CFTR structure and function: is there a role in the kidney?

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Abstract Cystic fibrosis (CF) is a lethal autosomal recessive genetic disease caused by mutations in the CF transmembrane conductance regulator (CFTR). Mutations in the CFTR gene may result in a defective protein processing that leads to changes in function and regulation of this chloride channel. Despite of the expression of CFTR in the kidney, patients with CF do not present major renal dysfunction, but it is known that both the urinary excretion of proteins and renal capacity to concentrate and dilute urine are altered in these patients. CFTR mRNA is expressed in all nephron segments of rat and human, and this abundance is more prominent in renal cortex and outer medulla renal areas. CFTR protein was detected in apical surface of both proximal and distal tubules of rat kidney but not in the outer medullary collecting ducts. Studies have demonstrated that CFTR does not only transport Cl^- but also ATP. ATP transport by CFTR could be involved in the control of other ion transporters such as Na^+ (ENaC) and K^+ (renal outer medullary potassium) channels, especially in TAL and CCD. In the kidney, CFTR also might be

involved in the endocytosis of low-molecular-weight proteins by proximal tubules. This review is focused on the CFTR function and structure, its role in the renal physiology, and its modulation by hormones involved in the control of extracellular fluid volume.

Keywords CFTR · Kidney · Nephron · TNR-CFTR · Chloride channel · ENaC · ROMK

Introduction

In mammals, one of the major roles of the kidneys is the maintenance of the extracellular sodium chloride (NaCl) concentration that regulates the extracellular fluid volume and blood pressure (Morales et al. 2000). Sodium and chloride are reabsorbed along the nephron, reaching over 99% of the filtered load under low salt diets. Chloride, the predominant anion in the glomerular ultrafiltrate, is reabsorbed along the nephron either by trans- or paracellular pathways (Brenner and Rector 1991). Transcellular transport of Cl^- involves several membrane proteins, including the channels (Morales et al. 2000).

Numerous chloride channels have been discovered in a variety of animal and plant cells, and their modulation and involvement in physiological processes are widely described in the literature. Some of these channels have been cloned, and mutations in their genes are associated with genetic diseases (Jentsch 1994; Lehmann-Horn and Jurkat-Rott 1999; Sasaki et al. 1994). Between the genetic diseases related to chloride channels dysfunction, we highlight cystic fibrosis (CF), a common lethal autosomal recessive disorder, caused by mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene, which encodes a multifunctional integral membrane protein, a

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chloride channel (member of ABC transporter family) expressed in a variety of epithelia, including the renal tubules (Riordan 1993).

What does it mean to be a member of the ABC family?

All ABC transporters members consist of integral membrane proteins that share unique topological characteristics: two membrane-spanning domains (MSD) and two nucleotide-binding domains (NBD; Higgins and Linton 2004; Holland and Blight 1999). Nearly all the members of the ABC superfamily perform active transport of substrates against cellular concentration gradients, utilizing ATP hydrolysis as the source of free energy (Chen and Hwang 2008).

Based on phylogenetic analysis, the human ABC superfamily has been classified into seven subfamilies (ABC-A to ABC-G; Chen and Hwang 2008). Mutations in 17 of the 48 human ABC family proteins members are linked to genetic diseases. These include Tangier disease (mutations in ABC-A1 transporter; Kolovou et al. 2006), Stargardt disease (mutation in ABC-A4 transporter; Koenekoop 2003), Dubin–Johnson syndrome (mutation in ABC-C2 transporter; Kubitz et al. 2005), familial persistent hyperinsulinemic hypoglycemia of infancy (mutation in ABC-C8 transporter; Thomas et al. 1995), and cystic fibrosis (mutation in ABC-C7; Riordan 2005).

The ABC proteins have a wide tissue distribution (Deeley and Cole 2006), and the substrates for different ABC proteins cover a broad spectrum of substances, including sugars, amino acids, drugs, polysaccharides, proteins, and ions (Chen and Hwang 2008). CFTR is known to be a chloride transporter expressed in different organs and different epithelia.

The core domains (two MSDs and two NBDs) of eukaryotic ABC transporters are usually encoded by a single gene (Chen and Hwang 2008). Among different members of the ABC superfamily, the MSDs are highly divergent in both amino acid sequence and topology, whereas the NBDs share significant sequence and structural homology (Chen and Hwang 2008). This is perhaps not surprising since the MSDs likely contain substrate binding sites that must be different in different transporters (Chen and Hwang 2008).

