

# Multiple Functions of Na,K-ATPase in Epithelial Cells

Sigrid A. Rajasekaran, Sonali P. Barwe, and Ayyappan K. Rajasekaran

The Na,K-adenosine triphosphatase (ATPase), or sodium pump, has been well studied for its role in the regulation of ion homeostasis in mammalian cells. Recent studies suggest that Na,K-ATPase might have multiple functions such as a role in the regulation of tight junction structure and function, induction of polarity, regulation of actin dynamics, control of cell movement, and cell signaling. These functions appear to be modulated by Na,K-ATPase enzyme activity as well as protein–protein interactions of the  $\alpha$  and  $\beta$  subunits. In this review we attempt to differentiate functions associated with enzyme activity and subunit interactions. In addition, the consequence of impaired Na,K-ATPase function or reduced subunit expression levels in kidney diseases such as cancer, tubulointerstitial fibrosis, and ischemic nephropathy are discussed.

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Na,K-adenosine triphosphatase (ATPase), also known as the sodium pump, is an ubiquitous plasma membrane protein complex extensively studied for its function in ion homeostasis. It couples the hydrolysis of 1 molecule of ATP to the translocation of 2 K<sup>+</sup> into and 3 Na<sup>+</sup> out of the cell against their electrochemical gradient. Thus, the Na,K-ATPase is crucial for maintaining the normally low Na<sup>+</sup> and high K<sup>+</sup> concentrations of eukaryotic cells (for comprehensive reviews see Kaplan<sup>1</sup> and Lingrel and Kuntzweiler<sup>2</sup>). The enzyme complex consists of ubiquitous and essential  $\alpha$  and  $\beta$  subunits<sup>3,4</sup> and an optional  $\gamma$  subunit whose expression is restricted to certain tissues.<sup>5</sup> The  $\alpha$  subunit is a polytopic membrane protein with the catalytic activity of the enzyme and the binding sites for Na<sup>+</sup>, K<sup>+</sup>, and ATP.<sup>4</sup> The  $\beta$  subunit has been shown to be important for the efficient translation of the  $\alpha$  subunit on the endoplasmic reticulum,<sup>6</sup> the correct folding and membrane insertion of the  $\alpha$  subunit, and the expression of the enzyme on the plasma membrane.<sup>7-9</sup> The  $\beta$  subunit, as well as the  $\gamma$  subunit, also has been suggested to be a modulator of the pump function.<sup>8,10,11</sup> In mammals, at least 4 isoforms of the  $\alpha$  subunit<sup>12-14</sup> and 3 isoforms of the  $\beta$  subunit<sup>2,15</sup> have been described. These isoforms are expressed in a tissue-specific manner and their functional prop-

erties differ. The isoforms predominantly expressed in kidney are  $\alpha_1$  and  $\beta_1$ .<sup>15,16</sup> The  $\alpha$  and  $\beta$  subunits referred to in this review are the  $\alpha_1$  and  $\beta_1$  isoforms of Na,K-ATPase.

The low intracellular Na<sup>+</sup> concentration maintained by the pump is crucial for the efficient functioning of various Na<sup>+</sup>-coupled transport systems and the sodium gradient provides the primary energy for uptake and extrusion of a wide variety of solutes. Furthermore, in epithelia the transepithelial Na<sup>+</sup> gradient generated by the basolaterally localized Na,K-ATPase is vital for the directional transport of solutes across the epithelial cell layer (vectorial transport). Although the transport function of the Na,K-ATPase has been investigated extensively, recent evidence suggests that the sodium pump might be a multifunctional protein involved in the induction of epithelial cell tight junctions and polarity,<sup>17-20</sup> cell motility,<sup>17</sup> cell signaling,<sup>21</sup> actin dynamics,<sup>17,18,20</sup> and cancer.<sup>22-25</sup> This review summarizes some of the recent work on these novel functions associated with Na,K-ATPase in kidney epithelial cells.

