

# Renewed interests in carbonic anhydrase IX in relevance to breast cancer treatment

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## Summary

The highly proliferating cancerous cells can form permissive accommodating milieu – the so-called tumor microenvironment (TME). During the initiation of solid tumors, hypoxia plays a key role in glycolysis, which can trigger the anomalous overexpression of several enzymes and transporters involved in the metabolism of glucose. Of these, carbonic anhydrases (CAs), especially CAIX, together with other molecular machinery involved in the production/trafficking of acidic byproducts, play key roles in the regulation of intracellular and extracellular pH. CAIX, along with other molecular machinery of cancer cells such as Na<sup>+</sup>/H<sup>+</sup> exchanger 1 (NHE1) and V-type H<sup>+</sup>-ATPase (V-ATPase), alkalinizes the tumor cells and maintains the acidic pH condition within the extracellular fluid of the TME. It facilitates the progression and metastasis of cancer and intensifies the migration and invasion of cancer cells. Thus, inhibition of CAIX can be considered a highly effective and promising therapeutic strategy in the treatment of aggressive tumors.

### Authors' Biosketch

**Dr. Mitra Dolatkhah** (Pharm.D.) is a Ph.D. candidate of Pharmaceutics at the Faculty of Pharmacy, Tabriz University of Medical Sciences. Her research activities are focused on targeted cancer drug delivery.



**Professor Yadollah Omid** (Pharm.D., Ph.D.) is a Professor of Pharmaceutical and Biomedical Sciences at TUOMS Faculty of Pharmacy, working on the development of advanced multifunctional drug delivery systems, biosensing, and tissue engineering. He has worked at Cardiff University and the University of Pennsylvania. Since 2004, Prof. Omid has published over 210 papers and 18 book chapters, supervised and trained over 100 graduate/postgraduate students/researchers.



Drug resistance mechanisms developed by cancer cells have been a major challenge of cancer therapy in the clinic. More recently, it has been recognized that tumor microenvironment (TME) can greatly influence the currently used anticancer therapies. As a result, it was proposed that the inhibition of extracellular ligand-receptor interactions and downstream pathways involved in the TME may be a solution to overcome drug resistance and improve therapeutic outcomes.<sup>1-3</sup>

It is well known that TME has unique physiological characteristics such as irregular acidic pH and hypoxia, which can cause the up- and/or down-regulation of certain biomolecules such as enzymes.<sup>2,4-6</sup> Because of abnormal proliferation of cancer cells and irregular vascular network in not being able to supply sufficient blood to all cancer cells, cancer cells experience hypoxia, and as a consequence, they show an increased dependency on glycolysis for the energy provision instead of oxidative

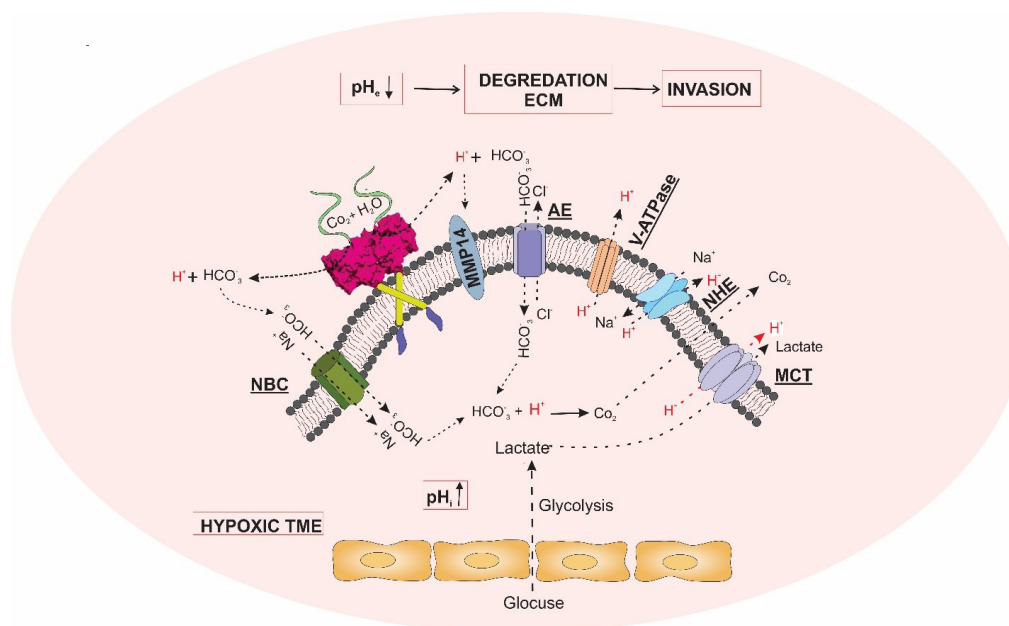
phosphorylation that is defined as Warburg effect.<sup>5,7,8</sup> This glycolysis pathway leads to the production of a large amount of lactate and H<sup>+</sup> ions. One of the adaptive mechanisms of cancer cells to adverse conditions is the activation of hypoxia-inducible factor-1 (HIF-1), which may result in the overexpression of the pH-regulating proteins, including isoforms II, IX, and XII of carbonic anhydrases (CAs), Na<sup>+</sup>/H<sup>+</sup> exchanger 1 (NHE1), and V-type H<sup>+</sup>-ATPase (V-ATPase). The expression levels of these molecular machinery pieces seem to be strongly enhanced under the hypoxic conditions via HIF-1 pathway concurrent with the glycolysis phenomenon.<sup>6,9</sup> Fig. 1 represents the molecular machinery involved in the pH dysregulation phenomenon in the TME. All these events result in the outward transportation of accumulated acidic byproducts into the extracellular fluid (ECF) in the TME, which makes the extracellular pH of solid tumors become more acidic compared to the