Like other ATP-binding proteins, the ABC transporters NBDs contain the characteristic Walker A (GxxGxGKS/T, x represents any amino acid) and Walker B (hhhhD, h represents a hydrophobic amino acid) nucleotide-binding motifs sequences (Walker et al. 1982). The signature sequence (LSGGQ), located in NBD, is unique in ABC superfamily, but the function of this sequence remains unclear despite the speculation that it serves to transduce signals from ATP binding/hydrolysis event to lead the

conformational changes in other domains of the ABC members during a transport cycle (Ames and Lecar 1992).

Although some early reports show that the NBDs are crystallized as a monomer (Karpowich et al. 2001), it is now generally accepted that there is a dynamic process for association (dimerization) and dissociation of the two NBDs from the same transporter, required for the protein function. Once a NBD dimer is formed, the two ATP-binding sites are buried at the dimer interface (Chen and Hwang 2008). The bound nucleotides as well as many amino acid residues from each NBD participating in nucleotide interactions is intimately involved in forming a stable dimer (Chen and Hwang 2008). Once the NBDs dimerize, the ATP-binding pocket is formed, and it is composed of the Walker A and B motifs from one NBD and the signature motif from the partner NBD. ATP hydrolysis by the dimers provides the energy to break the stable dimer formation (Ludewig et al. 1996; Moody et al. 2002). Consequently, during the normal ATP hydrolysis cycle, the NBD dimer is short-lived. This speedy dissociation of NBD dimers is an essential element for an efficient transport cycle (Chen and Hwang 2008). In this way, ATP-binding-triggered NBD dimerization may serve as the power stroke to promote mechanical structure changes in the protein. In addition, hydrolysis of the bound ATP drives the dissociation of the NBD dimer and subsequent release of the hydrolytic products, ADP and Pi, completing the transport cycle (Higgins and Linton 2004; Moody and Thomas 2005).

Although the exact stoichiometry between ATP hydrolysis cycle and transport cycle remains debatable for ABC transporters containing two hydrolysis-competent sites, in many of the ABC proteins (notably in ABC-C subfamily—including CFTR), the NH₂-terminal NBD (NBD1) lacks the catalytic glutamate adjacent to the Walker B motif. Thus, hydrolysis at one ATP-binding pocket has to be able to sustain the transport function for members in the ABCC subfamily.

Structure and function of CFTR

The CFTR is one of the few members of the ABC proteins that does not function as an active transporter and it works as chloride channel (Bear et al. 1992). Sulfonylurea receptor (SUR or ABC-C8) is another example, and like CFTR, it does not function as an active transporter.

The CFTR gene is localized in human chromosome 7, and its protein consists of 1,480 amino acids (Knowlton et al. 1985; Morales et al. 1999). Mutations of this transporter lead to a CF that is a lethal autosomal recessive disorder in which abnormal regulation of epithelial Cl[−] channels is associated with the pathophysiology of the disease (Morales et al. 1999; Riordan et al. 1989; Rommens et al.

1989). The hallmarks of CF include thick and dehydrated airway mucus, pancreatic insufficiency, bile duct obstruction, infertility in males, reduced fertility in females, high sweat Cl^- , intestinal obstruction, nasal polyp formation, and chronic sinusitis (Collins 1992).

The CFTR structure is composed of intracellular N-terminus followed by six transmembrane-spanning domains (TMD1) followed by a first nucleotide-binding domain (NBD1) containing Walker A and B consensus sequences that bind ATP (Morales et al. 1999; Collins et al. 1990). Flanking this site, a large regulatory domain (R) [rich in cAMP-dependent kinase (PKA) phosphorylation sites] is followed by a second set of six transmembrane-spanning domains (TMD2) and a second NBD2 (Fig. 1).

