MINI-REVIEW

Effects of Slc26a6 deletion and CFTR inhibition on HCO_3^- secretion by mouse pancreatic duct

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Abstract : Pancreatic duct epithelium secretes HCO_3^- -rich fluid, which is dependent on cystic fibrosis transmembrane conductance regulator (CFTR). HCO_3^- transport across the apical membrane is thought to be mediated by both SLC26A6 Cl^- - HCO_3^- exchange and CFTR HCO_3^- conductance. In this study we examined the relative contribution and interaction of SLC26A6 and CFTR in apical HCO_3^- transport. Interlobular pancreatic ducts were isolated from *slc26a6* null mice. Intracellular pH (pH_i) was measured by BCECF microfluorometry. Duct cells were stimulated with forskolin and alkalinized by acetate pre-pulse in the presence of HCO_3^- - CO_2 . Apical HCO_3^- secretion was estimated from the recovery rate of pH_i from alkaline load. When the lumen was perfused with high- Cl^- solution, the rate of apical HCO_3^- secretion was increased by luminal application of CFTRinh-172 in ducts from wild-type mice but it was decreased in ducts from *slc26a6* $-/-$ mice. This suggests that *slc26a6* and CFTR compensate/compete with each other for apical HCO_3^- secretion with high Cl^- in the lumen. With high HCO_3^- in the lumen, luminal CFTRinh-172 reduced the rate of apical HCO_3^- secretion in both wild-type and *slc26a6* $-/-$ ducts. This suggests that HCO_3^- conductance of CFTR mediates a significant portion of apical HCO_3^- secretion with high HCO_3^- in the lumen. J. Med. Invest. 56 Suppl. : 332-335, December, 2009

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SLC26A6 MEDIATES APICAL Cl^- - HCO_3^- EXCHANGE IN PANCREATIC DUCT

The pancreatic duct system of humans produces approximately 2.5 liters per day of isotonic fluid containing ~140 mM HCO_3^- , which is dependent on cystic fibrosis transmembrane conductance regulator (CFTR). The HCO_3^- -rich ductal secretion acts as both a vehicle for acini-derived digestive enzymes

and a buffer for duodenal acidity. We have been studying the mechanisms of HCO_3^- secretion by pancreatic duct cell by using interlobular duct segments isolated from guinea-pig pancreas (1). The pancreatic juice of guinea-pig contains ~140 mM HCO_3^- . Our data suggest that HCO_3^- secretion across the apical membrane is mediated by both Cl^- - HCO_3^- exchange and HCO_3^- conductance of CFTR and that the relative contribution of these 2 apical mechanisms varies depending on the anion composition of the luminal fluid (Fig. 1). A computer model suggested that ~75% of apical HCO_3^- secretion is mediated by Cl^- - HCO_3^- exchange in proximal pancreatic ducts close to acini where the lumen is filled with acini-derived Cl^- -rich fluid (2).

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Fig. 1 Hypothetical roles of SLC26A6 Cl⁻-HCO₃⁻ exchange and CFTR HCO₃⁻ conductance in HCO₃⁻ secretion by pancreatic duct. In proximal pancreatic ducts close to acini where the lumen is filled with acini-derived Cl⁻-rich fluid, SLC26A6 Cl⁻-HCO₃⁻ exchange and CFTR HCO₃⁻ conductance compensate/compete with each other for apical HCO₃⁻ secretion. The former provides a major route. As a result of HCO₃⁻ secretion across ductal epithelium, luminal [HCO₃⁻] gradually rises with distance along the duct (and luminal [Cl⁻] decreases). According to the changes in luminal anion composition, the relative contribution of Cl⁻-HCO₃⁻ exchange and HCO₃⁻ conductance was reversed. As luminal [HCO₃⁻] increases to 140 mM, Cl⁻-HCO₃⁻ exchanger of any stoichiometry (SLC26A3 and SLC26A6) approaches to their equilibrium and cannot support HCO₃⁻ secretion. Here HCO₃⁻ conductance of CFTR provides a major route for apical HCO₃⁻ secretion.

