

# Role of HIPK2 in kidney fibrosis

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Homeodomain interacting protein kinase 2 (HIPK2) functions as either a co-repressor or a co-activator of transcriptional regulators. Dysregulation of HIPK2 is associated with cancer and neurological disease. Recently, we found that HIPK2 is also an important driver of kidney fibrosis in the HIV-1 transgenic murine model, Tg26. HIPK2 protein levels are upregulated in the tubular epithelial cells of Tg26 mice as well as in kidney biopsies of patients with HIV-associated nephropathy, focal segmental glomerulosclerosis, diabetic nephropathy, and IgA nephropathy. We found that HIPK2 regulates pro-apoptotic, pro-fibrotic, and pro-inflammatory pathways including p53, transforming growth factor  $\beta$  (TGF- $\beta$ )-SMAD family member 3 (Smad3), Notch, Wingless and INT-1 (Wnt)/ $\beta$ -catenin, and nuclear factor kappa-light-chain-enhancer of activated B cells in renal tubular epithelial cells. Our data suggest that HIPK2 may be a potential target for antifibrotic therapy. As mice with germline deletion of *HIPK2* do not exhibit any phenotypic change under basal conditions, we do not expect significant side effects with specific HIPK2 inhibitors. However, potential effects of HIPK2 on tumor growth should be considered because of its tumor suppressor effects. Therefore, further understanding of structure-function relationships and post-translational modifications of HIPK2 are necessary to develop more specific drugs targeting the pro-fibrotic effects of HIPK2.

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## IDENTIFICATION OF HIPK2 AS A KEY PROTEIN KINASE IN KIDNEY FIBROSIS

A combined experimental and bioinformatic approach for identifying upstream protein kinases that are responsible for the regulation of downstream genes was used to define the regulation of gene expression in the diseased kidney of HIV-1 transgenic mouse (Tg26), which is a model for human HIV-associated nephropathy (HIVAN).<sup>1</sup> Using experimental data from DNA-protein interaction arrays and gene expression microarrays, we identified a list of transcription factors that are differentially regulated in HIV-1 transgenic mice, Tg26, as compared with their wild-type (WT) littermates (Figure 1). A systems biology approach to study signaling pathway activation was employed by taking into account protein-protein and protein-DNA interactions. Homeodomain interacting protein kinase 2 (HIPK2) was identified as a common regulator of signaling pathways activated in HIVAN. HIPK2 is known to mediate signaling through multiple pathways involved in apoptosis, epithelial-mesenchymal transition, and tubulointerstitial fibrosis. As it is technically challenging to screen protein kinase profiles, this method allowed us to deduce upstream protein kinases from microarray or mRNA-sequence data sets using a simple computational program called Enrichr as described in detail by Chen *et al.*<sup>2</sup> Subsequently, we confirmed that protein expression of HIPK2 was increased in the kidney of Tg26 mice as well as in human kidneys from patients with HIVAN, focal segmental glomerulosclerosis, diabetic nephropathy, and IgA nephropathy. *In vitro*, we found that HIPK2 induces apoptosis of renal tubular epithelial cells (RTECs) and expression of pro-fibrotic markers including smooth muscle actin, collagen I, and fibronectin. In addition, HIPK2 activates several pro-apoptotic (p53), pro-fibrotic (TGF- $\beta$  (transforming growth factor  $\beta$ )-Smad3 and Wnt-Notch pathways), and pro-inflammatory (NF- $\kappa$ B) pathways in RTEC. Using a gene knockout approach, we confirmed the role of HIPK2 in renal fibrosis in three animal models: unilateral ureteral obstruction, folic acid-induced nephropathy, and Tg26 mice. We also demonstrated that knockout of HIPK2 abolished the activation of pro-fibrotic signaling pathways and the expression of pro-fibrotic genes in these animal models of kidney fibrosis.<sup>1</sup>

## STRUCTURE AND FUNCTION OF HIPK2

HIPK2 is an evolutionarily conserved serine/threonine kinase.<sup>3,4</sup> It mainly localizes in subnuclear speckles, and