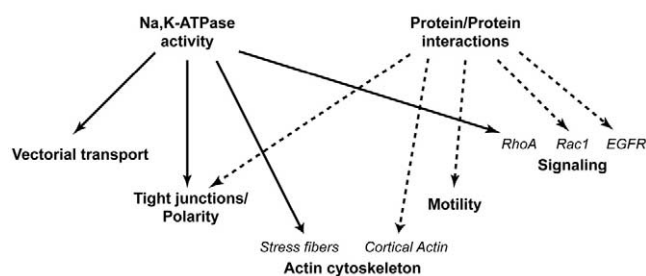
## Na,K-ATPase and the Establishment of Tight Junctions and Epithelial Polarity

Epithelial cells line the inner and outer surfaces of the body. Their functions include the formation of a barrier between 2 biological compartments and the control of the exchange of molecules between these compartments via regulated secretion and absorption. To serve these functions, epithelial cells have their plasma membrane divided into functionally and

From the Department of Pathology and Laboratory Medicine, UCLA David Geffen School of Medicine, Los Angeles, CA.

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Address reprint requests to Ayyappan K. Rajasekaran, PhD, Department of Pathology and Laboratory Medicine, Room 13-344 CHS, University of California, Los Angeles, CA 90095. E-mail: arajasekaran@mednet.ucla.edu



**Figure 1** Schematic presentation of the multiple functions of Na,K-ATPase in epithelial cells. Functions that are mediated by Na,K-ATPase activity are indicated by solid arrows. Dashed arrows indicate functions of Na,K-ATPase that possibly involve protein–protein interactions of Na,K-ATPase  $\alpha$  or  $\beta$  subunit with other cellular proteins.

biochemically distinct apical and basolateral domains separated by tight junctions. This unique structural phenotype also is referred to as a *polarized phenotype* or *well-differentiated phenotype*.<sup>26–28</sup>

Tight junctions are multiprotein complexes located at the boundary of apical and basolateral plasma membrane domains. Characterized by the close apposition of contiguous plasma membranes, they prevent the mixing of molecules between the apical and basolateral domains and regulate the passage of molecules across the paracellular space. Despite tight junctions being crucial for the epithelial phenotype and functioning of epithelia, the molecular mechanisms that regulate the formation of these specialized membrane microdomains are understood poorly (for comprehensive reviews see Anderson and Van Itallie,<sup>29</sup> Tsukita et al,<sup>30</sup> and Schneeberger and Lynch<sup>31</sup>). Although previous studies implicated the function of E-cadherin, a cell-adhesion molecule, in the formation and maintenance of junctional complexes and the epithelial phenotype,<sup>32</sup> studies from our laboratory revealed a crucial role for Na,K-ATPase in maintaining the integrity and functions of tight junctions.<sup>17,18,23</sup> Inhibition of Na,K-ATPase activity by ouabain (a specific inhibitor of Na,K-ATPase  $\alpha$  subunit) or by  $K^+$  depletion in Madin-Darby canine kidney (MDCK) cells subjected to a  $Ca^{2+}$ -switch experiment (an assay to study the formation of junctional complexes), prevented the formation of tight junctions. Consequently, the lack of functional tight junctions in these cells also prevented the establishment of polarity. Because the  $Na^+$  ionophore gramicidin that increases intracellular  $Na^+$  concentrations mimicked the effect of Na,K-ATPase inhibition, it is likely that the intracellular ionic gradient maintained by the sodium pump is involved in the assembly of tight junctions and generation of polarity in epithelial cells (Fig. 1).<sup>18</sup>

## Na,K-ATPase as a Regulator of Tight Junction Permeability

Molecular mechanisms involved in the formation of tight junctions might be distinct from the mechanisms required to maintain tight junctions. For example, although E-cadherin function is critical for the formation of tight junctions,<sup>32</sup> its

function appears dispensable once the cells have established tight junctions.<sup>33</sup> However, Na,K-ATPase function appears to be involved in both the formation and maintenance of tight junctions. Inhibition of Na,K-ATPase function in polarized monolayers of human retinal pigment epithelial cells resulted in increased tight junction permeability to both ions and nonionic molecules. These permeability changes were accompanied by a reduction in tight junction membrane contact points (kissing points) at the apicolateral plasma membrane border without complete destruction of the tight junctions.<sup>20</sup> Similar results were obtained in HPAF cells, a well-differentiated pancreatic tumor cell line that forms tight junctions in culture. Interestingly, inhibition of Na,K-ATPase resulted in an increase in the permeability of ionic and nonionic molecules without altering the localization of tight junction proteins (our unpublished data). It is possible that in epithelial cells with established tight junctions, Na,K-ATPase function is necessary to maintain tight junction membrane contact points and thus the permeability of tight junctions.