It is now generally accepted that apical Cl⁻-HCO₃⁻ exchange of epithelial tissues including pancreatic duct is mediated by members of SLC26 family of anion transporters (3). SLC26A3 and SLC26A6 have been identified in pancreatic duct cells (4), but SLC26A6 is likely the major Cl⁻-HCO₃⁻ exchanger in the apical membrane. This is because apical Cl⁻-HCO₃⁻ exchange is DIDS-sensitive (5) and SLC26A6 and CFTR are co-localized in the apical membrane of rat pancreatic intralobular ducts (5).

FUNCTIONAL CHARACTERISTICS OF SLC26A6 EXPRESSED IN THE HETEROLOGOUS SYSTEM

Heterologous expression studies have demonstrated that SLC26A6 operates in multiple transport modes: Cl⁻-formate exchange, Cl⁻-oxalate exchange, sulfate-oxalate exchange, Cl⁻-OH⁻ exchange, and Cl⁻-HCO₃⁻ exchange (6). Recently mouse *slc26a6* was reported to be electrogenic and mediate 1Cl⁻-2HCO₃⁻ exchange (7) and this stoichiometry/electrogenicity may help achieve higher HCO₃⁻ concentrations in the pancreatic juice. However, human SLC26A6 and mouse *slc26a6* share only 78% amino acid identity and exhibit significant functional differences (8).

PHENOTYPE OF SLC26A6 NULL MICE

SLC26A6 is expressed in the small intestine, pancreatic duct, kidney proximal tubule, and gastric parietal cells. *Slc26a6* null mice appeared healthy and exhibited normal growth, blood pressure, glomerular filtration rate, and serum electrolyte profile. In the duodenum, prostaglandin E₂-stimulated HCO₃⁻ secretion was reduced, whereas forskolin-stimulated component of HCO₃⁻ secretion was not affected (9). In kidney proximal tubule, oxalate-stimulated NaCl absorption and apical Cl⁻-HCO₃⁻ exchange were reduced. Calcium oxalate urolithiasis was frequently found, which was probably due to defective oxalate secretion in the small intestine (10).

FLUID AND HCO₃⁻ SECRETION BY PANCREATIC DUCT IN SLC26A6 NULL MICE

Two laboratories investigated the effects of *slc26a6* deletion on HCO₃⁻ transport in pancreatic duct cell by using interlobular pancreatic ducts isolated from *slc26a6* null mice. In our study, there were no effects on basal and forskolin-stimulated fluid secretion in sealed ducts and the effects on apical Cl⁻-HCO₃⁻ exchange in lumenally-microperfused ducts were opposite in 2 alternative directions (11). HCO₃⁻ efflux in exchange for luminal Cl⁻ was reduced, while HCO₃⁻ influx from the lumen in exchange for intracellular Cl⁻ was enhanced. The data suggested that *slc26a6* mediates Cl⁻-dependent HCO₃⁻ secretion and the unidirectionality is consistent with 1Cl⁻-2HCO₃⁻ stoichiometry (11). Another laboratory found that

the basal levels of $\text{Cl}^-/\text{HCO}_3^-$ exchange and HCO_3^- secretion were enhanced in sealed ducts from *slc26a6*^{-/-} mice and secretin failed to stimulate HCO_3^- secretion (12). The data suggest that *slc26a6* mediates most of cAMP-stimulated HCO_3^- secretion. Despite these discrepancies, volume and pH of the pancreatic juice collected *in vivo* under secretin stimulation was not affected by deletion of *slc26a6*. *Slc26a6* $\text{Cl}^-/\text{HCO}_3^-$ exchange may operate only in very proximal part of the duct system and/or the contribution of *slc26a6* to overall ductal HCO_3^- secretion may be small in mouse pancreas.

INTERACTION OF SLC26A6 AND CFTR IN HETEROLOGOUS SYSTEM

Heterologous expression studies have demonstrated that the activation of SLC26A6 $\text{Cl}^-/\text{HCO}_3^-$ exchange by cAMP largely depends on the presence of functional CFTR and that SLC26A6 reciprocally increases CFTR current (13). This mutual enhancement is achieved by physical interactions of 2 molecules through the STAS domain of SLC26A6 and the R domain of CFTR.

