

EDITORIAL

Gastric carbonic anhydrase IX deficiency: At base, it is all about acid

See related article: T. Li, X. Liu, B. Riederer, K. Nikolovska, A. K. Singh, K. A. Mäkelä, A. Seidler, Y. Liu, G. Gros, H. Bartels, K. H. Herzig, U. Seidler. 2017. Genetic ablation of carbonic anhydrase IX disrupts gastric barrier function via claudin-18 downregulation and acid backflux. *Acta Physiol* **222**, e12923.

In this issue of *Acta Physiol*, Taolang Li, et al sought to answer the question of how the stomach of CAIX knockout mice exhibits parietal cell loss and foveolar hyperplasia by careful time course observation from weaning to adult of the stomachs of CAIX knockout mice.¹ CAIX KO stomach had downregulation of the tight junction protein claudin-18A2 and upregulation of the inflammatory mediators IL-1 β and iNOS at 1 month of age, followed by parietal cell loss due to inflammation, with resultant hypergastrinemia, which ultimately promoted foveolar hyperplasia. The sequence that loss of acid secretion causes hypergastrinemia, which leads to foveolar hyperplasia, seems to be a common feature in models of genetic and chronic pharmacological acid suppression. Therefore, parietal cell loss is a key phenomenon associated with phenotypic changes in the stomach of CAIX KO mice.

Gastric mucosal defence mechanisms consist of striated mucus layers, high-resistance tight junctions between, and HCO₃⁻ secretion from gastric surface cells.^{2,3} The source of HCO₃⁻ for the so-called “alkaline tide” is parietal cells, which generate H⁺ and HCO₃⁻ through CO₂ hydration by intracellular CAII. The basolateral anion exchanger (AE)-2 exchanges intracellular HCO₃⁻ for extracellular Cl⁻ in parietal cells. In contrast, gastric surface cell ion transporters regulate intracellular pH (pH_i) by controlling HCO₃⁻ uptake via the Na⁺:HCO₃⁻ cotransporter (NBC) and H⁺ extrusion via the sodium hydrogen exchanger (NHE)-1 through the basolateral membranes.⁴ Subepithelial blood flow supplies HCO₃⁻ and removes H⁺ and CO₂ which is regulated by afferent nerve endings that express interstitial pH (pH_{int}) sensors.

CA, an evolutionarily old enzyme that catalyzes the conversion of CO₂ and H₂O into H⁺ and HCO₃⁻, enables all living beings to safely handle H⁺. CO₂ travels faster than H⁺ through the cytosol due to the protein trapping of H⁺. CO₂ traverses biological membranes without the need for channels (although aquaporin and rhesus glycoprotein are likely CO₂ channels that facilitate CO₂ permeation through the plasma membrane⁵), whereas H⁺ usually cannot rapidly traverse biological membranes by diffusion. Intracellular CA and membrane-bound CA combined with HCO₃⁻ transporters (as known as band 3 protein in red blood cells, which is AE-1; the interaction of CAII and

AE-1 was first described in 1998⁶) facilitate the virtual translocation of H⁺ across the plasma membrane by the Jacobs-Stewart cycle whereby H⁺ and HCO₃⁻ are converted to CO₂ and H₂O by membrane-bound CA; the CO₂ crosses the plasma membrane and is hydrated to H⁺ and HCO₃⁻ by cytosolic CA with HCO₃⁻ exported by an anion exchanger. This cycle is also observed in the duodenal epithelial cells during luminal acid and high CO₂ exposure at the apical membranes.⁷ Blood flow supplies HCO₃⁻ as well as O₂ and nutrients, and also removes H⁺ and CO₂ efficiently using the Jacobs-Stewart cycle at the basolateral membranes. Therefore, the presence of CAs helps to keep not only the epithelial pH_i but also pH_{int} constant. CAIX deficiency may disrupt rapid removal of H⁺ and the supply of HCO₃⁻ at the basolateral surface of the cells, affecting cellular functions, as CAIX is localized on the basolateral membranes of epithelial cells.

CAIX is a membrane-bound CA, whose expression is strongly induced under hypoxic conditions.⁸ CAIX expression is upregulated in tumours.⁸ With its enzymatically active domain facing the extracellular environment, CAIX is implicated in pH_i-control and extracellular buffering in the hypoxic and acidic tumour environment, thus enabling cell proliferation and tumour growth.⁹ With this concept of CAIX function in mind, how can the gradual loss of parietal cells be explained in the gastric mucosa, which predominantly expresses CAIX in the surface cell region?

The investigation of parietal cell number and acid secretory capacity from suckling pups to aged mice revealed no difference of both parameters between wild-type and CAIX KO stomach in suckling and juvenile mice. Nevertheless, gastric surface cells were more susceptible to intracellular acidification in KO mice stomach than in wild-type, and claudin-18A2 expression was already decreased in juvenile CAIX KO gastric mucosa,¹ suggesting that CAIX deficiency may alter gastric defence mechanisms by downregulation of claudin-18A2 prior to parietal cell loss.

One question is how CAIX deficiency reduces claudin-18A2 expression. Claudin-18A2 is a junctional protein with expression limited to the stomach,¹⁰ broadly localized to the tight junctions of surface mucus cells, parietal cells, chief cells and mucus neck cells comprising the gastric glands.¹¹ CAIX is also expressed on the basolateral membranes not only of surface cells, but also of parietal cells and chief cells in mice¹² and rats,¹³ suggesting that basolateral pH dysregulation may occur along with pH_i

dysregulation of gastric glands. An immunohistochemical study of alterations of claudin-18A2 expression will provide further details on the location of claudin-18A2 downregulation.

