Roles of Ionic Environments in Growth of Human Cancer Cell and Potentials of Ion Transporter Blockers in Cancer Therapies

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Abstract

Cancer cells produce a large amount of H⁺ due to high-rate glycolysis, however cancer cells keep the cytosolic pH (pHc) slightly higher than that of normal cells even under conditions provided by a large amount of H⁺ due to high-rate glycolysis. Further, interestingly cancer cells survive even under starvation conditions utilizing a self-nutrient-recycling autophagy system in lysosome. The intra-lysosomal pH is kept much lower than cytosolic pH, and this lowered intra-lysosomal pH is a key factor for keeping activity of lysosomal enzymes participating in autophagy function. It is notable that Cl⁻ plays an important role in regulation of pHc and intra-lysosomal pH. In this article, I discuss roles of H⁺ and Cl⁻ circumstances in cancer cell proliferation, and a possibility of drugs modifying H⁺ and Cl⁻ circumstances used as anti-cancer drugs.

Introduction

Cancer cells live in hypoxic, hypo-nutrient circumstances provided by insufficient angiogenesis due to fast rates of cancer cell proliferation [1,2]. These circumstances lead cancer cells via production of ATP as energy sources mainly via high rate glycolysis, while most normal cells produce ATP via oxidation of pyruvate in mitochondria with relatively low-rate glycolysis compared with cancer cells [3]. The high-rate glycolysis is forming lactate produces a large amount of H⁺, providing acidic microenvironments around cancer cells [4]. Interestingly, cancer cells keep the cytosolic pH (pHc) slightly higher than that of normal cells even under these environmental conditions provided by a large amount of H⁺ due to high-rate glycolysis [5,6]. From a viewpoint of cell cycle arrest, apoptosis and cell growth, pHc of tumor cells kept at a level slightly higher than that of normal cells is a key factor for prevention from cell cycle arrest and apoptosis even under acidic microenvironments around cancer cells [7,8]. These findings clearly indicate that cancer cells have to maintain their pHc at a level slightly higher than that of normal cells for survival even under acidic conditions with production of a large amount of H⁺. For maintenance of pHc at a level slightly higher even under acidic environmental conditions than normal one, expression and/or activity of H⁺ and/or HCO₃⁻ transporters and systems in cancer cells would be expected to be higher than normal cells. Further, it is notable that cancer cells utilize a self-nutrient-recycling autophagy system for their survival even under starvation conditions [9-11]. The self-nutrient-recycling autophagy occurs in lysosome, and the intra-lysosomal pH is much lower than the cytosolic one [12,13]. Cl⁻ also plays a key role in regulation of pHc and intra-lysosomal pH. In this article, I discuss roles of ionic circumstances in growth and lysosomal function in cancer cells.

Expression of ion transporters regulating pHc in cancer cells

Cancer cells have been reported to express four major ion transporters participating in keeping pHc at a normal or slightly higher level even under acidic environmental conditions than normal one [14-16]. These four major ion transporters regulating pHc are classified into two categories [14-16]: i) H⁺ transporters, and ii) HCO₃⁻ transporters. H⁺ transporters, Na⁺/H⁺ exchanger (NHE) and H⁺ pumps (V-type H⁺-ATPase, etc.), directly extrude H⁺ from the cytosolic space to the extracellular or into the intra-lysosomal one keeping high pHc. On the one hand, HCO₃⁻ transporters, Na⁺/HCO₃⁻ cotransporter (NBC), Na⁺/H⁺/Cl⁻ co-transporter (NDCBE), and Cl⁻/HCO₃⁻ exchanger (AE) participate in HCO₃⁻ movement across the plasma membrane regulating pHc. Unlike H⁺ transporters these HCO₃⁻ transporters do not participate in unidirectional transport of HCO₃⁻; i.e., NBC and NDCBE contribute to uptake of HCO₃⁻ into the cytosolic space using the electrochemical gradient of Na⁺ for elevation of pHc, but AE extrudes HCO₃⁻ from the cytosolic space to the extracellular one for a decrease in pHc instead of elevation of the cytosolic Cl⁻ concentration under general conditions.

Roles of NHE and its Inhibitor in Growth of Cancer Cells and Ionic Circumstances in Cancer Cells

