COMMENTARY

Is CFTR Dysfunction in Cystic Fibrosis Compensated by MRPs?

Commentary on the article by Hurbain *et al.* on page 627

SHINYA ITO

Division of Clinical Pharmacology and Toxicology, Department of Pediatrics, Hospital for Sick Children, University of Toronto, Toronto, Ontario, M5G 1X8, Canada

A disease caused by a single defective gene often shows a wide range of phenotypes. Cystic fibrosis, the most common lethal autosomal recessive disease in children of European descent, is no exception. The disease is caused by mutations in a gene located on chromosome 7, which encodes a membrane glycoprotein with chloride channel activity, called *cystic fibrosis transmembrane conductance regulator* (CFTR) (1–3). A deletion of a phenylalanine residue at position 508 ($\Delta F508$) of the CFTR protein is the consequence of the most frequent mutation found in nearly 70% of patients. As a result of faulty protein folding and posttranslational modification, the defective gene product does not undergo a normal trafficking process to the apical membrane (*i.e.* luminal side) of epithelia, although if inserted into the membrane correctly $\Delta F508$ -CFTR protein shows chloride channel activity (4). The lack of the chloride channel function on the surface epithelia is the pathognomonic feature of the cystic fibrosis.

If these patients homozygous for $\Delta F508$ invariably lack functional membrane CFTR, where does the variation of the clinical course come from? A recent study has shown membrane expression of CFTR $\Delta F508$ protein in airway submucosal glands of some of these patients (5). In addition, studies suggested positive correlations between the chloride channel activity in nasal epithelia and a milder clinical course of the airway disease in cystic fibrosis patients with $\Delta F508$ mutation (6–8). Apparently, the chloride channel activity is somehow preserved to some extent in some cystic fibrosis patients.

The report by Hurbain *et al.* in this issue of the Pediatric Research provides another piece of information to this important aspect of cystic fibrosis heterogeneity (9). Given the recent observation that induction of membrane proteins called *multi-drug resistance-associated proteins* (MRP) may compensate deficient CFTR function (10), and functional overlap between CFTR and MRPs, the authors speculated that different expression levels of MRPs may be the reason for the variation in the

clinical pictures of those patients. They compared the MRP 1–5 mRNA expressions between healthy control and cystic fibrosis patients with $\Delta F508$ mutation. In addition, they studied association between MRP gene expression levels and a chloride conductive activity of these patients. They found lower MRP1 and 5 expressions in patients with $\Delta F508$ mutation than control, positive correlation between MRP 1 and 5 in controls and patients, and lower MRP1 transcript levels in cystic fibrosis patients with lower basal (non-CFTR) chloride conductive activity. In summary, they showed that transcriptions of MRP, specifically MRP1 and MRP5, in nasal epithelia of $\Delta F508$ -homozygous cystic fibrosis patients correlate with milder airway disease and non-CFTR chloride conduction activity. Do the MRPs compensate the defective CFTR function by improving chloride ion conductance?

CFTR belongs to the ATP-binding cassette (ABC) transporter superfamily, which includes MRPs, MDR1 P-glycoprotein, and so on so forth (11). These transporters bind ATP to drive the across-the-membrane transport of various molecules, ranging from chloride ion to xenobiotics. These ABC transporter genes are further divided into 7 subfamilies (from A to G) according to genomic organization, and sequence homology in the ATP binding and transmembrane domains. CFTR and MRPs are classified into the C subfamily (ABCC) that consists of 12 proteins: CFTR; 2 sulfonylurea receptors; and 9 MRPs. Hence, CFTR and MRPs are related at least from a genomics point of view. What about function, then?

CFTR is a chloride channel activated by the ATP binding, and by the cAMP-mediated phosphorylation of serine residues in its regulatory domain. Recent data also suggest that CFTR plays a role in transmembrane fluxes of organic anions such as glutathione, one of the important extracellular antioxidants in the lung (12, 13). Speculation is that reduced glutathione in airway surface fluid of cystic fibrosis patients may be due to the lack of glutathione transport through CFTR (13). Of the 9 MRPs, MRP1, MRP2, MRP3, and MRP6 mediate transport of amphipathic anionic compounds and conjugates such as leukotriene C₄, various cancer chemotherapeutic agents, glutathione S-conjugates, oxidized glutathione, and sulfate and gluc-

Received May 8, 2003; accepted May 20, 2003.