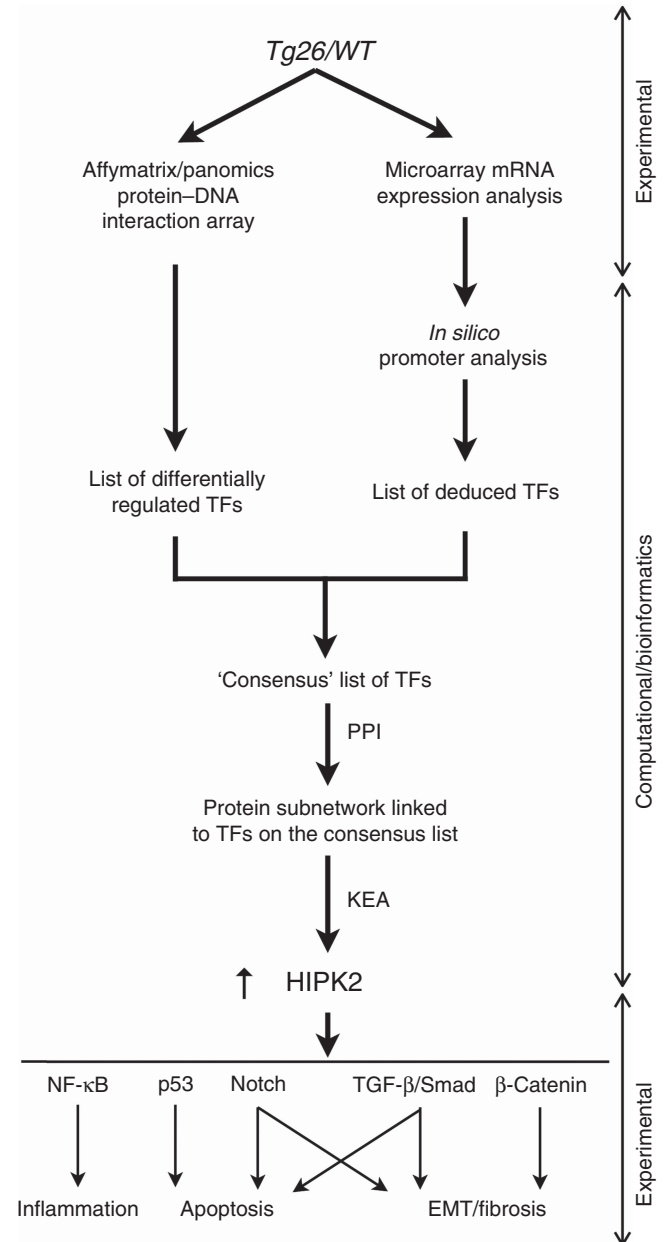
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only a small proportion is found in the nucleoplasm or cytosol.<sup>5</sup> HIPK2 comprises an N-terminal region containing a sumoylation site at Lysine 25<sup>6,7</sup> and a 330 amino-acid Ser/Thr kinase domain, in which K221 is the key catalytic site.<sup>8</sup> A protein–protein interaction region called Homeobox-interacting domain located adjacent to the kinase domain is followed by a speckle-retention signal, which is essential for the subcellular localization of HIPK2 in nuclear bodies (NBs).<sup>3</sup> A sumoylation (SUMO) interacting motif has been identified within the speckle-retention signal. The SIM is critical for HIPK2 localization to NBs and also for its recruitment to promyelocytic leukemia NBs.<sup>5</sup> There are two PEST motifs overlapped with a noncovalent binding site of SUMO. The ubiquitylation site (K1182) within auto-inhibitory domain at the terminal domain is cleaved by caspases for full activation of HIPK2.<sup>9</sup> There are short repeats of S, Q, or A at the C-terminal domain (Figure 2).

HIPK2 acts as a co-regulator of transcription factors and modulates many different cellular processes such as growth, development, morphogenesis, and cell death in multiple organisms and cell types.<sup>10–13</sup> HIPK2 is also involved in the regulation of gene transcription in response to DNA damage as triggered by UV light, ionizing radiation, and chemotherapeutic drug treatment.<sup>14–16</sup>

