Editorial Focus: Going with the Wnt? Focus on “Hyperaldosteronism, hypervolemia, and increased blood pressure in mice expressing defective APC”

Armin Just
Physiologisches Institut I, Albert-Ludwigs-Universität Freiburg, Freiburg, Germany

THE WNT SIGNALING SYSTEM consists of ancient pathways with highly conserved core components tracing back more than 700 million years including insects and nematodes (20). Wnt signals classically play a crucial role in embryonic development concerning cell fate, motility, and polarity, as well as embryonic axis formation and the generation of limbs and organs (10, 16, 29; see also www.stanford.edu/~rnusse/wntwindow.html). However, more recent findings indicate that the Wnt cascades, or at least some of their core components, also take part in important functions in postnatal life, such as cancer and metastasis, schizophrenia and Alzheimer’s disease, type II diabetes and obesity, or recovery from ischemic renal failure (11, 16). Bhandaru et al. (3) explore a completely new aspect of Wnt signaling probing an involvement of one of its core components, adenomatous polyposis coli (APC) protein, in the regulation of sodium transport and volume homeostasis.

Wnt Signaling

The denomination Wnt is derived from the upstream extracellular signaling proteins wingless in drosophila and its homolog integrated in vertebrates. The family of Wnts consists of seven members in flies and 19 in mice and humans. Upon binding of these lipid-modified glycopeptides to their plasma membrane receptors, Frizzled and low-density lipoprotein-related protein, the cytoplasmic phosphoprotein Disheveled (Dsh) is activated (10, 11, 16) (Fig. 1). This triggers one or more of at least three different cascades: the canonical Wnt pathway, the planar cell polarity (PCP) pathway, and/or the Wnt/Ca2+ pathway. In the canonical pathway, the activation and membrane translocation of Dsh recruits the so-called β-catenin destruction complex to the plasma membrane, which leads to degradation of the complex and release of β-catenin. The complex consists, among other proteins, of the APC protein, Axin, GSK3, and casein kinase 1 (CK1), and binds β-catenin. In the absence of Wnt signaling it promotes the destruction of β-catenin by phosphorylation through CK1 and GSK3, which predisposes β-catenin for degradation in ubiquitin-mediated proteolysis. Activation of the Wnt receptors thus leads to release and cytosolic accumulation of β-catenin. The latter is then shuttled to the nucleus, where it associates with T-cell factor 4 and lymphoid enhancer factor to up- or downregulate the transcription of various genes including c-jun, c-myc, cyclin D1, axin 2, survivin, and others (22). While the β-catenin destruction complex is a pivotal component of the Wnt signaling system, several parts seem to be involved in signalling functions even in the absence of Wnt proteins. β-Catenin can also associate with β-cadherins on the plasma membrane and thereby escape the destruction complex and contribute to signaling in cell-cell adhesion (15). Other functions of β-catenin include smooth muscle proliferation (7) as well as formation, maintenance, and plasticity of central synapses (1). APC is a large multidomain protein capable of interacting with numerous other proteins. The 2800 amino acids of APC comprise an oligomerization domain, two nuclear localization signals, several nuclear export signals, binding sites for β-catenin (armadillo repeat), Axin (SAMP repeats), and microtubules, as well as a PDZ binding motif (8). As implied in its name, loss of function mutations of APC have been found in familial adenomatous tumors in the colon, indicating its role in Wnt-typical proliferation responses (8). Under normal conditions, the same function of APC seems to be involved in the regulation of epithelial cell renewal of the colonic crypts, since APC expression increases from the bottom of the crypt to the top of the villi in parallel to the mitotic activity (23). In addition, more active functions of APC than “simple” suppression of growth have also been described, such as cell migration, cell adhesion, mitotic chromosome segregation, apoptosis, and neuronal differentiation, functions that might play out when APC is not degraded by Wnt-signaling (8). However, more recent observations suggest that APC may not only be involved in cell growth and differentiation but possibly also in the regulation of epithelial transport. In mice with a spontaneous mutation in the APC gene (APC<sup>min/+</sup>) enhanced expression of the ENaC and potassium channel activity was observed in the mucosa of the colon (17). However, because the animals had severe polyposis with rectal bleeding, those authors tended to ascribe these observations to the chronic blood loss (17). Nevertheless, similar results were obtained by Bhandaru et al. (3) in that they had found enhanced H+−K+−ATPase activity in the gastric mucosa of the same strain of mice (18).