There is strong evidence that CFTR's two NBDs form a head-to-tail dimer similar to those found in other ABC transporters discussed before (Vergani et al. 2005). The two ATP-binding pockets (ABP) for CFTR are defined as follows: ABP1, formed by Walker A and B motifs of NBD1 and the signature sequence of NBD2; ABP2, formed by Walker A and B motifs of NBD2 and the signature sequence of NBD1. The amino acids sequences of CFTR's two NBDs show significant differences even in those conserved motifs (Chen and Hwang 2008). For example, the glutamate residue adjacent to the Walker B motif, found in most ABC members, is replaced by a serine in NBD1. A histidine residue that has been shown to play an important role in ATP hydrolysis in other ABC proteins (Zaitseva et al.

2005) is also replaced by a serine in NBD1. In addition, the signature sequence in CFTR's NBD2 is degenerated (LSHGH instead of LSGGQ). This structural asymmetry of two NBDs in CFTR likely accounts for the observation that only ABP2, but not ABP1, hydrolyses ATP (Aleksandrov et al. 2002; Basso et al. 2003; Stratford et al. 2007).

Phosphorylation of many consensus serine residues in R domain is prerequisite for CFTR function. Not only phosphorylation of consensus sites by PKA regulates CFTR activity; some findings suggest regulation of its activity also by protein kinase C (Chen and Hwang 2008).

Once ATP binds to homologous nucleotide-interacting motifs in the two NBDs; these domains are thought to approach each other closely, sandwiching two ATPs in the NBD1–NBD2 interface (Gadsby et al. 2006). Upon this intramolecular heterodimer-like interaction, a signal would be transmitted through cytoplasmic-linking domains to open the gate in the transmembrane domain (Gadsby et al. 2006). That channel-opening signal would be sustained until hydrolysis of one of the ATPs leads to disruption of the NBD1–NBD2 interface and separation of the NBDs (Gadsby et al. 2006). Loss of the signal allows the channel gate to close, terminating anion flow until ATP again binds to the NBDs (Gadsby et al. 2006).

CFTR function is not only important for the chloride transport through its structure, but this channel can interact with other transporters inhibiting or increasing their ion transportation, and this fact could be important for

Fig. 1 Schematic view of CFTR protein into the plasma membrane. *N* N-terminus domain, *C* carboxy-terminus domain, *TMD1* transmembrane-spanning domain 1, *TMD2* transmembrane-spanning domain 2, *NBD1* nucleotide-binding domain 1, *NBD2* nucleotide-binding domain 2, *R DOMAIN* regulatory domain

epithelia involved in an intense transport of ions and fluid such as the ones found in lung and kidneys. For example, CFTR function leads to a stimulation of outward rectifying chloride channels—ORCC (Schwiebert et al. 1998; Fulmer et al. 1995) and an inhibition of epithelial sodium channels—ENaC (Kunzelmann et al. 2001).

Renal epithelia have an enormous amount of different membrane transporters, and their expression differ along different nephron segments. Once CFTR is abundantly expressed in kidneys (Souza-Menezes et al. 2008; de Andrade Pinto et al. 2007a; Morales et al. 2001; Morales et al. 1996, 2000), is there an important function for this transporter in renal physiology? Is there an interaction of CFTR with other transporters expressed in renal epithelia?

CFTR and kidney

Since the discovery of the gene encoding CFTR, an impressive number of studies have been performed to elucidate the role of this protein in the organs affected by the CF (Barriere et al. 2004). All these studies have converged toward the conclusion that CFTR is a small linear Cl^- channel regulated by cAMP and that the ΔF508 mutation (present in 70% of the CF patients) is associated with a loss of this cAMP sensitivity. However, although the vital role of CFTR in secretory epithelia is now widely accepted, its role in reabsorbing epithelia, that includes the kidney, remains not completely understood (Wang 1999).

It is well known that CFTR is abundantly expressed in the kidney. CFTR mRNA is detected in all nephron segments of rat and human, and this abundance is more prominent in renal cortex and outer medulla renal areas comparing with inner medulla (Morales et al. 1996). In agreement with mRNA finds, CFTR protein was detected, by immunostaining, at the apical surface of both proximal and distal tubules of rat kidney but not in the outer medullary collecting ducts (Crawford et al. 1991). Studies in mouse kidney (Jouret et al. 2007) revealed that CFTR is mainly expressed in the apical area of proximal tubular (PT) cells (pars recta, S3 segment; Jouret and Devuyst 2008) with a subcellular distribution compatible with endosomes, as shown before for ClC-5 transporter and vacuolar H^+ -ATPase (V-ATPase) in Rab5a (a common component of the apical and basolateral endocytic machinery in polarized epithelial cells) enriched fractions (Jouret and Devuyst 2008). In the human kidney, CFTR protein expression was detected in the PT, thin limbs of Henle's loop, distal tubules, and collecting ducts (Morales et al. 1996; Crawford et al. 1991; Devuyst et al. 1996). CFTR is also expressed in the branching ureteric bud during early nephrogenesis (Devuyst et al. 1996). Besides its location in the plasma membrane, CFTR is located in intracellular organelles along the endocytic and secretory pathways in PT