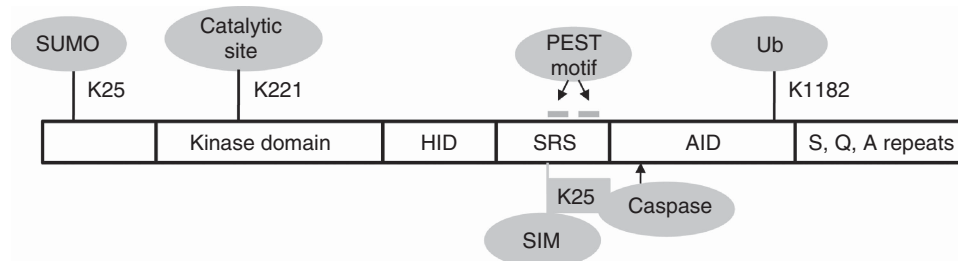
**HIPK2-MEDIATED SIGNALING PATHWAYS**

It is known that HIPK2 interacts with transcription factors and functions as both a co-repressor and a co-activator.<sup>4</sup> HIPK2 has been implicated in the activation of multiple downstream signaling pathways including p53-mediated DNA damage signaling pathways,<sup>16,17</sup> TGF-β/Smad3,<sup>18,19</sup> Wnt/β-catenin,<sup>20</sup> bone morphogenetic proteins (BMPs),<sup>21</sup> and Notch<sup>122</sup> pathways to mediate cell growth, apoptosis, and development. HIPK2 has a critical role in the initiation of DNA double-stranded break (DSB) repair signaling.<sup>17,23</sup> HIPK2 serves as an important activator of P53, which is a transcription factor that has an essential role in DNA damage signaling.<sup>8,24,25</sup> A recent study reported that HIPK2 targets WIP1 (wild-type p53-induced phosphatase 1) for phosphorylation and proteasomal degradation in response to DSBs.<sup>26</sup> Consistently, we found that p53 is activated in kidneys of Tg26 mice, and knockout of HIPK2 abolished HIV-induced activation of p53 in RTEC and kidney cortices.<sup>1</sup> HIPK2 can also function as a transcriptional co-repressor that directly interacts with Smads, including Smad1, Smad2, and Smad3, to modulate Smad-dependent gene expression.<sup>18,19,27</sup> HIPK2 regulates the survival and apoptosis of midbrain dopamine neurons during programmed cell death by interacting with TGF-β.<sup>28</sup> In our prior study, we found that HIPK2 interacts with TGF-β/Smad3 pathway to promote the expression of pro-fibrotic markers, and deletion of HIPK2 suppressed the activation of TGF-β/Smad3 in the diseased kidney.<sup>1</sup> HIPK2 has also been found to have an important role in the negative regulation of BMP-induced transcriptional activation. HIPK2 regulates the BMP signaling pathway in the maintenance of enteric neurons



**Figure 1 | Schema on the combined experimental and computational approach to characterize signaling pathway activation in HIV-associated nephropathy.** EMT, epithelial-mesenchymal transition; HIPK2, homeodomain interacting protein kinase 2; KEA, kinase enrichment analysis; NF-κB, nuclear factor kappa-light-chain-enhancer of activated B cells; PPI, protein-protein interactions; TFs, transcription factors; TGF, transforming growth factor; WT, wild type.

and glia. Loss of HIPK2 increases BMP signaling, with an increased expression of phosphorylated Smad1/5/8.<sup>21</sup> Our unpublished data suggest that HIPK2 negatively regulates BMP7 phosphorylation in RTEC, which is of potential relevance, given BMP7 is considered to mediate an antifibrotic pathway. It has also been shown that HIPK2 activates Wnt/β-catenin and Notch signaling pathways by antagonizing the global co-repressor Groucho during development.<sup>29</sup> We



**Figure 2 | Schematic summary of HIPK2-interacting domains.** AID, auto-inhibitory domain; HID, homeobox-interacting domain; KD, kinase domain; PEST, a peptide sequence that is rich in proline (P), glutamic acid (E), serine (S), and threonine; SIM, SUMO-interacting motif; SRS, speckle-retention signal; SUMO, sumoylation; Ub, ubiquitylation.

found that knockout of HIPK2 inhibited HIV infection-induced expression of Wnt/ $\beta$ -catenin and Notch targeted genes in RTEC and in kidneys of Tg26 mice.<sup>1</sup> HIPK2 can either activate the Wnt/ $\beta$ -catenin pathway through phosphorylation of transcription factor 3 (TCF3), a transcriptional repressor or inhibit Wnt-mediated transcription by phosphorylation of lymphoid enhancer-binding factor 1.<sup>30</sup> Our studies showed that HIPK2 overexpression increased TCF3 phosphorylation without affecting lymphoid enhancer-binding factor 1 phosphorylation in RTEC,<sup>1</sup> suggesting that HIPK2 is an activator of the Wnt pathway in the kidney.

Through unbiased screening, we found that HIPK2 also mediates expression of NF- $\kappa$ B and stat3 target genes. These findings indicate that HIPK2 may also be involved in the regulation of inflammatory responses, observations that are relevant, given that inflammation is also a key component of kidney fibrosis.

#### POST-TRANSLATIONAL MODIFICATION OF HIPK2

HIPK2 is regulated by phosphorylation, ubiquitination, SUMOylation, and acetylation. HIPK2 steady-state levels are regulated primarily through proteasome-dependent degradation.<sup>31</sup> In unstressed cells, HIPK2 is unstable because of the constant degradation by the ubiquitin-proteasome system, which is carried out by the ubiquitin E3 ligases such as Siah (Seven in absentia homolog) family including Siah1, Siah2,<sup>32</sup> MDM2 (p53 inhibitor mouse double minute 2),<sup>33</sup> and WSB-1 (WD40-repeat/SOCS box protein).<sup>34</sup> HIPK2 stabilized and accumulated in response to DNA damage when ubiquitylation-mediated targeting of HIPK2 by Siah, MDM2, or WSB-1 was abolished.<sup>17,32,34</sup> Consistently, we found that Siah1 expression is suppressed in mouse and human diseased kidneys leading to the upregulation of HIPK2 protein level.<sup>1</sup> Therefore, Siah1 is a potential drug target for kidney fibrosis.