Given the ubiquitous presence of Na,K-ATPase in mammalian cells and its fundamental role in cell functions, it is tempting to speculate that the sodium pump might have a conserved role in the regulation of tight junction structure and functions in epithelial cells. Indeed, Genova and Fehon<sup>34</sup> reported an essential role of Na,K-ATPase in the barrier function of septate junctions in *Drosophila*. Septate junctions in *Drosophila* generally are considered analogous to tight junctions in mammalian cells.<sup>35</sup> In *Drosophila*, both Na,K-ATPase  $\alpha$  subunit (*Atp $\alpha$* ) and Nervana 2 (*Nrv2*) (which encodes the  $\beta$  subunit of Na,K-ATPase) were identified as essential for the barrier function of the septate junction. Similarly, Paul et al<sup>36</sup> reported that mutations in *Atp $\alpha$*  and *Nrv2* disrupt the stable formation of septate junctions in *Drosophila*. These recent studies identified an essential function of Na,K-ATPase in establishing and maintaining the primary paracellular barrier in invertebrate as well as in mammalian epithelial cells.

## Na,K-ATPase and the Cortical Actin Cytoskeleton

The actin cytoskeleton plays an important role in the structure and functions of epithelial cells. The interaction of the actin cytoskeleton with cell adhesion molecules is thought to strengthen cell–cell contacts, providing a scaffolding platform for signaling molecules involved in epithelial polarization and to promote further the assembly of junctional complexes in epithelial cells including adherens junctions and tight junctions.<sup>37,38</sup> These junctions are associated with the cortical actin cytoskeleton also referred to as the *perijunctional actomyosin ring*. Earlier studies showed that tight junction structure and permeability are regulated by the perijunctional actomyosin ring,<sup>29,39,40</sup> and it has been proposed that contraction of perijunctional actin filaments and the resulting centrifugal traction on tight junction membrane regulates tight junction permeability.<sup>39</sup>

Inhibition of Na,K-ATPase pump function did not alter

visually the perijunctional actin ring in epithelial cells.<sup>18,20</sup> However, the  $\beta$  subunit itself appears to have a role in the organization and stabilization of this cortical actin ring in epithelial cells. In Moloney sarcoma virus–transformed (MSV)-MDCK cells that express highly reduced levels of both E-cadherin and the  $\beta$  subunit, repletion of E-cadherin alone did not establish a continuous cortical actin ring. However, expression of Na,K-ATPase  $\beta$  subunit in E-cadherin expressing MSV-MDCK cells resulted in the formation of a continuous submembranous cortical actin ring comparable with parental MDCK<sub>wt</sub> cells.<sup>17</sup> McNeill et al<sup>41</sup> showed that E-cadherin–mediated cell–cell contact is involved in establishing the cortical actin cytoskeleton. Our results showed that E-cadherin expression alone is not sufficient to stabilize this cortical actin cytoskeleton and that the  $\beta$  subunit expression also is required to establish a stable cortical actin cytoskeleton. In fact, we have shown that expression of the  $\beta$  subunit was associated with increased stabilization of E-cadherin on the plasma membrane as determined by Triton-X-100 solubility assay.<sup>17</sup>

How exactly expression of the  $\beta$  subunit results in stabilizing the cortical actin ring is not yet known. It is possible that the  $\beta$  subunit itself functions as a cell–cell adhesion molecule and acts as a scaffold for the recruitment of either actin-binding proteins or actin molecules to the subplasma membrane region. Consistent with this notion, MSV-MDCK cells expressing the  $\beta$  subunit alone formed cell aggregates in an *in vitro* cell–cell adhesion assay.<sup>17</sup> Alternatively, the  $\beta$  subunit via its interaction with the  $\alpha$  subunit that is known to associate with ankyrin, spectrin,<sup>42–44</sup> and cofilin<sup>45</sup> might recruit more actin and actin-binding proteins to the subplasma membrane region. Thus, protein–protein interactions between the Na,K-ATPase subunits and actin-binding proteins might be involved in recruiting actin and stabilizing the cortical actin ring at the plasma membrane. Because inhibition of Na,K-ATPase activity did not show a significant change in the organization of the cortical actin,<sup>18,20</sup> it is possible that protein–protein interactions of the Na,K-ATPase subunits might be a major factor contributing to the organization and stabilization of the cortical actin ring in epithelial cells (Fig. 1). Future identification of proteins interacting with the  $\alpha$  and  $\beta$  subunits should provide valuable information regarding the role of Na,K-ATPase subunits in actin organization.