where it might act as a pH regulator by importing Cl^- into endocytic vesicles equilibrating H^+ accumulation caused by de transport via H^+ -ATPase (Bradbury 1999). For this reason, CFTR, a well-known regulator of other membrane transporters, could also work in kidney as a regulator of intracellular pathways.

CFTR and proximal tubule endocytosis of low-molecular-weight proteins

The distribution of CFTR in the apical endosomes of mouse PT cells (Jouret et al. 2007) revealed its possible involvement in renal endocytosis. This hypothesis was recently supported by using CFTR KO mouse models to characterize the role of CFTR in the kidney (Jouret and Devuyst 2008). Plasma and urine analyses revealed that baseline renal function was normal in *Cftr*^{-/-} mice (Jouret and Devuyst 2008). However, the urinary excretion of low-molecular-weight (LMW) proteins, such as the Clara cell secretory protein 16 (CC16, protein of 16 kDa; which leaks from the respiratory tract, and it is known to be filtrated by glomeruli) was significantly increased in *Cftr*^{-/-} mice compared to controls, reflecting the possible defect in proximal tubule cell apical endocytosis (Jouret and Devuyst 2008). This was supported by the demonstration of a significant decrease in renal uptake of radiolabeled ¹²⁵I- β_2 -microglobulin and aminoglycosides in *Cftr*^{-/-} mice compared to wild-type mice, both known to be endocytosed by proximal tubules (Jouret et al. 2007).

The endocytic uptake of aminoglycosides and LMW proteins, like CC16 and β_2 -microglobulin, is mediated by the multiligand receptors, megalin and cubilin (Christensen and Birn 2002). Cubilin is a highly conserved membrane glycoprotein with little structural homology to other well-known endocytic receptors, and it is characterized by the absence of a transmembrane domain (Birn and Christensen 2006). High-affinity binding of purified megalin to cubilin N-terminal region has been shown in vitro, suggesting that megalin participates not only in the endocytosis, intracellular trafficking of cubilin but also in cubilin anchoring in plasma membranes (Moestrup et al. 1998). Interestingly, there was a selective decrease of cubilin expression in the straight (S3) segment of PT of *Cftr*^{-/-} mice and an increase of urinary excretion of cubilin ligands such as transferrin and CC16 (Jouret et al. 2007). Further investigations demonstrated that the lack of CFTR in the kidney was not associated with changes in the biosynthesis of cubilin but, rather, with a significant increase in excretion of cubilin in urine of *Cftr*^{-/-} mice (Jouret and Devuyst 2008). No significant changes in kidney and urine abundance of megalin were observed (Jouret and Devuyst 2008). Taken together, these data suggest that the lack of CFTR in renal

PT cells could induce instability of cubilin at the brush border, leading to its accelerated shedding into urine (Jouret and Devuyst 2008). Although the entire process remains unclear, at this moment, we cannot reject the possibility that CFTR can work together with other proteins in the acidification of endosomal vesicles in the PT cells. Disturbance in this acidification mechanism is already well reported, causing impaired endocytosis of LMW protein in PT cells as described in Dent's disease where mutation in CIC-5 transporter leads to high endosomal pH and consequently LMW proteinuria (Jentsch 2008; Souza-Menezes et al. 2007).

