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WNK kinases and the control of blood pressure

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Abstract

The WNK kinases are a small group of serine/threonine kinases with unique catalytic domains that lack the lysine residue used in other kinases to co-ordinate ATP (hence, With No K [WNK]). Their closest homologues are found within the mitogen-activated protein kinase (MAPK) pathway suggesting a role in signalling. Two WNK isoforms, WNK1 and WNK4, have been identified as the disease genes for a rare monogenic hypertension syndrome (Gordon's syndrome or pseudohypoaldosteronism type 2 [PHA2]) implicating them in salt homeostasis by the kidney. This is supported by recent data showing widespread expression of WNK1 and WNK4 in mammalian transporting epithelia. Within the kidney, WNKs probably regulate the surface expression of several proteins involved in ion transport, including the sodium-chloride cotransporter (NCCT) and the potassium channel renal outer medullary potassium channel (ROMK), based on co-expression studies in *Xenopus* oocytes. WNKs, especially WNK4, have been suggested as candidate genes for essential hypertension itself, but evidence for this is lacking. Some of the effects of the WNKs are independent of their kinase function, suggesting that they are dependent on specific protein-protein interactions. It seems likely that the WNKs are part of much larger protein scaffolds in cells and have effects in cells beyond ion transport. However, because of their effect on expression of the NCCT they are attractive drug targets for the development of novel antihypertensive agents. These agents could potentially offer the efficacy of a thiazide diuretic, but without the metabolic side effects usually seen with this class of antihypertensive therapy.

Keywords: WNK; Kinases; Genetic syndrome; Essential hypertension; Thiazide diuretic; Cotransporters; Ion transport; Kidney

Abbreviations: ACE, angiotensin converting enzyme; CCD, cortical collecting duct; DCT, distal convoluted tubule; ENaC, epithelial sodium channel; ERK epidermal growth factor receptor kinase; HEK, human embryonic kidney; MAPK, mitogen-activated protein (MAP) kinase; NCCT, sodium-chloride cotransporter; NHE, sodium hydrogen exchanger; NKCC, sodium-potassium-chloride cotransporter; PHA2, pseudohypoaldosteronism type 2; PRKWNK, protein kinase WNK; PT, proximal tubule; ROMK, renal outer medullary potassium channel; SGK, serum and glucocorticoid responsive kinase; SHR, spontaneously hypertensive rat; SPAK, **S**te-20 related **P**roline-**A**lanine-rich **K**inase; WKY, Wister-Kyoto rat; WNK, With No K (K=lysine)

Contents

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1. Introduction

The WNK kinases are recently classified members of the human kinome functioning as serine/threonine kinases. Although they form a discrete branch on the current dendrogram for the kinome (Manning et al., 2002), they are structurally most closely related to the sterile-20 (STE-20) kinases with ~30% homology (Dan et al., 2001). This has attracted particular attention because most of their members activate mitogen-activated protein kinase (MAPK) cascades. MAPK cascades are crucial to a number of cellular events, transmitting signals from external stimuli such as cytokines and growth factors to internal transcription factors and the regulation of gene transcription.

2. The discovery and structure of WNK kinases

2.1. WNK discovery

The WNK1 gene was first isolated in 2000 from a rat brain cDNA library during a screen for novel members of the MEK kinase family of proteins (Xu et al., 2000). The acronym reflects the highly unusual character of its catalytic domain, which lacks the ubiquitous lysine residue present within other kinase domains for co-ordinating ATP, hence With No K=lysine (WNK, see Section 2.2). Database searches have identified 3 other kinase genes within the human genome that show the same domain structure as WNK1. They share >95% sequence homology over their catalytic domains with WNK1, although homology is less well conserved outside the kinase region (see Table 1). The highly conserved consensus sequence within sub-domain I+II is present in WNK1-like kinases identified from a variety of multicellular organisms (see Table 2). Yeast and bacterial genomes appear to contain no WNK-like kinases, and outside of mammals only the *Fugu* genome contains paralogues for all 4 WNK kinases. Hence, WNK2–4 are phyllogenically recent proteins associated with complex eukaryotic organisms.

2.2. The atypical WNK kinase domain

WNK kinases possess a serine/threonine catalytic domain that is structurally distinct from other kinases. Almost all known kinases have an invariant catalytic lysine residue in subdomain II (beta strand 3) that corresponds to position 250 in WNK1. In WNK1 this residue is a cysteine, and the catalytic lysine residue is actually contributed from the adjacent subdomain I (beta strand 2). This lysine (K233 in WNK1) is conserved across all the WNKs and replaces a glycine residue in the phosphate anchor ribbon of beta strand 2. Mutation from lysine in WNK1 (K233A) causes complete loss of kinase activity, and mutating C250 to lysine does not restore kinase activity (Xu et al., 2000), i.e. the catalytic lysine will not function if switched to its location in non-WNK kinases. This is intriguing, because the complementary experiment of switching the catalytic lysine in a non-WNK kinase (EKR2) to the phosphate ribbon does produce a functional protein. It suggests that the steric constraints on the catalytic lysine are very different within the 2 kinase groups (Xu et al., 2002).

Kinases are often regulated by autoinhibition, which suppresses kinase activity until an appropriate activation signal releases the autoinhibitory domain. There is evidence that a similar control exists for the WNKs and involves its autophosphorylation. Wild-type WNK1 autophosphorylates serine residues both in vitro and in vivo, and the key residues

Table 1
Amino acid alignment of catalytic subdomains I+II for the 4 human WNKs and the WNK1 orthologues in *Caenorhabditis elegans* (c) and *Drosophila melanogaster* (d)

hWNK1	LKFDIEIGRGSF**K**TVY—KGLDTETTVEVAWCELQ
hWNK2	LKFDIELGRGSF**K**TVY—KGLDTETWVEVAW**C**ELQ
hWNK3	LKFDIELGRGAF**K**TVY—KGLDTETWVEVAW**C**ELQ
hWNK4	LQFDIEIGRGSF**K**TVY—KGLDTETTVKVAW**C**ELQ
dCG717	FKYDKEVGRGSF**K**TVY—KGLDTLTGVPVAW**C**ELL
cC46C2.1	LKFDEELGRGSF**K**TVF—RGLDTETGVAVAW**C**ELQ
Concensus	GRG-F**K**TV—GLDT-T-V-VAW**C**EL

The normal catalytic lysine is a cysteine (**C**) in WNKs and the alternative lysine used by these kinases for ATP co-ordination is in bold font (**K**).