## Na,K-ATPase in the Regulation of Stress Fibers

Stress fibers are dynamic actin filaments implicated in cell migration.<sup>46</sup> Although inhibition of Na,K-ATPase activity had no apparent effect on the organization of the cortical actin ring in MDCK and retinal pigment epithelial cells, the pump activity seems to be involved in the organization of stress fibers.<sup>18,20</sup> In Ca<sup>2+</sup> switch assays of MDCK cells, inhibition of the sodium pump prevented the formation of stress fiber bundles that appear transiently during epithelial polarization in control cells. In confluent monolayers of retinal pigment epithelial cells, stress fibers disappeared on inhibition of Na,K-ATPase activity. Consistent with a role in stress

fiber dynamics, Na,K-ATPase appears to be an upstream regulator of RhoA guanosine triphosphatase (GTPase). RhoA GTPase (a small GTP-binding protein) has been implicated in the regulation of actin stress fiber formation in fibroblasts and in epithelial cells.<sup>47,48</sup> In Na,K-ATPase activity–inhibited MDCK cells the failure to form stress fiber bundles was accompanied by reduced RhoA activity. Exogenous overexpression of wild-type RhoA GTPase bypassed the inhibitory effect of Na,K-ATPase on stress-fiber formation. These effects of Na,K-ATPase inhibition on RhoA activity seem to be specific for RhoA because neither levels nor activity of Rac1 (another member of the Rho family) were altered in Na,K-ATPase–inhibited MDCK cells.<sup>18</sup> The molecular mechanisms of how Na,K-ATPase is involved in the regulation of RhoA GTPase activity are not known. However, in MDCK cells treated with gramicidin, an inhibitory effect on RhoA activity was observed, indicating that intracellular ion homeostasis regulated by Na,K-ATPase function is involved in the regulation of stress fibers through RhoA (Fig. 1).<sup>18</sup>

Although less prominent, stress fibers projecting from the perijunctional actin ring interface at the cytoplasmic surface of tight-junction membrane contact points.<sup>49,50</sup> In Na,K-ATPase–inhibited cells reduced tight junction membrane contact points and increased tight junction permeability correlated with the reduced stress-fiber content. Consistent with these results, dominant-negative RhoA disrupted tight-junction structure and permeability functions,<sup>51</sup> indicating a role for stress fibers in the assembly and functions of tight junctions. It is tempting to speculate that tight-junction membrane contact points are in a dynamic equilibrium with stress fibers regulated by Na,K-ATPase and loss of or reduced Na,K-ATPase activity might lead to loss of stress fibers and increased tight-junction permeability.

In fibroblasts, stress fibers are more prominent than in polarized epithelial cells. Although in MDCK cells tremendous amounts of stress fibers appear during the early stages of polarization, tight polarized monolayers of MDCK cells show only small amounts of actin stress fibers. However, upon loss of the epithelial phenotype during epithelial-mesenchymal transition (EMT), a pathologic process involved in the progression of cancer, stress fibers reappear. MSV-MDCK cells have lost their epithelial phenotype and have abundant actin stress fibers. These cells are highly motile and express low levels of Na,K-ATPase  $\beta$  subunit.<sup>17</sup> Ectopic expression of Na,K-ATPase  $\beta$  subunit in these cells resulted in the loss of stress fibers. Interestingly, this stress-fiber loss was accompanied by an increase in Rac1 activity, whereas the activity of RhoA was not altered (our unpublished data). Expression of Na,K-ATPase  $\beta$  subunit resulted in increased  $\alpha$  subunit levels and increased enzyme activity.<sup>6,17</sup> However, inhibition of Na,K-ATPase activity in the  $\beta$  subunit expressing MSV-MDCK cells did not alter Rac1 activity (our unpublished data). It is tempting to speculate that Na,K-ATPase modulates RhoA and Rac1 by independent mechanisms. RhoA activity seems to be regulated by intracellular ion homeostasis whereas Rac1 activity might be modulated by protein–protein interactions (Fig. 1). Future research should provide more insights into how Na,K-ATPase is involved in the reg-

ulation of the Rho GTPase family members and actin dynamics in epithelial cells.

