

## MINI-REVIEW

# Effects of Slc26a6 deletion and CFTR inhibition on HCO<sub>3</sub><sup>-</sup> secretion by mouse pancreatic duct

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**Abstract :** Pancreatic duct epithelium secretes HCO<sub>3</sub><sup>-</sup>-rich fluid, which is dependent on cystic fibrosis transmembrane conductance regulator (CFTR). HCO<sub>3</sub><sup>-</sup> transport across the apical membrane is thought to be mediated by both SLC26A6 Cl<sup>-</sup>-HCO<sub>3</sub><sup>-</sup> exchange and CFTR HCO<sub>3</sub><sup>-</sup> conductance. In this study we examined the relative contribution and interaction of SLC26A6 and CFTR in apical HCO<sub>3</sub><sup>-</sup> transport. Interlobular pancreatic ducts were isolated from slc26a6 null mice. Intracellular pH (pH<sub>i</sub>) was measured by BCECF microfluorometry. Duct cells were stimulated with forskolin and alkalinized by acetate pre-pulse in the presence of HCO<sub>3</sub><sup>-</sup>-CO<sub>2</sub>. Apical HCO<sub>3</sub><sup>-</sup> secretion was estimated from the recovery rate of pH<sub>i</sub> from alkaline load. When the lumen was perfused with high-Cl<sup>-</sup> solution, the rate of apical HCO<sub>3</sub><sup>-</sup> secretion was increased by luminal application of CFTRinh-172 in ducts from wild-type mice but it was decreased in ducts from slc26a6 <sup>-/-</sup> mice. This suggests that slc26a6 and CFTR compensate/compete with each other for apical HCO<sub>3</sub><sup>-</sup> secretion with high Cl<sup>-</sup> in the lumen. With high HCO<sub>3</sub><sup>-</sup> in the lumen, luminal CFTRinh-172 reduced the rate of apical HCO<sub>3</sub><sup>-</sup> secretion in both wild-type and slc26a6 <sup>-/-</sup> ducts. This suggests that HCO<sub>3</sub><sup>-</sup> conductance of CFTR mediates a significant portion of apical HCO<sub>3</sub><sup>-</sup> secretion with high HCO<sub>3</sub><sup>-</sup> in the lumen. *J. Med. Invest.* 56 Suppl. : 332-335, December, 2009

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## SLC26A6 MEDIATES APICAL Cl<sup>-</sup>-HCO<sub>3</sub><sup>-</sup> EXCHANGE IN PANCREATIC DUCT

The pancreatic duct system of humans produces approximately 2.5 liters per day of isotonic fluid containing ~140 mM HCO<sub>3</sub><sup>-</sup>, which is dependent on cystic fibrosis transmembrane conductance regulator (CFTR). The HCO<sub>3</sub><sup>-</sup>-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<sub>3</sub><sup>-</sup> 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<sub>3</sub><sup>-</sup>. Our data suggest that HCO<sub>3</sub><sup>-</sup> secretion across the apical membrane is mediated by both Cl<sup>-</sup>-HCO<sub>3</sub><sup>-</sup> exchange and HCO<sub>3</sub><sup>-</sup> 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<sub>3</sub><sup>-</sup> secretion is mediated by Cl<sup>-</sup>-HCO<sub>3</sub><sup>-</sup> exchange in proximal pancreatic ducts close to acini where the lumen is filled with acini-derived Cl<sup>-</sup>-rich fluid (2).

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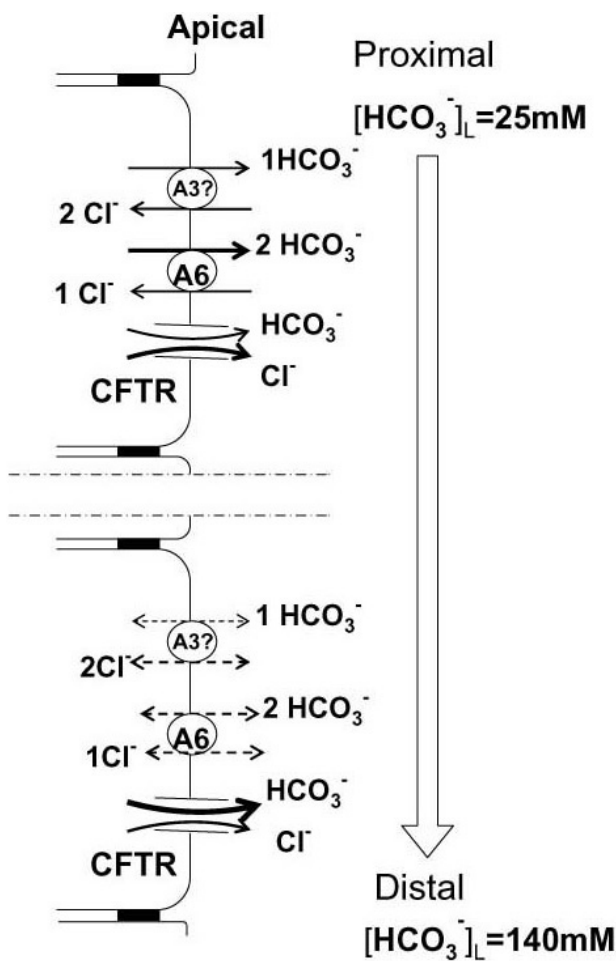


