poor prognosis^[26].

These results indicate that AQPs play important roles in the tumorigenesis and progression of esophageal cancer and suggest that especially, AQP3 and 5 can become potential therapeutic targets against esophageal cancer.

PH REGULATORS IN ESOPHAGEAL CANCER

Anion exchanger (AE) proteins facilitate the electroneutral exchange of Cl^- for HCO_3^- across the plasma membrane of mammalian cells, and their basic role is to maintain intracellular pH. Sarosi *et al* showed that acid increased MAPK-mediated proliferation in Barrett's esophageal adenocarcinoma cells via intracellular acidification through AE^[27]. The sodium-hydrogen exchanger (NHE) mediates a coupled counter-transport of one H^+ ion in exchange for one Na^+ ion and thus contributes to regulation of intracellular pH. Guan *et al* have shown that NHE1 was highly expressed in esophageal adenocarcinoma tissues, and that knockdown of NHE1 suppressed viability and induced apoptosis in esophageal cancer cells^[28]. Vacuolar H^+ -ATPases (V-ATPases) are the specific proton pump of the cell and play a crucial role in maintaining intracellular pH. Huang *et al* have shown that V-ATPase was over-expressed in ESCC cells, and that its expression was associated with pathological grade, TNM stage and tumor metastasis^[29].

The carbonic anhydrases (CAs) are a family of zinc metalloenzymes, participate in various physiological process by contributing to pH homeostasis. To date, 15 active isoforms of CAs, 12 of which are catalytically active has been identified in mammals. In esophageal cancer, CA IX expression is correlated with poor prognosis and malignant phenotype in both adenocarcinoma^[30] and SCC^[31]. Schoppmann *et al* have demonstrated that CA IX overexpression is associated with diminished patients' prognosis and correlates with expression of HER-2 in esophageal cancer^[32,33]. Further, they showed that CA IX-expressing tumor stroma is associated with poor prognosis in esophageal cancer^[34].

These reports suggest that pH regulators, such as AEs, NHEs, V-ATPases and CAs are potentially key therapeutic targets and the silencing of their expression could provide a new therapeutic approach for esophageal cancer.

REGULATION OF OSMOLALITY

Several previous studies have indicated the cytotoxic effects of hypotonic stress on cancer cells and the efficacy of peritoneal lavage with distilled water (DW) during surgery^[35-38]. Recently, we have analyzed the changes in the cellular morphology and volume of esophageal cancer cells subjected to hypotonic stress using several unique methods and apparatus^[39]. Video recordings by high-speed digital camera have demonstrated that hypotonic stress with DW induces cell swelling followed by cell rupture, and measurements of cell volume changes using a high-resolution flow cytometer indicate that severe hypotonicity with DW increases broken fragments of esophageal cancer cells within 5 min^[39]. In addition, we treated the esophageal cancer cells with a Cl⁻ channel blocker, 5-nitro-2-3-phenylpropylamino)-benzoic acid (NPPB), to enhance the cytotoxic effects by increasing cell volume during the hypotonic stress via the inhibition of regulatory volume decrease (RVD)^[40,41]. After hypotonicity-induced cell swelling, RVD occurs by activation of ion channels and transporters, which cause effluxes of K⁺, Cl⁻, and H₂O, leading to cell shrinkage. In the TE5, TE9 and KYSE170 cells, treatment with NPPB increases cell volume by inhibiting RVD and enhanced the cytotoxic effects of the hypotonic solution. We have found similar phenomena in gastric^[42], colorectal^[43] and pancreatic cancer cells^[44]. Recently, we performed an *in vivo* evaluation and demonstrated the safeness of a peritoneal injection of DW^[45]. Furthermore, the development of dissemination nodules from cancer cells was prevented by a preincubation with or peritoneal injection of DW^[45].

These findings demonstrate the cytotoxic effects of hypotonic stress on esophageal cancer cells, and suggest that these effects are enhanced by the regulation of ion transport. A deeper understanding of ion transport mechanisms in esophageal cancer cells during a change of osmolality could lead us to the development of novel therapeutic approaches.

CONCLUSION

We systematically reviewed the current knowledge on progress

in cellular physiological researches on esophageal cancer (Table 1). This review shows a variety of ion transporters, AQP and pH regulators are expressed in human esophageal cancer cells and tissues. Pharmacological manipulation and gene silencing affect their activities and fundamental cellular functions that would be involved in the progression of esophageal cancer. Results of these researches suggest that ion, water transporters and pH regulators are functional biomarkers and therapeutic targets in esophageal cancer. A deeper understanding of molecular mechanisms may lead us to the discovery of these cellular physiological strategies, such as regulation of ion channels, water channels, intracellular pH and osmolality as a novel therapeutic approach for esophageal cancer.

CONFLICT OF INTERESTS

There are no conflicts of interest with regard to the present study.

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Table 1 Cellular physiological researches on esophageal cancer.

			Histological type	Author, year
Ion channel and transporter	Potassium	Eag1	SCC	Ding <i>et al</i> 2008 ^[7]
		hERG1	SCC, Adenocarcinoma	Ding <i>et al</i> 2008 ^[8] , Lastraioli <i>et al</i> 2006 ^[9]
	Chloride	NKCC1	SCC	Shiozaki <i>et al</i> 2014 ^[16]
		KCC3	SCC	Shiozaki <i>et al</i> 2014 ^[18]
	Calcium	T-type Ca ²⁺ channel	SCC	Lu <i>et al</i> 2008 ^[19]
		SOCE	SCC	Zhu <i>et al</i> 2014 ^[20]
TRPC6		SCC	Shi <i>et al</i> 2009 ^[21] , Zhang <i>et al</i> 2013 ^[22]	
TRPV2		SCC	Zhou <i>et al</i> 2014 ^[23]	
Water channel	AQP3	SCC	Kusayama <i>et al</i> 2011 ^[24] , Liu <i>et al</i> 2013 ^[26]	
	AQP5	SCC	Shimizu <i>et al</i> 2014 ^[25] , Liu <i>et al</i> 2013 ^[26]	
pH regulator	AE	Adenocarcinoma	Sarosi <i>et al</i> 2005 ^[27]	
	NHE1	Adenocarcinoma	Guan <i>et al</i> 2014 ^[28]	
	V-ATPase	SCC	Huang <i>et al</i> 2012 ^[29]	
	CAIX	SCC, Adenocarcinoma	Driessen <i>et al</i> 2006 ^[30] , Tanaka <i>et al</i> 2008 ^[31] , Schoppmann <i>et al</i> 2012 ^[32] , Birner <i>et al</i> 2011 ^[33] , Jomrich <i>et al</i> 2014 ^[34]	
Regulation of osmolality		SCC	Kosuga <i>et al</i> 2011 ^[39]	

Eag: ether a go-go; hERG: human ether-a-go-go-related gene; NKCC: Na⁺/K⁺/2Cl⁻ cotransporter; KCC: K⁺-Cl⁻ cotransporter; SOCE: storeoperated Ca²⁺ entry channel; TRPC: transient receptor potential canonical; TRPV: transient receptor potential vanilloid; AQP: aquaporin; AE: anion exchanger; NHE: sodium-hydrogen exchanger; V-ATPase: vacuolar H⁺-ATPases; CA: carbonic anhydrase; SCC: squamous cell carcinoma.

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