## Na,K-ATPase and Cell Motility

Cell motility is regulated by a balance between formation and disassembly of actin filaments, and members of the Rho family of small GTPases are known to control these actin dynamics. RhoA has been implicated in the formation of actin-filament bundles (stress fibers) and Rac1 induces the formation of lamellipodia and membrane ruffles.<sup>47,48</sup> Our studies revealed a role of Na,K-ATPase as an upstream regulator of RhoA and Rac1 activities (see earlier). Interestingly, expression of Na,K-ATPase  $\beta$  subunit in highly motile MSV-MDCK reduced their motility as determined in 2 independent assays: a wound assay and a Transwell motility assay.<sup>17</sup> This reduced motility correlated with increased levels of active Rac1 in these cells (our unpublished results). Previous studies have shown that phosphoinositide 3-kinase is involved in the activation of Rac1<sup>52,53</sup> and, interestingly, the regulatory subunit of phosphoinositide 3-kinase has been shown to bind to the Na,K-ATPase  $\alpha$  subunit.<sup>54</sup> Whether Na,K-ATPase-mediated inhibition of cell motility involves phosphoinositide 3-kinase-dependent activation of Rac1 still is to be determined.

## Na,K-ATPase and Cell Signaling

Epidermal growth factor receptor (EGFR), also called *ErbB1*, was the first identified member of a subfamily of tyrosine kinase receptors that includes ErbB2/Neu, ErbB3, and ErbB4. EGFR can be activated by various ligands such as EGF, transforming growth factor- $\alpha$  (TGF- $\alpha$ ), and heparin-binding EGF-like growth factor<sup>55</sup> that bind to the extracellular domain of EGFR. Ligand binding results in receptor dimerization and stimulation of the intrinsic tyrosine kinase activity, leading to receptor autophosphorylation and subsequent phosphorylation of numerous cellular substrates.<sup>55,56</sup> In addition, EGFR can be activated by ligand-independent mechanisms<sup>57,58</sup> known as transactivation solely achieved by intracellular events.<sup>59</sup> Recent studies have shown that binding of ouabain to Na,K-ATPase results in the transactivation of EGFR independent of changes in intracellular Na<sup>+</sup> and K<sup>+</sup> concentrations.<sup>60</sup> Transactivation of EGFR leads to activation of the Ras/MAPK signaling cascade and the accumulation of Na,K-ATPase  $\alpha$  subunit and EGFR in caveoli, a membrane microdomain involved in cell signaling in mammalian cells (Fig. 1).<sup>60,61</sup>

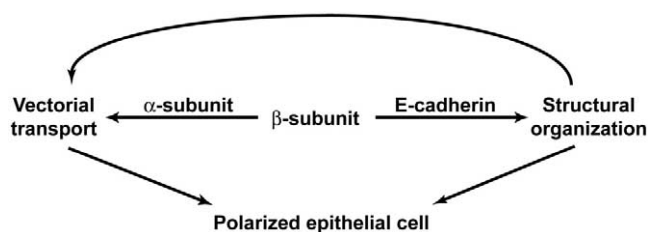
Interactions between ligands and receptors are central to communication between cells and tissues. However, the segregation of receptor and ligand is crucial for the fine regulation of receptor activation and subsequent signaling process in epithelial cells. In polarized kidney epithelial cells this segregation is accomplished by tight junctions in 2 ways: (1) tight junctions form a diffusion barrier between apical and basolateral plasma membranes and therefore prevent the considerable amounts of EGF present in the urine to activate EGFR localized to the basolateral membrane,<sup>24,62,63</sup> and (2)

tight junctions form a permeability barrier for luminal EGF to diffuse to the basolateral intercellular space. Because the function of Na,K-ATPase is crucial for the integrity of tight junctions, reduced Na,K-ATPase activity might lead to a non-polarized localization of EGFR, with EGFR having access to luminal EGF. Alternatively, leaky tight junctions might allow for EGF to permeate to the basolateral intercellular space and activate EGFR located to the basolateral membrane. Thus, Na,K-ATPase might be involved in the regulation of both ligand-independent and ligand-dependent activation of EGFR.