CFTR and regulation of ROMK channel activity

In the kidney, the renal outer medullary potassium (ROMK) channel plays an important role in K^+ recycling in the lumen of thick ascending limbs of Henle's loop (TAL) and K^+ secretion in the cortical collecting duct (CCD) by its expression in luminal membrane of both nephron segments (Wang et al. 1997). Potassium recycling across the apical membrane of the TAL is important for NaCl reabsorption that leads to concentration of solutes in the renal medulla (Greger 1985; Hebert and Andreoli 1984). Three important renal phenomena are connected with the K^+ recycling across the apical membrane of TAL (Giebisch 1998). First, K^+ recycling hyperpolarizes the cell membrane potential and consequently provides the driving force for Cl^- diffusion across the basolateral membrane. Second, K^+ recycling is essential for the lumen-positive potential, which is the driving force for the transepithelial Na^+ reabsorption. Third, K^+ recycling provides an adequate supply of K^+ for the Na–K–Cl cotransporter. Mutations in ROMK channel are associated with Barter's syndrome where the capacity of urine and renal medulla concentration is dismissed.

In the CCD, the K^+ secretion takes place by K^+ entering the cell across the basolateral membrane via Na–K–ATPase and then secreting into the lumen through the apical K^+ channels. It is believed that the ROMK channel provides the major route for K^+ movement across the apical membrane of the CCD (Wang 1999).

Previous studies have demonstrated that SUR inhibition by glibenclamide inhibits ROMK2 activity probably by increasing local ATP concentration or by direct interaction between SUR and ROMK2 (Inagaki et al. 1995). Consequently, an accessory protein is required for the complete activity of ROMK2. The SUR1 and SUR2 (ABC family members), involved in the interaction with ROMK2, are not expressed in the kidney (Aguilar-Bryan et al. 1998). Since CFTR (Fuller and Benos 1992) expression is abundant in the renal epithelia, several groups have explored the

possibility that CFTR may couple to the ROMK channels to form the functional renal low-conductance ATP-sensitive K^+ channel. A previous work (McNicholas et al. 1996) has reported that when CFTR is coexpressed together with ROMK2, application of sulfonylurea agents, such as glibenclamide, blocks the activity of ROMK2 channel. The studies carried out by the same group have further suggested that a functional CFTR–NBD1 is required for CFTR–ROMK2 interaction (McNicholas et al. 1997). The observation that coupling the ROMK channel with CFTR is essential for restoring the response to sulfonylurea agents has also been reported (Ruknudin et al. 1998). In addition, using voltage clamp technique in *Xenopus* oocytes to study the ATP regulation of ROMK1, it was found that millimolar concentrations of Mg-ATP had no significant effect on channel activity. However, when ROMK1 was coexpressed with CFTR, the K^+ channel became sensitive to ATP (Ruknudin et al. 1998), suggesting that CFTR may be required to increase this sensitivity. These data suggest that CFTR could play an important role in the kidney and other organs where ROMK channels are expressed.

CFTR and regulation of ENaC activity

A large body of evidence indicates that CFTR is a cAMP-regulated Cl^- channel as well as a conductance regulator, which is colocalized with ENaC in airway, colonic, and other epithelial tissues (Kunzelmann et al. 2000; Schwiebert et al. 1999). It has been proposed that when cAMP activates CFTR, ENaC is inhibited, and this inhibitory effect of CFTR has been used as an argument to explain the pathophysiology of cystic fibrosis in airway epithelia (Mall et al. 1998; Stutts et al. 1995). In *Xenopus* oocytes expressing ENaC, coexpression of CFTR reduces the ENaC-mediated Na^+ current (Briel et al. 1998), an effect that might be attributed to a direct effect of CFTR on ENaC activity. In addition, Kunzelmann and collaborators (Konig et al. 2001; Kunzelmann 2003) have shown that the inhibitory effect of CFTR can be mimicked by coexpression of other anion channels (CIC-0 and CIC-2) or treatment with amphotericin B, and this effect can be explained by an increase of Cl^- currents or an intracellular Cl^- ($[Cl^-]_i$). Some authors have been shown that an increase in $[Cl^-]_i$ inhibits ENaC activity in FL-MDCK cells (MDCK cells that had been transfected with rat ENaC subunits containing the FLAG epitope in their extracellular loops; Morris and Schafer 2002); however, this effect cannot explain the effect of Cl^- secretion on Na^+ absorption with cAMP treatment because stimulation of Cl^- secretion by cAMP results in a fall rather than a rise in $[Cl^-]_i$ (Xie and Schafer 2004). Moreover, in the *Xenopus* oocyte expression system, CFTR activation inhibits ENaC at a

continuous holding potential at which CFTR mediates inward currents corresponding to Cl^- efflux (Konstas et al. 2003). Under these conditions, activation of CFTR will not result in an increase in $[\text{Cl}^-]_i$. Therefore, a change in $[\text{Cl}^-]_i$ is unlikely to fully explain the observed reciprocal regulation of ENaC and CFTR.