Fig. 1 Hypothetical roles of SLC26A6 Cl-HCO<sub>3</sub><sup>-</sup> exchange and CFTR HCO<sub>3</sub><sup>-</sup> conductance in HCO<sub>3</sub><sup>-</sup> secretion by pancreatic duct. In proximal pancreatic ducts close to acini where the lumen is filled with acini-derived Cl-rich fluid, SLC26A6 Cl-HCO<sub>3</sub><sup>-</sup> exchange and CFTR HCO<sub>3</sub><sup>-</sup> conductance compensate/compete with each other for apical HCO<sub>3</sub><sup>-</sup> secretion. The former provides a major route. As a result of HCO<sub>3</sub><sup>-</sup> secretion across ductal epithelium, luminal [HCO<sub>3</sub><sup>-</sup>] gradually rises with distance along the duct (and luminal [Cl] decreases). According to the changes in luminal anion composition, the relative contribution of Cl-HCO<sub>3</sub><sup>-</sup> exchange and HCO<sub>3</sub><sup>-</sup> conductance was reversed. As luminal [HCO<sub>3</sub><sup>-</sup>] increases to 140 mM, Cl-HCO<sub>3</sub><sup>-</sup> exchanger of any stoichiometry (SLC26A3 and SLC26A6) approaches to their equilibrium and cannot support HCO<sub>3</sub><sup>-</sup> secretion. Here HCO<sub>3</sub><sup>-</sup> conductance of CFTR provides a major route for apical HCO<sub>3</sub><sup>-</sup> secretion.

It is now generally accepted that apical Cl-HCO<sub>3</sub><sup>-</sup> 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-HCO<sub>3</sub><sup>-</sup> exchanger in the apical membrane. This is because apical Cl-HCO<sub>3</sub><sup>-</sup> 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<sup>-</sup> exchange, and Cl-HCO<sub>3</sub><sup>-</sup> exchange (6). Recently mouse *slc26a6* was reported to be electrogenic and mediate 1Cl-2HCO<sub>3</sub><sup>-</sup> exchange (7) and this stoichiometry/electrogenicity may help achieve higher HCO<sub>3</sub><sup>-</sup> 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 E<sub>2</sub>-stimulated HCO<sub>3</sub><sup>-</sup> secretion was reduced, whereas forskolin-stimulated component of HCO<sub>3</sub><sup>-</sup> secretion was not affected (9). In kidney proximal tubule, oxalate-stimulated NaCl absorption and apical Cl-HCO<sub>3</sub><sup>-</sup> exchange were reduced. Calcium oxalate urolithiasis was frequently found, which was probably due to defective oxalate secretion in the small intestine (10).

### FLUID AND HCO<sub>3</sub><sup>-</sup> SECRETION BY PANCREATIC DUCT IN SLC26A6 NULL MICE

Two laboratories investigated the effects of *slc26a6* deletion on HCO<sub>3</sub><sup>-</sup> 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-HCO<sub>3</sub><sup>-</sup> exchange in lumenally-microperfused ducts were opposite in 2 alternative directions (11). HCO<sub>3</sub><sup>-</sup> efflux in exchange for luminal Cl<sup>-</sup> was reduced, while HCO<sub>3</sub><sup>-</sup> influx from the lumen in exchange for intracellular Cl<sup>-</sup> was enhanced. The data suggested that *slc26a6* mediates Cl-dependent HCO<sub>3</sub><sup>-</sup> secretion and the unidirectionality is consistent with 1Cl-2HCO<sub>3</sub><sup>-</sup> stoichiometry (11). Another laboratory found that