Corresponding: Shinya Ito, M.D., Division of Clinical Pharmacology and Toxicology, Department of Pediatrics, Hospital for Sick Children, 555 University Avenue, Toronto, Ontario, CANADA M5G 1X8; e-mail: shinya.ito@sickkids.ca Supported by a grant from the Canadian Institute of Health Research

uronide conjugates (14, 15). Substrates for MRP4 and 5 include nucleotide analogues. Taken together, functions of CFTR and MRP may overlap, compensating each other for their transport activity, although whether MRPs confer chloride channel activity directly or indirectly remains to be proven. The findings by Hurbain *et al.* (9) in this issue appear to fit this yet-to-be-proven theoretical scheme of mutually complementing CFTR and MRPs, showing that MRP1 (and MRP5 to a lesser extent) expression, at least at mRNA level, is positively associated with better respiratory function and increased non-CFTR chloride channel activity in $\Delta F508$ -homozygous cystic fibrosis patients. However, we have a problem.

Existing data on cellular localization of MRPs in epithelial tissues do not fully support the above notion. When expressed in polarized renal tubular cell lines, MRP1 and MRP5 are routed to the basolateral membranes, whereas MRP2 (also known as cMOAT) is targeted to the apical membranes (14, 16, 17). In accordance with the basal membrane localization and its unidirectional transport function from inside to outside of the cells, the MDCK canine renal tubular cells transfected with MRP5 show increased apical-to-basal vectorial flux of S-(2,4,-dinitrophenyl)glutathione and glutathione (17). In nasal mucosa of healthy individuals, CFTR and MRP1 show different cellular localization. Specifically, CFTR is located on the apical membrane, whereas MRP1 is expressed in the cytoplasm (18). These data suggest differences in protein sorting processes of these transporters in epithelia. If the MRPs are not expressed on the apical membranes, how do they compensate the deficient chloride channel activity of $\Delta F508$ -CFTR on the apical membrane? Are airway epithelia different from other epithelia, expressing MRP1 and MRP5 on the apical (*i.e.* luminal) membrane? Do airway epithelial cells of cystic fibrosis patients with the $\Delta F508$ mutation target MRP1 and MRP5 to the apical membranes? Do MRPs on the basal membranes somehow influence the apical chloride channel activity? Although highly speculative, Hurbain *et al.* (9) suggested that MRP1 on the basal membranes transports glutathione into interstitium, which then diffuses into the airway lining fluid *via* para-cellular pathways.

Clearly, we need more data on a role of MRPs in chloride conduction, including protein expression and cellular localization of MRPs in airway epithelia of $\Delta F508$ -homozygous cystic

fibrosis patients. Overall, the findings by Hurbain *et al.* (9) need to be confirmed. If their observations hold true, we will have a plenty of interesting questions to address, which would eventually bring new treatment strategies in the management of CF patients.