It is possible that downregulation of claudin-18A2 with consequent leaky intercellular junctions observed with CAIX deficiency may alter parietal cell number. As the gastric stem cell zone, termed the isthmus, is located near the surface of the mucosa, pH_{int} dysregulation at the gastric surface may influence basolateral pH in the isthmus. The stomach of claudin-18 KO mice displays parietal cell loss on the first day after birth,¹⁰ also supporting this hypothesis. A similar phenomenon of progressive parietal cell loss is also observed in mice deficient for occludin that also alters claudin-18 expression,¹⁴ further supporting this hypothesis.

Most likely, CAIX KO mice may have diminished surface cell HCO_3^- secretion, in parallel with lower acid handling at the basolateral surface, supported by lower surface pH despite a normal acid secretory response. As blood flow supplies HCO_3^- and removes H^+/CO_2 from the basolateral surface of surface cells, both regulated by rapid interconversion of H^+ and H_2O catalyzed by basolateral CAIX, CAIX KO impairs HCO_3^- loading into and H^+ extrusion from the surface cells across the basolateral surface, with a lower rate of HCO_3^- secretion, lower surface pH and rapid intracellular acidification (lower pH_i). This may damage the surface cells, affecting claudin-18A2 expression. Increased expression of IL-1 β and iNOS at the same time may damage the surface cells before or after claudin-18A2 downregulation. Enhanced stimulated gastric acid secretion observed at 1 month in CAIX KO stomach prior to parietal cell loss¹ may be also explained by disrupted HCO_3^- secretion during stimulated acid secretion.

Further study is necessary to determine which occurs first, claudin-18A2 downregulation or upregulation of IL-1 β and iNOS. Mild proton pump inhibitor (PPI) treatment partially reversed claudin-18A2 expression, but had no effect on iNOS upregulation,¹ suggesting that CAIX KO may induce iNOS expression before H^+ back diffusion occurs. iNOS expression under CAIX inhibition in wild-type stomach or iNOS inhibition in CAIX KO stomach will clarify this possibility.

As gastric acid secretion occurs only after birth and is fully activated after weaning, this carefully performed study provides essential insight into the complex interplay of pH regulation, tight junctional permeability, inflammation, proliferation and injury. We hope to see studies in the future in which this interplay is related to cancer and other important diseases.

CONFLICT OF INTEREST

All authors declare that they have no conflict of interests.

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REFERENCES

- Li T, Liu X, Riederer B, et al. Genetic ablation of carbonic anhydrase IX disrupts gastric barrier function via claudin-18 downregulation and acid backflux. *Acta Physiol* 2018;222:e12923.
- Kaunitz JD. Barrier function of gastric mucus. *Keio J Med*. 1999;48:63-68.
- Laine L, Takeuchi K, Tamawski A. Gastric mucosal defense and cytoprotection: bench to bedside. *Gastroenterology*. 2008;135:41-60.
- Stuarttilley A, Sardet C, Pouyssegur J, Schwartz MA, Brown D, Alper SL. Immunolocalization of anion exchanger AE2 and cation exchanger NHE-1 in distinct adjacent cells of gastric mucosa. *Am J Physiol*. 1994;266:C559-C568.
- Endeward V, Cartron JP, Ripoche P, Gros G. RhAG protein of the Rhesus complex is a CO_2 channel in the human red cell membrane. *FASEB J*. 2008;22:64-73.
- Vince JW, Reithmeier RA. Carbonic anhydrase II binds to the carboxyl terminus of human band 3, the erythrocyte C1 - HCO_3^- exchanger. *J Biol Chem*. 1998;273:28430-28437.
- Mizumori M, Meyerowitz J, Takeuchi T, et al. Epithelial carbonic anhydrases facilitate PCO_2 and pH regulation in rat duodenal mucosa. *J Physiol*. 2006;573:827-842.
- Ivanov S, Liao SY, Ivanova A, et al. Expression of hypoxia-inducible cell-surface transmembrane carbonic anhydrases in human cancer. *Am J Pathol*. 2001;158:905-919.
- Swietach P, Hulikova A, Vaughan-Jones RD, Harris AL. New insights into the physiological role of carbonic anhydrase IX in tumour pH regulation. *Oncogene*. 2010;29:6509-6521.
- Hayashi D, Tamura A, Tanaka H, et al. Deficiency of claudin-18 causes paracellular H^+ leakage, up-regulation of interleukin-1 β , and atrophic gastritis in mice. *Gastroenterology*. 2012;142:292-304.
- Shinozaki A, Ushiku T, Morikawa T, et al. Epstein-Barr virus-associated gastric carcinoma: a distinct carcinoma of gastric phenotype by claudin expression profiling. *J Histochem Cytochem*. 2009;57:775-785.
- Gut MO, Parkkila S, Vernerova Z, et al. Gastric hyperplasia in mice with targeted disruption of the carbonic anhydrase gene Car9. *Gastroenterology*. 2002;123:1889-1903.
- Akiba Y, Ghayouri S, Takeuchi T, et al. Carbonic anhydrases and mucosal vanilloid receptors help mediate the hyperemic response to luminal CO_2 in rat duodenum. *Gastroenterology*. 2006;131:142-152.
- Saitou M, Furuse M, Sasaki H, et al. Complex phenotype of mice lacking occludin, a component of tight junction strands. *Mol Biol Cell*. 2000;11:4131-4142.