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**Fig. 1.** Molecular machinery involved in the pH dysregulation phenomenon in the TME. Monocarboxylate transporter (MCT), V-type H<sup>+</sup>-ATPase (V-ATPase), Na<sup>+</sup>/H<sup>+</sup> exchanger (NHE), bicarbonate co-transport (NBC), anion exchanger (AE), and carbonic anhydrase IX (CA IX) are involved in pH regulation in the TME. The degradation of ECM is triggered by the dysregulation of pH and activation of MMP14 by CAIX, facilitating invasion and metastasis of solid tumors. MMP14: Matrix metalloproteinase 14; HCO<sub>3</sub><sup>-</sup>: bicarbonate; CO<sub>2</sub>: carbon dioxide; H<sup>+</sup>: proton; ECM: extracellular matrix. MCT: monocarboxylate transporter; AE: anion exchanger; NBC: electrogenic Na<sup>+</sup>/HCO<sub>3</sub><sup>-</sup> cotransporter; TME: tumor microenvironment.

normal tissues. Such an acidic condition of the TME can influence the integrity of the extracellular matrix (ECM), and hence, remodel the TME by the degradation of the ECM in favor of further metastasis and progression of cancer.<sup>10</sup> The acidic condition in the serial extracellular fluid (ECF) of solid tumors may provoke the exocytosis in cancer cells. All these incidences create a complex dynamic situation within TME, which result in capricious cacophonous functions in cancer cells along with other stromal cells such as the immune system cells. Under such circumstances, the rebellious cancer cells develop defense mechanisms against anticancer agents and circumvent the immunosurveillance functionalities. The emergence of such complexity makes the treatment of solid tumors very problematic.<sup>2,3,11</sup>

Recent developments in cancer therapy have generated renewed interest in targeting molecular machinery (genes and proteins), involved in the pH dysregulation within the TME, as platforms for the development of anti-tumor regimens. Of these molecular machinery pieces, CAs have gained much attention in tumor pH regulatory studies as a valuable therapeutic target, in large part because of their cardinal role in maintaining the intracellular pH homeostasis. The CAs are transmembrane zinc-binding enzymes that catalyze the reversible conversion of CO<sub>2</sub> molecules. This function can impede the pH homeostasis between the intracellular fluid (ICF) and ECF, resulting in the acidification of ECF by protons. Further, bicarbonate molecules are directly transported to the cytoplasm by bicarbonate transporters, which favor the alkalization of

the ICF.

To date, fifteen CA isoforms have been characterized in human.<sup>12,13</sup> Recent studies offer some evidence of increased expression of CAIX in a variety of solid cancers, including breast cancer.<sup>14</sup> CAIX, as a pH-regulating enzyme, plays a key role in maintaining an acidic extracellular pH under hypoxic conditions. Hypoxia is a major trigger for CAIX expression in cancer cells. Moreover, CAIX can be induced in normoxia by high cell-density-mediated pseudohypoxia and by hypoxia-independent mechanisms such as lactate and redox-mediated stabilization of HIF-1. The induction of CAIX might associate with the cell adhesion, migration, and invasion proteins (e.g., MMP14). Such phenomena indicate an intricate role of CAIX in tumor invasiveness, degradation of ECM, distant metastasis, and poor survival in patients. It should be noted that the overexpression of CAIX is associated with poor prognosis.<sup>12,15</sup>

Several pre-clinical studies have clearly highlighted the useful effect of addition of CAIX inhibitors to standard cancer-therapy regimens. A number of researches have suggested that CAIX inhibitors, as co-treatments, may increase the effects of chemotherapy agents and/or anti-angiogenic drugs, and also enhance the response of tumors to radiotherapy.<sup>16-18</sup> Compelling data suggest that the inhibition of CAIX might restrict cancer cell proliferation, migration, and invasion. Furthermore, some *in vivo* studies exhibited that metastatic growth could be limited by such treatment modality.<sup>19</sup> A number of studies indicate that the anti-tumor effects of the inhibition of CAIX may occur through (i) the intrusion in the pH

regulation in cancer cells, (ii) the interaction with other pH-independent mechanisms, and (iii) the association with some signaling pathways involved in cancer cells progression and metastasis. Thus, all these biological events may influence the response of cancer cells to the treatment modalities administered.<sup>11</sup> In fact, some CAIX inhibitors are already under consideration in different clinical studies. Small interfering RNAs, therapeutic antibodies, or small molecule inhibitors (e.g., sulfonamide, sulfamate, sulfamide, and coumarin types substances) can be used to investigate the tumor therapeutic potential of CAIX.<sup>20</sup> Taken all, the up-regulation of CAIX appears to play several key roles in the progression and invasion of cancer cells, and therefore, it could potentially be targeted in cancer therapy. Further, the hallmarks of cancer must be taken into consideration for the treatment of solid tumors such as breast cancer.

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There is none to be stated.

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#### Competing interests

No competing interests to be disclosed.

#### Authors' contribution

MD and YO gathered the data and drafted the manuscript. YO finalized the manuscript.

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