INTERACTION OF SLC26A6 AND CFTR IN PANCREATIC DUCT

The activation of apical $\text{Cl}^-/\text{HCO}_3^-$ exchange by cAMP depends on the presence of functional CFTR in pancreatic duct cell similarly to the findings in heterologous expression studies, whereas SLC26A6 seems to have a tonic inhibitory effect on CFTR activity in basal unstimulated condition. Activation of apical $\text{Cl}^-/\text{HCO}_3^-$ exchange by cAMP was abolished in main pancreatic duct from ΔF cystic fibrosis mice (14). When human pancreatic duct cell-line CFPAC-1 cells, which lack functional CFTR, were transfected with wild-type CFTR, mRNA expression of SLC26A6 was enhanced and apical $\text{Cl}^-/\text{HCO}_3^-$ exchange activity was increased (4).

Recently we investigated the functional interaction of SLC26A6 $\text{Cl}^-/\text{HCO}_3^-$ exchange and CFTR (once they are expressed and activated) in cAMP-stimulated HCO_3^- secretion across the apical membrane using interlobular ducts isolated from guinea pig pancreas (15). Inhibition of CFTR by luminal application of CFTRinh-172 enhanced both apical $\text{Cl}^-/\text{HCO}_3^-$ exchange activity (probably mediated by SLC26A6) and Cl^- -dependent HCO_3^- secretion. The

data suggest that $\text{Cl}^-/\text{HCO}_3^-$ exchange by SLC26A6 and HCO_3^- conductance of CFTR compensate or compete with each other for HCO_3^- secretion with Cl^- -rich fluid in the lumen. Membrane hyperpolarization upon removal of luminal Cl^- support the $1\text{Cl}^-/2\text{HCO}_3^-$ stoichiometry proposed for SLC26A6. SLC26A6 and CFTR are possibly coupled functionally via changes in membrane potential.

EFFECTS OF SLC26A6 DELETION AND CFTR INHIBITION ON HCO_3^- SECRETION BY THE MOUSE PANCREATIC DUCT

To examine the relative contribution and interaction of SLC26A6 and CFTR in apical HCO_3^- transport under different anion composition in the lumen, we examined apical HCO_3^- secretion in interlobular pancreatic ducts (diameter: $\sim 100\ \mu\text{m}$) isolated from *slc26a6* null mice. The lumen was perfused either with high- Cl^- (125 mM Cl^- -25 mM HCO_3^-) or high- HCO_3^- (25 mM Cl^- -125 mM HCO_3^-) solution. Intracellular pH (pH_i) was measured by microfluorometry in duct cells loaded with BCECF. Duct cells were stimulated with forskolin and alkalinized by acetate pre-pulse in the presence of $\text{HCO}_3^-/\text{CO}_2$. Apical HCO_3^- secretion was estimated from the recovery rate of pH_i from alkaline load in the presence of dihydro-DIDS in the bath to inhibit basolateral HCO_3^- flux. When the lumen was perfused with the high- Cl^- solution, luminal application of CFTRinh-172 enhanced HCO_3^- secretion in ducts from wild-type mice. This finding is consistent with our previous data on guinea-pig pancreatic ducts (15). When ducts from *slc26a6*^{-/-} mice were luminally-perfused with the high- Cl^- solution, the rate of apical HCO_3^- secretion was significantly faster compared with wild-type, and it was significantly inhibited by luminal CFTRinh-172. The data suggest that *slc26a6* $\text{Cl}^-/\text{HCO}_3^-$ exchange and CFTR HCO_3^- conductance compensate/compete with each other for apical HCO_3^- secretion in proximal pancreatic ducts close to acini where the lumen is filled with acini-derived Cl^- -rich fluid (Fig. 1). We repeated the same protocol in ducts luminally-perfused with the high- HCO_3^- solution. In this condition, luminal application of CFTRinh-172 inhibited apical HCO_3^- secretion both in wild-type and *slc26a6*^{-/-} ducts. This suggests that HCO_3^- conductance of CFTR mediates a significant portion of apical HCO_3^- secretion in distal pancreatic ducts where the luminal HCO_3^- concentration is already high due to ductal HCO_3^-

secretion (Fig. 1).