Among the pHc-regulating ion transporters mentioned above, NHE is the major regulator of pHc ubiquitously expressed in most cells including cancer cells, and 10 isoforms of NHEs have been identified [17-21]. NHE1, an isoform of NHE, is the most ubiquitously expressed one among 10 isoforms [22-28]. Various types of growth factors, integrins, tyrosine phosphatases and cytokines regulate activity of NHE1 [22-28]. NHE1, in particular NHE1, in cancer cells plays a crucial role in maintenance of pHc at a level slightly higher than normal one [29]. Proliferation and migration of many cancer cells require pHc slightly higher than normal one, which depends on NHE activity [19-21]. Therefore, NHE inhibitors should be investigated as anticancer drugs inhibiting proliferation of cancer cells under acidic micro-environmental conditions. Indeed, many researchers have tried to investigate action of NHE inhibitors on proliferation of cancer cells in detail [30]. Recently, we [31] have also reported that 5-(N-ethyl-N-isopropyl) amiloride (EIPA, an NHE inhibitor [32]) inhibits the proliferation of human gastric cancer cells. It is generally thought that inhibition of NHE decreases pHc. However, our report [31] indicates that inhibition of NHE has no influence on pHc, but decreases the cytosolic Cl⁻ concentration ([Cl⁻]). The role of cytosolic Cl⁻ in cancer cell proliferation has been also investigated; when [Cl⁻] is experimentally
such as mitochondria utilizing this autophagy system [49-51]. The autophagy system is induced by starvation-caused poverty of nutrients, mainly amino acids, for cells to survive utilizing reproduced nutrients originally contained in cells themselves [9]. Recycled amino acids reproduced by autophagy are utilized for new proteins synthesis [9]. Autophagy ability in cancer cells is much higher than normal cells, since cancer cells have to survive under hypoxic, hypo-nutrient micro environments utilizing recyclable nutrition such as amino acids [53]. It has been reported that Atg5 or Atg7 plays a key role in autophagy function; lack of Atg5 or Atg7 impairs the autophagy system and apoptosis in cancer cells [56-58]. Lysosomal machineries catabolize cell components, indicating that lysosome is a key organelle producing autophagy function in degradation of various compounds [11]. It is well known that the intra-lysosomal pH is lower than pH7 [12,13], and that this lower intra-lysosomal pH is a key factor to maintain the digesting activity of lysosomal enzymes; i.e., lysosomal enzymes require low pH to maintain their enzymatic activities [59]. The V-type H+ -ATPase (proton pump) generates the intra-lysosomal low pH operating with CIC-7 located on the lysosome membrane [60,61]. CIC-7 has stoichiometry of 2Cl-/1H+ exchange, and is assumed to primarily behave as a Cl- permeation pathway across the lysosomal membrane [60], although there is still some contradictory observations (e.g., [62]). Mutation of CIC-7 impairs lysosomal function, which is detected as abnormal accumulation of proteins into the intra-lysosomal space [63], meaning that the function of CIC-7 as a Cl- permeation pathway would contribute to lysosomal function (protein degradation) via maintenance of low intra-lysosomal pH. Further, knock down of CIC-7 impairs lysosomal acidification [60], and inhibits cell proliferation associated with abnormal accumulation of proteins in lysosomes [64]. These studies [60,63,64] suggest that CIC-7 would be essential as a Cl- permeation pathway contributing to lysosomal function (protein degradation) via maintenance of low intra-lysosomal pH and autophagy-dependent cell proliferation. Although these studies [60,63,64] suggest the importance of CIC movement/transport for maintenance of low intra-lysosomal pH and autophagy-dependent cell proliferation, our study [65] suggests that the presence of Cl- itself plays an essential role in autophagy (Figure 1).

**Autophagy and Intra-Lysosomal Ionic Circumstances in Cancer Cells**

Autophagy ability in cancer cells is much higher than normal cells, since cancer cells have to survive under hypoxic, hypo-nutrient micro environments utilizing recyclable nutrition such as amino acids [53]. It has been reported that Atg5 or Atg7 plays a key role in autophagy function; lack of Atg5 or Atg7 impairs the autophagy system and apoptosis in cancer cells [56-58]. Lysosomal machineries catabolize cell components, indicating that lysosome is a key organelle producing autophagy function in degradation of various compounds [11]. It is well known that the intra-lysosomal pH is lower than pH7 [12,13], and that this lower intra-lysosomal pH is a key factor to maintain the digesting activity of lysosomal enzymes; i.e., lysosomal enzymes require low pH to maintain their enzymatic activities [59]. The V-type H+ -ATPase (proton pump) generates the intra-lysosomal low pH operating with CIC-7 located on the lysosome membrane [60,61]. CIC-7 has stoichiometry of 2Cl-/1H+ exchange, and is assumed to primarily behave as a Cl- permeation pathway across the lysosomal membrane [60], although there is still some contradictory observations (e.g., [62]). Mutation of CIC-7 impairs lysosomal function, which is detected as abnormal accumulation of proteins into the intra-lysosomal space [63], meaning that the function of CIC-7 as a Cl- permeation pathway would contribute to lysosomal function (protein degradation) via maintenance of low intra-lysosomal pH. Further, knock down of CIC-7 impairs lysosomal acidification [60], and inhibits cell proliferation associated with abnormal accumulation of proteins in lysosomes [64]. These studies [60,63,64] suggest that CIC-7 would be essential as a Cl- permeation pathway contributing to lysosomal function (protein degradation) via maintenance of low intra-lysosomal pH and autophagy-dependent cell proliferation. Although these studies [60,63,64] suggest the importance of CIC movement/transport for maintenance of low intra-lysosomal pH and autophagy-dependent cell proliferation, our study [65] suggests that the presence of Cl- itself plays an essential role in autophagy (Figure 1).

**Conclusion**

Some drugs modifying activity of ion transporters involved in cytosolic Cl- environments would be useful for anticancer therapies (Figure 1).  

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