Additionally, extracellular ATP has been shown to attenuate amiloride-sensitive Na^+ absorption in a variety of tissues, including airways (Devor and Pilewski 1999; Iwase et al. 1997; Inglis et al. 1999) and renal epithelia (Inglis et al. 1999; McCoy et al. 1999; Cuffe et al. 2000). Similarly, activation of CFTR not only inhibits ENaC but also increases Cl^- secretion in native epithelial tissues (Stutts et al. 1995; Kunzelmann and Schreiber 1999). The influence of CFTR on other transporters activity could be explained by the possible release of ATP through CFTR, and this phenomenon may happen along the nephron segments where both ENaC and CFTR are coexpressed (Stutts et al. 1995; Schwiebert et al. 1995; Sugita et al. 1998). In a previous work, the endogenous release of ATP by the FL-MDCK monolayers into the apical but not in basolateral solution under basal conditions [consistent with measurements in cortical CD; Schwiebert and Kishore 2001] was showed. This release was augmented more than threefold by cAMP treatment (Xie and Schafer 2008).

All this together showed a possible role of CFTR in the regulation of ENaC, suggesting a possible interaction between these two channels in renal epithelia where they are coexpressed.

CFTR expression regulation by hormones involved in extracellular volume regulation

Hormonal modulation of ions transporters in the kidney and intestinal epithelia is well known to be important in extracellular volume regulation (Novaira et al. 2006). Classical extracellular volume-regulating hormones such as aldosterone and atrial natriuretic peptide (ANP) induce ion transport not only in the kidney but also in the distal colon (Novaira et al. 2006; Grotjohann et al. 1999; Coric et al. 2004). These hormones have been shown to regulate the expression of chloride channels in the renal tissues (Morales et al. 2001; Jentsch et al. 2002; Ornellas et al. 2002) and distal part of the intestine, particularly in the proximal and distal colon (Novaira et al. 2006; Schroeder et al. 2000; Estevez et al. 2001).

We previously showed that arginine vasopressin (AVP; an extracellular tonicity involved hormone; Morales et al. 2001) was able to modulate CFTR expression in kidney. In this work, it was showed that AVP was able to increase the expression of CFTR mRNA expression in rat renal tissues and in MDCK1 cell line. Also, water-deprived rats

presented higher expression of CFTR in the kidney. In vitro, AVP but not high osmolality cell medium was able to increase CFTR expression in MDCK1 cell line (Morales et al. 2001), suggesting the direct effect of AVP in the kidney.

In the same line of investigation, we explore the role of the ANP in the modulation of CFTR, and it was observed that ANP was able to increase CFTR mRNA expression in both rat proximal colon and in human intestinal epithelial cell line (CaCo-2; Novaira et al. 2006).

Although thyroid hormones (THs) generally are not regarded as classical extracellular fluid volume regulators, specific receptors for T3 are present in kidney epithelium, suggesting the potential for a direct action of this hormone (Shahrara et al. 1999). Indeed, the thyroid hormones, thyroxine (T4) and triiodothyronine (T3), affect renal morphology as well as the activity of ion transporters via alteration of their function and/or expression along the nephron (de Andrade Pinto et al. 2007b). Thus, depending on their plasma levels, THs can regulate renal NaCl excretion (Azuma et al. 1996). In hypothyroidism, both decreases in glomerular filtration rate and in urine concentrating capacity are observed (Katz et al. 1975). Recently, we showed that hypothyroid rats presented low expressions of CFTR and its renal alternative splicing, TNR-CFTR (de Andrade Pinto et al. 2007b). In the same work, the authors showed that rats subjected to hyperthyroidism presented higher expression of CFTR and TNR-CFTR (de Andrade Pinto et al. 2007a).

The modulation of CFTR by AVP, ANP, and THs suggests the participation of this channel in the mechanism of ion transport related to extracellular volume regulation.