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REFERENCES

1. Ishiguro H, Steward MC, Sohma Y, Kubota T, Kitagawa M, Kondo T, Case RM, Hayakawa T, Naruse S : Membrane potential and bicarbonate secretion in isolated interlobular ducts from guinea-pig pancreas. *J Gen Physiol* 120 : 617-628, 2002
2. Sohma Y, Gray MA, Imai Y, Argent BE : HCO_3^- transport in a mathematical model of the pancreatic ductal epithelium. *J Membr Biol* 176 : 77-100, 2000
3. Steward MC, Ishiguro H, Case RM : Mechanisms of bicarbonate secretion in the pancreatic duct. *Annu Rev Physiol* 67 : 377-409, 2005
4. Greeley T, Shumaker H, Wang Z, Schweinfest CW, Soleimani M : Downregulated in adenoma and putative anion transporter are regulated by CFTR in cultured pancreatic duct cells. *Am J Physiol Gastrointest Liver Physiol* 281 : G1301-G1308, 2001
5. Shcheynikov N, Wang Y, Park M, Ko SB, Dorwart M, Naruse S, Thomas PJ, Muallem S : Coupling modes and stoichiometry of $\text{Cl}^-/\text{HCO}_3^-$ exchange by *slc26a3* and *slc26a6*. *J Gen Physiol* 127 : 511-524, 2006
6. Mount DB, Romero MF : The SLC26 gene family of multifunctional anion exchangers. *Pflugers Arch* 447 : 710-721, 2004
7. Xie Q, Welch R, Mercado A, Romero MF, Mount DB : Molecular characterization of the murine *Slc26a6* anion exchanger : functional comparison with *Slc26a1*. *Am J Physiol Renal Physiol* 283 : F826-F838, 2002
8. Chernova MN, Jiang L, Friedman DJ, Darman RB, Lohi H, Kere J, Vandorpe DH, Alper SL : Functional comparison of mouse *slc26a6* anion exchanger with human SLC26A6 polypeptide variants : differences in anion selectivity, regulation, and electrogenicity. *J Biol Chem* 280 : 8564-8580, 2005
9. Tuo B, Riederer B, Wang Z, Colledge WH, Soleimani M, Seidler U : Involvement of the anion exchanger SLC26A6 in prostaglandin E_2 -but not forskolin-stimulated duodenal HCO_3^- secretion. *Gastroenterology* 130 : 349-358, 2006
10. Jiang Z, Asplin JR, Evan AP, Rajendran VM, Velazquez H, Nottoli TP, Binder HJ, Aronson PS : Calcium oxalate urolithiasis in mice lacking anion transporter. *Nat Genet* 38 : 474-478, 2006
11. Ishiguro H, Namkung W, Yamamoto A, Wang Z, Worrell RT, Xu J, Lee MG, Soleimani M : Effect of *Slc26a6* deletion on apical $\text{Cl}^-/\text{HCO}_3^-$ exchanger activity and cAMP-stimulated bicarbonate secretion in pancreatic duct. *Am J Physiol* 292 : G447-G455, 2007
12. Wang Y, Soyombo AA, Shcheynikov N, Zeng W, Dorwart M, Marino CR, Thomas PJ, Muallem S : *Slc26a6* regulates CFTR activity *in vivo* to determine pancreatic duct HCO_3^- secretion : relevance to cystic fibrosis. *EMBO J* 25 : 5049-5057, 2006
13. Ko SB, Zeng W, Dorwart MR, Luo X, Kim KH, Millen L, Goto H, Naruse S, Soyombo A, Thomas PJ, Muallem S : Gating of CFTR by the STAS domain of SLC26 transporters. *Nat Cell Biol* 6 : 343-350, 2004
14. Lee MG, Choi JY, Luo X, Strickland E, Thomas PJ, Muallem S : Cystic fibrosis transmembrane conductance regulator regulates luminal $\text{Cl}^-/\text{HCO}_3^-$ exchange in mouse submandibular and pancreatic ducts. *J Biol Chem* 274 : 14670-14677, 1999
15. Stewart AK, Yamamoto A, Nakakuki M, Kondo T, Alper SL, Ishiguro H : Functional coupling of apical exchange with CFTR in stimulated secretion by guinea pig interlobular pancreatic duct. *Am J Physiol* 296 : G1307-G1317, 2009