## E-Cadherin and Na,K-ATPase $\beta$ Subunit Function Synergistically in Epithelial Cells

E-cadherin is a cell-adhesion molecule that mediates homophilic Ca<sup>2+</sup>-dependent interactions and has been implicated in tissue development and development of epithelial polarity.<sup>64-66</sup> We have proposed earlier a functional synergism between E-cadherin and Na,K-ATPase  $\beta$  subunit in the induction of polarity based on the following observations.<sup>1</sup> In transformed MSV-MDCK cells that express low levels of E-cadherin and Na,K-ATPase  $\beta$  subunit, expression of E-cadherin alone is not sufficient to rescue the epithelial phenotype. However, expression of both E-cadherin and Na,K-ATPase  $\beta$  subunit was sufficient to induce tight junctions and epithelial polarity.<sup>2</sup> The association of E-cadherin with the actin cytoskeleton was stable only when the  $\beta$  subunit was expressed, too.<sup>3</sup> Individual expression of E-cadherin or the  $\beta$  subunit reduced cell motility of MSV-MDCK cells. However, only expression of both these proteins reduced the cell motility to levels comparable with MDCK<sub>wt</sub> cells.<sup>4</sup> Finally, expression of both the  $\beta$  subunit and E-cadherin abolished the invasiveness of MSV-MDCK cells in collagen invasion assays.<sup>17</sup>

Proper regulation of cell adhesion is essential during early development and during organogenesis and cell-cell adhesions are rearranged dynamically during tissue development. E-cadherin is expressed from the fertilized egg onward but the expression levels underlie constant up- and down-regulation during organogenesis.<sup>64-66</sup> The transcription factor Snail, a member of the Zinc finger family of proteins, seems to be a key regulator of E-cadherin levels. Snail has been shown to bind to E-boxes of the E-cadherin promoter and suppress E-cadherin expression.<sup>67-70</sup> Interestingly, Snail also suppresses the expression of Na,K-ATPase  $\beta$  subunit in carcinoma cells and ectopic expression of Snail in well-differentiated epithelial cell lines reduced the protein levels of both E-cadherin and  $\beta$  subunit. Furthermore, Na,K-ATPase  $\beta$  subunit levels correlated positively with E-cadherin levels and inversely with Snail levels in carcinoma cells.<sup>25</sup> Down-regulation of Na,K-ATPase  $\beta$  subunit and not the  $\alpha$  subunit with Snail in carcinoma cells further provides a molecular mechanism for the synergistic role of E-cadherin and the  $\beta$  subunit in maintaining the polarized phenotype of epithelial



**Figure 2** The central role of Na,K-ATPase  $\beta$  subunit in epithelial polarity and vectorial transport. The vectorial transport function of epithelial cells depends on both a functional  $\alpha$  subunit and a polarized phenotype. Na,K-ATPase  $\beta$  subunit may provide a molecular link between epithelial cell function and cell structure because of its dual roles in the synthesis and plasma membrane expression of the catalytic  $\alpha$  subunit and in establishing epithelial cell polarity together with E-cadherin.

cells. E-cadherin and Na,K-ATPase have been shown to be in the same complex,<sup>71</sup> however, a direct interaction of Na,K-ATPase subunits with E-cadherin has not been shown. Whether such interaction occurs and is critical for establishment of polarity in epithelial cells currently is under investigation.

We suggest that the  $\beta$  subunit of Na,K-ATPase plays a crucial role in the regulation of the well-differentiated phenotype of epithelial cells. Our hypothesis is that the  $\beta$  subunit of Na,K-ATPase functions as a molecular link between the structure and function of polarized epithelial cells. The  $\beta$  subunit is essential for the synthesis and plasma membrane expression of the  $\alpha$  subunit, which in turn is crucial for the vectorial transport function of epithelial cells. Vectorial transport is dependent on the structural organization (polarized phenotype) of epithelial cells, which appears to be regulated by both E-cadherin and the  $\beta$  subunit. Thus, the  $\beta$  subunit of Na,K-ATPase is a potential candidate molecule to link the structure and function of polarized epithelial cells (Fig. 2). With this central role in epithelial polarity it is conceivable that reduced Na,K-ATPase  $\beta$  subunit expression might be a key factor associated with loss of the polarized phenotype of epithelial cells.<sup>25,72</sup>