Kidney and cystic fibrosis

The kidney of CF patients does not display major changes in renal function (Wang 1999). This absence of an obvious renal phenotype is a priori paradoxal because CFTR protein is expressed in kidneys (de Andrade Pinto et al. 2007a; Morales et al. 1996, 2000, 2001; Souza-Menezes et al. 2008), and it is involved in the regulation of many reabsorption and secretion mechanisms, as discussed before. In the absence of functional CFTR in the kidney, as observed for CF, other Cl^- channel can compensate the CFTR function, but it does not seem to happens because CFTR is not only involved with Cl^- transport but also with regulation of other channels by apparent mechanisms other than chloride transport.

Morales et al. (1996) showed that CFTR pre-mRNA in renal medulla can form a splice variant called TNR-CFTR that is highly abundant in renal medulla comparing to wild-type CFTR expression. At a genomic level, TNR-CFTR

mRNA lacks 145 bp corresponding to segments of exons 13 and 14, which encode the last part (7%) of the regulatory (R) domain of CFTR molecule. This deletion causes a shift in the reading frame, leading to creation of premature termination codon in exon 14. This alternative structure contains only the first transmembrane domain, the first NBD, and the regulatory domain and it was named TNR-CFTR (Morales et al. 1996).

In the kidney, TNR-CFTR is expressed in a tissue-specific manner primarily in renal medulla and conserves the functional characteristics of wild-type CFTR (Morales et al. 1996). This CFTR form is also found in human kidney medulla, but not in human kidney cortex (Morales et al. 1996). In 2007, de Andrade Pinto et al. showed the modulation of CFTR and TNR-CFTR mRNA and protein expressions by thyroid hormone (de Andrade Pinto et al. 2007a). This work suggests that the same factors that are able to regulate CFTR expression are also able to modulate TNR-CFTR expression. In 1996, Morales et al. (1996) proposed that the TNR-CFTR is an alternative splicing of wild-type CFTR pre-mRNA by homology of cDNA molecules forming TNR-CFTR and CFTR, and this hypothesis was supported by Souza-Menezes et al. (2008) showing that small nuclear RNAs U11 and U12 (small nuclear RNAs present in the composition of spliceosomes that participate in alternative splicing mechanisms) probably are involved in the production of TNR-CFTR mRNA using a primary transcript from CFTR gene. In this same work, the authors showed that the pattern of U11 and U12 RNA expression in rat kidneys is similar to that observed for TNR-CFTR in rat kidneys, i.e., high levels of expression in renal medulla and low levels of expression in renal cortex (Souza-Menezes et al. 2008). In addition, Souza-Menezes et al. showed that when U11 and U12 RNA expressions were suppressed in proximal tubule cell line, it was possible to observe a significant reduction in TNR-CFTR mRNA expression (Souza-Menezes et al. 2008).

The functional significance of TNR-CFTR remains to be elucidated, but Morales et al. (1996) raised two hypothesis. Since several related members of ATP-binding cassette family, to which CFTR belongs, function in intracellular organelles as half molecules with TMD1-NBD1 (Mosser et al. 1993; Valle and Gartner 1993), there is a possibility that TNR-CFTR may have a basic function in intracellular organelles rather than in plasma membrane. Another hypothesis is based on the fact that this unusual form of mRNA processing occurs only in renal medulla, a portion of kidney with high osmolality. Once this half molecule of CFTR works as a chloride channel and also regulates other conductances (such as ORCC; Morales et al. 1996), its high expression, especially in renal medulla, could protect the kidney from functional defects observed in cystic fibrosis.

Conclusions

Despite that mutations in CFTR gene have been associated with some renal disturbances (such as endocytosis of low-molecular-weight proteins by the proximal tubules), no expressive changes in renal function could be observed in CF patients or CFTR knockout mice. This is a controversial observation because CFTR is expressed along different nephron segments with possible role in the modulation of different epithelial conductances, such as channels for sodium (ENaCs), potassium (ROMK2), and chloride (ORCCs). So far, there are no conclusive data that justify this controversial findings, but one possibility is the replacement of CFTR function by other transporters or proteins where CFTR is absent or not functioning correctly. One candidate for this is TNR-CFTR, an alternative splice of CFTR, which, in vitro, functions as wild-type CFTR, although further studies are crucial to understand the CFTR role in renal physiology.

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