SUMOylation affects the functional properties of many substrates in the regulation of cellular processes. Recent reports indicate a crucial role of sumoylation in kidney diseases, suggesting that targeting of SUMOylation would be of considerable interest for novel therapies.<sup>35,36</sup> HIPK2 is covalently modified by SUMO1 at lysine 25 preceding the catalytic kinase domain.<sup>6,7</sup> In addition, retention of HIPK2 in NBs critically depends on a SUMO-interacting motif in the

C-terminal part. Deletion or mutation of the SIM domain prevents SUMO binding and precluded localization of HIPK2 in nuclear speckles, thus precipitating localization of HIPK2 to the entire cell in response to DNA damage signals.<sup>5</sup> In addition, SUMOylation of HIPK2 controls its acetylation.<sup>37,38</sup> SUMOylation is frequently associated with recruitment of histone deacetylases (HDACs), and HDAC inhibitors have positive effect on kidney fibrosis, inflammation and epithelial-mesenchymal transition.<sup>39,40</sup> Therefore, understanding of sumoylation and acetylation of HIPK2 will provide us with a better understanding of this protein kinase in human disease, thereby developing more specific and effective drugs for therapy.

#### HIPK2 AND DISEASES

Previous studies suggest that HIPK2 functions as a tumor suppressor<sup>41,42</sup> and HIPK2 is inactivated in tumor cells through multiple mechanisms, including downregulation, mutation, or mislocalization.<sup>42-44</sup> Genetic loss at the HIPK2 locus was found in human thyroid follicular cells,<sup>45</sup> as well as in radiation-induced thymic lymphomas.<sup>46</sup> Mechanistically, it has been shown that HIPK2 induces tumor cell apoptosis by activating the p53 pathway.<sup>8,10,11,47</sup>

In addition, HIPK2 has a critical role in the nervous system. A pro-apoptotic function of HIPK2 has been shown in primary neuronal cells.<sup>48,49</sup> Recent studies reported that beta amyloid-induced HIPK2 degradation may contribute to the progression of Alzheimer's disease.<sup>50,51</sup> In addition, HIPK2 is essential for TGF- $\beta$ -mediated survival of midbrain DA neurons.<sup>18</sup> However, the exact role of HIPK2 in neurological disease remains to be determined.

#### HIPK2 AS A POTENTIAL DRUG TARGET

Drugs targeting individual pro-fibrotic pathways such as TGF- $\beta$  or connective tissue growth factor have been developed and tested as antifibrotic therapies. However, the efficiency of these drugs has not been proved clinically. HIPK2 functions as a master regulator of multiple pro-fibrotic pathways and could be a better drug target for antifibrotic therapy. Protein kinases are readily targeted by small pharmacologic agents. There is a growing interest in synthesizing active protein kinase inhibitors, and there is an increasing number of drugs in this category approved for

clinical use.<sup>52</sup> Protein kinases are the second most important group of drug targets, after G-protein-coupled receptors.<sup>53,54</sup> HIPK2 global knockout mice do not exhibit an abnormal phenotype, suggesting that inhibitors of HIPK2 might have low toxicity under normal physiological conditions.

As HIPK2 serves as a potential tumor suppressor,<sup>42–44</sup> pro-oncogenic effects of HIPK2 suppression will need to be monitored. HIPK2 also has a critical role in the peripheral nervous system.<sup>18,49</sup> Therefore, HIPK2 inhibition may also result neurological side effects, and these potential problems have to be addressed before HIPK2 inhibition can be tried in fibrosis.

In conclusion, our studies strongly suggest a critical role of HIPK2 in kidney fibrosis. HIPK2 activates several pro-apoptotic, pro-fibrotic, and pro-inflammatory pathways leading to kidney fibrosis. Therefore, inhibition of HIPK2 should be an effective antifibrotic therapy. However, a major concern is the potential tumor suppressor effects of HIPK2. Therefore, further understanding of structure, post-translational modifications, and function of HIPK2 will help us to develop more specific inhibitors of HIPK2 to reduce renal fibrosis and hence progressive renal injury.

#### DISCLOSURE

All the authors declared no competing interests.

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