Aldosterone Signaling

A major determinant of sodium reabsorption in the kidney and thereby of volume homeostasis and arterial pressure is the mineralocorticoid hormone aldosterone (19). Aldosterone promotes Na⁺ reabsorption in the aldosterone-sensitive distal nephron (i.e., connecting tubule and cortical collecting duct) through upregulation and activation of the ENaC (19), together with stimulatory effects on Na⁺−K⁺−ATPase (6) and ROMK potassium channels (27). Aldosterone is also an important contributor to the regulation of potassium homeostasis (27). With major contributions from Bhandaru et al. (3), strong evidence has been accumulated today that the upregulation of ENaC by aldosterone is communicated in large part through serum and glucocorticoid-inducible kinase 1 (SGK1) (14, 24, 26). By binding to the cytosolic mineralocorticoid receptor, aldosterone enhances expression and activation of SGK1 (24, 26). SGK1 phosphorylates the ubiquitin ligase Nedd4-2 (neural precursor cell expressed, developmentally downregulated), and
thereby inhibits the action of Nedd4-2 to retract the channel from the membrane (24, 26). SGK1 is a serine/threonine protein kinase of the AGC family showing ~50% homology to Akt/protein kinase B (28). As its name implies, its expression is stimulated by adrenocorticosteroid hormones (28). For adequate function, however, SGK1 needs to be phosphorylated, which is probably achieved through phosphatidylinositol-3-kinase (PI3K) and PI3K-dependent kinases PDK1 and PDK2 (9). The importance of SGK1 is most impressively demonstrated in knockout mice deficient for SGK1 (30); while these animals show normal arterial pressure, glomerular filtration rate, and Na-excretion under a standard diet, their ability to retain sodium during a low-salt diet is severely impaired compared with their wild-type controls (30).

A Connection Between Wnt and Aldosterone?

The strength of the present work by Bhandaru et al. (3) is to bring these seemingly unrelated fields together. In a previous study, the authors had observed enhanced SGK1 expression in mice with a spontaneous APC mutation (18), suggesting that SGK1 might be a target of canonical Wnt-signaling. A previous microarray study in a colon cancer cell line with enhanced Wnt-signaling due to a mutation in β-catenin had increased expression of SGK1 when the Wnt-pathway was further activated by small interfering RNA against APC (5). However, results from another tumor cell line found enhanced SGK1 protein expression but unchanged or lower mRNA levels in response to inducing a more Wnt-resistant β-catenin (13). Furthermore, an analysis of expression patterns in murine and human colon tumors found SGK1 not up- but downregulated consistently in all adenomas (21). Nevertheless, the observations may indicate that SGK1 could be one of the target genes of Wnt-signaling at least under some conditions. On the basis of this premise and on the clear contribution of SGK1 to the effects of aldosterone, the authors proposed that Wnt-signaling or at least APC could be involved in epithelial transport. To follow this extremely intriguing hypothesis, the authors studied APCmin/+ mice (min = multiple intestinal neoplasms). These animals carry a truncating heterozygous mutation in the APC gene, which leads to multiple colorectal polyps (12). To assess the predicted involvement of SGK1 the authors used an elegant genetic approach by crossing the APCmin/+ mice with animals deficient for SGK1. The chronic metabolic investigations indeed revealed reduced renal sodium excretion, enhanced renal potassium excretion, and elevated plasma volume and systolic arterial pressure in the APCmin/+ mice. All of these effects were virtually normalized by additional elimination of SGK1. Taken together, these results are well in line with the hypothesis. One might argue that plasma aldosterone levels were elevated in the APCmin/+ animals, which would induce exactly the same results. However, this simple explanation would not hold true in the case of the animals with additional loss of SGK1, as the abnormalities were normalized despite even higher levels of aldosterone. What remains unclear, however, is why aldosterone levels were increased and whether this might indicate a role of APC also in the regulation of aldosterone secretion. Another noteworthy result is the 40% reduction of glomerular filtration rate in APCmin/+ regardless of the presence or absence of SGK1. This is of particular importance given the high levels of aldosterone in both animals and the known involvement of aldosterone (4) and possibly also SGK1 (2, 25) in renal fibrosis. Although it remains unclear whether the hyperfiltration has developmental or postnatal causes, the data would suggest that these causes do not require SGK1. Overall, the present work proposes intriguing new connections between signaling pathways, although many open questions
remain. Exciting new opportunities lie ahead for Wnt, APC, and catenin signals to be found entangled in these and other new functions.

REFERENCES