Leveraging unique structural characteristics of WNK kinases to achieve therapeutic inhibition

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ABSTRACT

The with-no-lysine (K) WNK kinases are master regulators of the Na^+-(K^+)-Cl^- cotransporters, including the renal-specific NCC and NKCC2 cotransporters. The discovery of WNK463, an orally bioavailable pan-WNK kinase inhibitor that exploits unique structural properties of the WNK catalytic domain to achieve high affinity and kinase selectivity, illustrates a strategy of leveraging distinct kinase features to develop specific inhibitors and validates the genetic predictions of the in vivo pharmacology of WNK inhibition.

Protein kinases are pathogenically mutated in multiple human diseases, including cancer, neuropsychiatric disorders, inflammatory conditions, infectious diseases, and cardiovascular diseases (*1*). Approximately one-third of all protein targets under investigation in the pharmaceutical industry are kinases; over 30 kinase inhibitors are approved for clinical use; and dozens of other kinase inhibitors are actively under investigation in clinical trials (*2*). Despite this, the available armamentarium of clinically used kinase inhibitors covers less than 15% of the kinome, and these are used overwhelming for oncological conditions (*2*). Compared to cancer in which lack of treatment is often lethal, the bar is set much higher for kinase drug discovery in chronic conditions, because exquisite target selectivity and a minimum of side effects are required to compete with existing therapies (*1*).

Mutations in the serine-threonine kinases of the with-no-lysine (K) WNK family WNK1 (encoded by *PRKWNK1*) and WNK4 (encoded by *PRKWNK4*) cause an autosomal dominant form of thiazide-sensitive and NaCl-sensitive hypertension with hyperkalemia termed Gordon's Syndrome, or pseudohypoaldosteronism type II (PHAII; OMIM # 614496) (*3*). The WNK kinases regulate blood pressure and electrolyte homeostasis by phosphorylating and activating two related Ste20-type kinases termed STE20/SPS1-related proline/alanine-rich kinase (SPAK) and oxidative stress-responsive kinase 1 (OSR1), collectively referred to as SPAK/OSR1. Activated SPAK/OSR1 phosphorylate and stimulate the activities of two related cation-Cl^- cotransporters in the kidney in the aldosterone-sensitive part of the nephron, the Na^+-Cl^- cotransporter NCC and the Na^+-K^+-$2Cl^-$ cotransporter NKCC2 (**Figure 1A**). NCC is inhibited by hydrochlorothiazide and NKCC2 is inhibited by the furosemide, respectively. These are two of the most commonly used drugs (and well tolerated) in clinical medicine for the treatment of hypertension and edema (fluid retention in tissues). WNKs also regulate NKCC1 and KCC2, cation-Cl- cotransporters critical for establishing Cl- homeostasis in the nervous system, and implicated in multiple diseases featuring neuronal excitability due to GABA disinhibition(*4*). These actions of the WNKs make them attractive candidates for the development of novel kinase inhibitors.

Kinase of the WNK (with no lysine) family are different from other kinases, because of the unusual placement of the catalytic lysine residue in WNK isoforms (Lys^{233} of WNK1) compared the active site lysine in all other protein kinases (*5*). This peculiarity could theoretically be exploited to create WNK-specific ATP-competitive kinase inhibitors. Indeed, Yamada *et al*. exploited these unique structural features to conduct a high throughput screen for inhibitors of WNK1 catalytic activity (*6*). They discovered the first orally bioavailable pan-WNK kinase inhibitor, WNK463, which exhibits both low nanomolar affinity and high kinase selectivity. By solving the X-ray -crystal structure of WNK463 with the kinase-dead mutant WNK1 S382A at 1.65Å resolution, Yamada *et al*. showed WNK463 contacts the hinge region of the ATP binding site by burrowing through a narrow tunnel to the back pocket of WNK1, which occurs because of the nonstandard placement of the catalytic Lys^{233} in the glycine-rich loop (Fig. 1A).

Inhibition of WNK kinases promotes both diuresis and vasodilation, therefore these kinases have garnered much attention as potential targets for the development of antihypertensive agents. Mutations in WNK1 (encoded by *PRKWNK1*) and WNK4 (encoded by *PRKWNK4*) cause an autosomal dominant form of hypertension that is also associated with hyperkalemia termed Gordon's Syndrome or pseudohypoaldosteronism type II (PHAII; OMIM # 614496) (*3*). The WNK kinases regulate blood pressure and electrolyte homeostasis by phosphorylating and activating two related Ste20-type kinases termed STE20/SPS1-related proline/alanine-rich kinase (SPAK) and oxidative stress-responsive kinase 1 (OSR1), collectively referred to as SPAK/OSR1. Activated SPAK/OSR1 phosphorylate and stimulate the activities of two related cation-Cl^- cotransporters in the kidney in the aldosterone-sensitive part of the nephron, the Na^+-Cl^- cotransporter NCC and the Na^+-K^+-$2Cl^-$ cotransporter NKCC2 (Fig. 1B).