REFERENCES

1. Kerem B, Rommens JM, Buchanan JA, Markiewicz D, Cox TK, Chakravarti A, Buchwald M, Tsui LC 1989 Identification of the cystic fibrosis gene: genetic analysis. *Science* 245:1073–1080
2. Riordan JR, Rommens JM, Kerem B, Alon N, Rozmahel R, Grzelczak Z, Zielenski J, Lok S, Plavsic N, Chou JL, et al 1989 Identification of the cystic fibrosis gene: cloning and characterization of complementary DNA.[erratum: *Science* 1989;245:1437]. *Science* 245:1066–1073
3. Rosenstein BJ, Zeitlin PL 1998 Cystic Fibrosis. *Lancet* 351:277–282
4. Li C, Ramjeeasingh M, Reyes E, Jensen T, Chang X, Rommens JM, Bear CE 1993 The cystic fibrosis mutation (delta F508) does not influence the chloride channel activity of CFTR. *Nat Genet* 3:311–316
5. Kalin N, Claass A, Sommer M, Puchelle E, Tummeler B 1999 DeltaF508 CFTR protein expression in tissues from patients with cystic fibrosis. *J Clin Invest* 103:1379–1389
6. Sermet-Gaudelus I, Vallee B, Urbain I, Torossi T, Marianovski R, Fajac A, Feuillet MN, Bresson JL, Lenoir G, Bernaudin JF, Edelman A 2002 Normal function of the cystic fibrosis conductance regulator protein can be associated with homozygous (Delta)F508 mutation. *Pediatr Res* 52:628–635
7. Thomas SR, Jaffe A, Geddes DM, Hodson ME, Alton EW 1999 Pulmonary disease severity in men with deltaF508 cystic fibrosis and residual chloride secretion. *Lancet* 353:984–985
8. Bronsveld I, Mekus F, Bijman J, Ballmann M, de Jonge HR, Laabs U, Halley DJ, Ellemunter H, Mastella G, Thomas S, Veeze HJ, Tummeler B 2001 Chloride conductance and genetic background modulate the cystic fibrosis phenotype of Delta F508 homozygous twins and siblings. *J Clin Invest* 108:1705–1715
9. Hurbain H, Sermet-Gaudelus I, Vallee B, Feuillet M-N, Lenoir G, Bernaudin J-F, Edelman A, Fajac A 2003 Evaluation of MRP1–5 gene in cystic fibrosis patients homozygous for the $\Delta F508$ mutation. *Pediatr Res* 54:627–634
10. Lallemand JY 1997 Induction by antitumoral drugs of proteins that functionally complement CFTR: a novel therapy for cystic fibrosis? *Lancet* 350:711–712
11. Dean M, Rzhetsky A, Allikmets R 2001 The human ATP-binding cassette (ABC) transporter superfamily. *Genome Res* 11:1156–1166
12. Linsdell P, Hanrahan JW 1998 Glutathione permeability of CFTR. *Am J Physiol Cell Physiol* 275:C323–C326
13. Gao L, Kim KJ, Yankaskas JR, Forman HJ 1999 Abnormal glutathione transport in cystic fibrosis airway epithelia. *Am J Physiol Lung Cell Mol Physiol* 277:L113–L118
14. Evers R, Zaman GJR, van Deemter L, Jansen H, Calafat J, Oomen LCJM, Oude Elferink RPJ, Borst P, Schinkel AH 1996 Basolateral localization and export activity of the human multidrug resistance-associated protein in polarized pig kidney cells. *J Clin Invest* 97:1211–1218
15. Gottesman MM, Fojo T, Bates SE 2002 Multidrug resistance in cancer: role of ATP-dependent transporters. *Nat Rev Cancer* 2:48–58
16. Evers R, Kool M, van Deemter L, Janssen H, Calafat J, Oomen LC, Paulusma CC, Oude Elferink RPJ, Baas F, Schinkel AH, Borst P 1998 Drug export activity of the human canalicular multispecific organic anion transporter in polarized kidney MDCK cells expressing cMOAT (MRP2) cDNA. *J Clin Invest* 101:1310–1319
17. Wijnholds J, Mol CA, van Deemter L, de Haas M, Scheffer GL, Baas F, Beijnen JH, Scheper RJ, Hatse S, De Clercq E, Balzarini J, Borst P 2000 Multidrug-resistance protein 5 is a multispecific organic anion transporter able to transport nucleotide analogs. *Proc Natl Acad Sci USA* 97:7476–7481
18. Wioland MA, Fleury-Feith J, Corlieu P, Commo F, Monceaux G, Lacau-St-Guily J, Bernaudin JF 2000 CFTR, MDR1, and MRP1 immunolocalization in normal human nasal respiratory mucosa. *J Histochem Cytochem* 48:1215–1222