the basal levels of Cl-HCO<sub>3</sub><sup>-</sup> exchange and HCO<sub>3</sub><sup>-</sup> secretion were enhanced in sealed ducts from slc26a6<sup>-/-</sup> mice and secretin failed to stimulate HCO<sub>3</sub><sup>-</sup> secretion (12). The data suggest that slc26a6 mediates most of cAMP-stimulated HCO<sub>3</sub><sup>-</sup> 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 Cl-HCO<sub>3</sub><sup>-</sup> exchange may operate only in very proximal part of the duct system and/or the contribution of slc26a6 to overall ductal HCO<sub>3</sub><sup>-</sup> 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 Cl-HCO<sub>3</sub><sup>-</sup> 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 Cl-HCO<sub>3</sub><sup>-</sup> 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 Cl-HCO<sub>3</sub><sup>-</sup> exchange by cAMP was abolished in main pancreatic duct from  $\Delta F$  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 Cl-HCO<sub>3</sub><sup>-</sup> exchange activity was increased (4).

Recently we investigated the functional interaction of SLC26A6 Cl-HCO<sub>3</sub><sup>-</sup> exchange and CFTR (once they are expressed and activated) in cAMP-stimulated HCO<sub>3</sub><sup>-</sup> 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 Cl-HCO<sub>3</sub><sup>-</sup> exchange activity (probably mediated by SLC26A6) and Cl-dependent HCO<sub>3</sub><sup>-</sup> secretion. The

data suggest that Cl-HCO<sub>3</sub><sup>-</sup> exchange by SLC26A6 and HCO<sub>3</sub><sup>-</sup> conductance of CFTR compensate or compete with each other for HCO<sub>3</sub><sup>-</sup> secretion with Cl-rich fluid in the lumen. Membrane hyperpolarization upon removal of luminal Cl<sup>-</sup> support the 1Cl<sup>-</sup>:2HCO<sub>3</sub><sup>-</sup> 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<sub>3</sub><sup>-</sup> SECRETION BY THE MOUSE PANCREATIC DUCT

To examine the relative contribution and interaction of SLC26A6 and CFTR in apical HCO<sub>3</sub><sup>-</sup> transport under different anion composition in the lumen, we examined apical HCO<sub>3</sub><sup>-</sup> secretion in interlobular pancreatic ducts (diameter: ~100  $\mu$ m) isolated from slc26a6 null mice. The lumen was perfused either with high-Cl<sup>-</sup> (125 mM Cl<sup>-</sup>-25 mM HCO<sub>3</sub><sup>-</sup>) or high-HCO<sub>3</sub><sup>-</sup> (25 mM Cl<sup>-</sup>-125 mM HCO<sub>3</sub><sup>-</sup>) solution. Intracellular pH (pH<sub>i</sub>) 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 HCO<sub>3</sub><sup>-</sup>-CO<sub>2</sub>. Apical HCO<sub>3</sub><sup>-</sup> secretion was estimated from the recovery rate of pH<sub>i</sub> from alkaline load in the presence of dihydro-DIDS in the bath to inhibit basolateral HCO<sub>3</sub><sup>-</sup> flux. When the lumen was perfused with the high-Cl<sup>-</sup> solution, luminal application of CFTRinh-172 enhanced HCO<sub>3</sub><sup>-</sup> 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<sup>-/-</sup> mice were luminally-perfused with the high-Cl<sup>-</sup> solution, the rate of apical HCO<sub>3</sub><sup>-</sup> secretion was significantly faster compared with wild-type, and it was significantly inhibited by luminal CFTRinh-172. The data suggest that slc26a6 Cl-HCO<sub>3</sub><sup>-</sup> exchange and CFTR HCO<sub>3</sub><sup>-</sup> conductance compensate/compete with each other for apical HCO<sub>3</sub><sup>-</sup> secretion in proximal pancreatic ducts close to acini where the lumen is filled with acini-derived Cl<sup>-</sup>-rich fluid (Fig. 1). We repeated the same protocol in ducts luminally-perfused with the high-HCO<sub>3</sub><sup>-</sup> solution. In this condition, luminal application of CFTRinh-172 inhibited apical HCO<sub>3</sub><sup>-</sup> secretion both in wild-type and slc26a6<sup>-/-</sup> ducts. This suggests that HCO<sub>3</sub><sup>-</sup> conductance of CFTR mediates a significant portion of apical HCO<sub>3</sub><sup>-</sup> secretion in distal pancreatic ducts where the luminal HCO<sub>3</sub><sup>-</sup> concentration is already high due to ductal HCO<sub>3</sub><sup>-</sup>

secretion (Fig. 1).

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