Yamada *et al*. tested WNK463 in a rat hypertension model (*6*). In spontaneously hypertensive rats, orally administered WNK463 significantly decreased blood pressure, facilitated a brisk diuresis, and reduced the phosphorylation of SPAK and OSR1. This proof-of-biology study is important, because it establishes the importance of the WNK kinase catalytic domain in blood pressure and electrolyte homeostasis and confirms predictions made by human and rodent genetics about the in vivo pharmacology of WNK kinase inhibition (*7*). Moreover, WNK463 will be an important research reagent that will help illuminate CCC regulation and the role of this pathway in physiological processes.

The development of WNK463 as a potential therapeutic was discontinued due to other unspecified effects beyond those reported in the cardiovascular and renal systems when administered to the rats at higher concentrations (*6*). This is perhaps not surprising, because the WNK kinases (including WNK2 and WNK3, kinases that not mutated in PHAII) are present throughout the body, although some isoforms exhibit restricted distribution. For example, the HSN2 isoform of WNK1 is almost exclusively detected in the nervous system, including the spinal cord dorsal horn, and inactivating HSN2 mutations cause hereditary sensory and autonomic neuropathy type IIA (HSANII; OMIM #201300) (*8*). Pan-WNK inhibition might, therefore, affect physiological process beyond those of blood pressure and electrolyte homeostasis.

With regard to alternative means to inhibit the WNK pathway, where to go from here? The WNK substrates SPAK and OSR1 play a critical role in controlling blood pressure, and SPAK-deficient mice have markedly reduced blood pressure yet are otherwise healthy. Genome-wide association studies of essential hypertension show a strong association with common variants of SPAK. The strategy of targeting the ATP-binding site of the SPAK/OSR1 raises concern regarding the ability to develop sufficiently selective inhibitors that do not suppress other kinases. The development of Closantel and STOCK1S-14279, ATP insensitive inhibitors, has introduced the possibility of developing inhibitors

of WNKs signaling by binding to constitutively active or WNK-sensitive (T233E)SPAK(*9*) (Fig. 1B).

Another approach is to target protein-protein interaction sites. SPAK possess a specific docking CCT domain that mediates the interaction with RFxV motifs present in WNKs, NCC, and NKCC2 (*10*) (Fig. 1B). A high resolution three-dimensional structure of this domain complexed to the RFxV motif has been solved (*11*) (Fig. 1A). Screens have identified inhibitors that disrupt the CCT-RFxV interaction (*12*) and, chemical modulation of these first generation inhibitors might yield compounds that could be used in vivo. Because the CCT domain is unique to SPAK and OSR1, targeting this interaction site would not be expected to inhibit other kinases, which could provide sufficient specificity and safety for use in the treatment of a chronic condition, such as hypertension. Other strategies that antagonize tissue-specific WNK isoforms, for example by targeting the unique HSN2 isoform to treat neuropathic pain (*13*), could minimize unwanted side effects in other organ systems.

Major challenges remain for the development of therapeutically effective kinase inhibitors in non-oncological diseases, including the identification and validation of driver kinases in these conditions and the discovery of drugs with adequate selectivity and safety (*2*). Targeting kinases that, when mutated, disrupt human physiology (even in rare inherited forms of disease) is a good strategy for the development of personalized treatments (*14*). With eight members of the WNK kinase signaling pathway, including their upstream regulators (the E3 ubiquitin ligases CUL3-KLHL3) and downstream targets (SPAK and OSR1 and cation-Cl^- cotransporters) are mutated in Mendelian forms of renal and central nervous system pathology (*7*), continued efforts at drug discovery targeting this pathway are most certainly warranted.

Figure 1. Domains and sites important for regulation of and signaling through the WNK-SPAK/OSR1 pathway. (A) Left: The structure of WNK463 bound to the kinase-dead mutant WNK1 S382A (PDB ID: 5DRB). Right: The structure of the SPAK CCT domain bound to the RFxV motif. (**B**) Proteins with slashes indicate that multiple isoforms have the same properties. For SPAK/OSR1, the residue numbering above the protein represents SPAK, and the residue numbering below represents OSR1. Kinase X refers to a yet unidentified kinase that is regulated by WNKs and mediates the direct phosphorylation and inhibition of Site-1 on the KCCs. STOCK1S-50699 is a small-molecule inhibitor that blocks the interaction between SPAK/OSR1 and WNK by binding to the CCT domain (*12*); Closantel and STOCK1S-14279 are SPAK (ATP insensitive) inhibitors, able to bind to constitutively active or WNK-sensitive(T233E)SPAK (*9*); and WNK463 inhibits of WNK1 catalytic activity (*6*).

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