## Implications in Kidney Diseases

### Cancer

The Snail family of transcription factors has been implicated in EMT, the phenotypic conversion of epithelial cells to mesenchymal cells in the development of cancer. A direct correlation has been observed between Snail induction and the acquisition of metastatic properties such as increased motility and invasiveness in human tumor cell lines of different epithelial origin.<sup>67-70</sup> Studies from our laboratory showed that Na,K-ATPase  $\beta$  subunit levels are reduced in a variety of carcinoma cell lines and we showed that the transcription factor Snail is involved in the repression of the  $\beta$  subunit in these cell lines. The repression of the  $\beta$  subunit by Snail was specific to this subunit because the  $\alpha$  subunit levels were not affected significantly.<sup>25</sup> Interestingly, in clear-cell renal cell

carcinoma, an invasive form of kidney cancer, the  $\beta$  subunit levels were reduced drastically in tumor tissues as compared with autologous normal kidney tissue whereas the  $\alpha$  subunit levels did not show such a reduction.<sup>22</sup> These studies suggested that  $\alpha$ - and  $\beta$  subunit levels could be regulated independently in carcinoma cells. It is tempting to speculate that the  $\beta$  subunit might have functions independent from its role in regulating Na,K-ATPase activity in epithelial cells. Reduced  $\beta$  subunit expression in poorly differentiated carcinoma and its down-regulation by Snail is consistent with a hypothesis that loss of  $\beta$  subunit expression might play a role in the conversion of the epithelial phenotype to the mesenchymal phenotype during EMT.

### Tubulointerstitial Fibrosis

Renal interstitial fibrosis is caused by a variety of progressive injuries, including urinary tract obstruction, chronic inflammation, and diabetes leading to chronic renal failure. The chronic inflammatory changes of the interstitium are associated with EMT of the renal tubules to myofibroblasts and synthesis of extracellular matrix leading to interstitial fibrosis.<sup>73,74</sup>

TGF- $\beta$  is a multifunctional cytokine transforming growth factor and is a potent inducer of EMT in several tissues. Emerging evidence suggests that TGF- $\beta$  initiates the transition of renal tubular epithelial cells to myofibroblasts, the cellular source of extracellular matrix deposition.<sup>75,76</sup> Interestingly, Snail has been identified as an immediate-early gene target of the TGF- $\beta$  pathway. Neutralizing antibodies against TGF- $\beta$  block the expression of Snail and interference with Snail expression blocks EMT.<sup>77</sup> With Na,K-ATPase  $\beta$  subunit expression being suppressed by Snail<sup>25</sup> and  $\beta$  subunit's role in epithelial polarity,<sup>17,18,25</sup> it is tempting to speculate that the  $\beta$  subunit might play an important role during EMT of renal tubules in interstitial fibrosis. It is essential to identify molecular events involved in the induction of EMT in renal fibrosis and we suggest that Na,K-ATPase is a candidate molecule in the pathogenesis of renal fibrotic disorders. Experiments in our laboratory are in progress to understand further the role of Na,K-ATPase function and the role of the  $\beta$  subunit in this disease process.

### Ischemic Nephropathy

Ischemic events in kidney manifest as a progressive loss of kidney function and kidney atrophy. Interestingly, ischemia in kidney has been associated with the loss of tight junctions,<sup>78-80</sup> and reduced Na,K-ATPase surface levels have been reported in ischemia-induced acute renal failure<sup>81</sup> and during posts ischemic renal injury.<sup>82</sup> Furthermore, during renal ischemia the ATP content of the affected epithelial cells is depleted rapidly, leading to inhibition of Na,K-ATPase function.<sup>83-85</sup> The role of Na,K-ATPase in establishing epithelial tight junctions and regulation of tight-junction function might have important clinical implications during the recovery from ischemic injury and re-establishing adequate Na,K-ATPase function/levels during recovery from ischemic injury

might prove to be an important factor to consider in the management of ischemic events in kidney.

## Conclusions

Since its discovery, Na,K-ATPase has been characterized extensively as a pump involved in ion transport. As described in this review, Na,K-ATPase might have multiple functions such as in epithelial polarization, cell motility, and cell signaling. Evidence suggests that the  $\beta$  subunit of Na,K-ATPase might have new functions associated with the regulation of epithelial cell structure and functions. It is plausible that these functions are independent of its role in Na,K-ATPase activity. It is time to rethink that a fundamental protein such as Na,K-ATPase might have multiple functions in the cell. Deciphering these functions by a concerted effort of biochemists, cell biologists, physiologists, and clinicians should provide valuable information on the role of Na,K-ATPase in epithelial cell diseases such as cancer, tubulointerstitial fibrosis, and ischemic nephropathy. Recent advances in cell and molecular biology and bioinformatics should be used as tools to further explore and validate the multifunctional nature of Na,K-ATPase.

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