HIPK2 modification code for cell death and survival

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Keywords: apoptosis, caspase cleavage, DNA damage response, DNA repair, HIPK2, phosphorylation, SUMOylation, ubiquitination

Abbreviations: ADR, adriamycin; AMPKα, adenosine monophosphate-activated kinase α; ATF, activating transcription factor; ATM, ataxia-telangiectasia mutated; ATR, ATM- and RAD3-related; CREB, cAMP response element binding protein; DSB, double strand break; HDAC, histone deacetylase; HIPK2, homeodomain-interacting protein kinase 2; IR, ionizing radiation; Mdm2, mouse double minute 2; ROS, reactive oxygen species; Siah, seven in absentia homolog; SUMO, small ubiquitin-like modifier; WIP1, wild-type p53-inducible phosphatase 1; WSB-1, WD40 domain and suppressor of cytokine signaling (SOCS) box protein-1

Introduction

Our understanding of protein post-translational modification (PTM) has come a long way over the past decades since protein phosphorylation was first recognized as a key regulatory mechanism of diverse signaling pathways. Advances in biochemical techniques including mass spectrometry have enabled researchers to map the sites of modification in physiological conditions as well as in vitro. Protein functions are regulated by differential modifications in response to diverse intrinsic or extrinsic cellular stimuli. Protein modification by small molecules (such as phosphorylation, acetylation, methylation) and by peptides (such as ubiquitination, SUMOylation, ISGylation) occurs dynamically depending on cellular context, even in response to different levels of the same stimulus. Therefore, analysis of the PTM dynamics of key regulatory proteins in diverse signaling pathways is pivotal to understanding the real-time regulation of cellular activity and signal networking in a spatiotemporal manner.

Homeodomain-interacting protein kinase 2 (HIPK2) is a member of a nuclear serine/threonine kinase family containing 4 proteins (HIPK1-HIPK4).1-3 HIPKs were first identified as Nkx1.2-interacting proteins in yeast 2-hybrid screening. HIPKs contain a conserved protein kinase domain separated from a WD40 domain and suppressor of cytokine signaling (SOCS) box protein-1. Hence, HIPK2 modification code differentially contributes to cellular homeostasis and determination of cell fate depending on cellular context.

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Submitted: 06/08/2014; Revised: 07/07/2014; Accepted: 07/08/2014
http://dx.doi.org/10.1080/23723548.2014.955999
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neurogenesis, myogenesis, angiogenesis, fat development, and hematopoiesis. A representative phosphorylation target of HIPK2 is p53. HIPK2 phosphorylates p53 at the Ser46 residue, which is crucial in p53-mediated induction of apoptosis. To date, more than 20 proteins have been identified as HIPK2 phosphorylation targets. HIPK2 participates in the coordination of diverse developmental signaling pathways including TGF-β, Notch, Wnt, Hedgehog, and Hippo signaling. Most studies on HIPK2 phosphorylation of downstream effectors and HIPK2-mediated regulation of target genes at a transcriptional level were interpreted by the simple concept of turning HIPK2 on or off at a switchboard. Recent studies, however, indicate that HIPK2 is an integrating sensor for diverse cellular signaling events such as genotoxic stresses, hypoxia, and reactive oxygen species (ROS). Integration of different doses of signaling cues and damage stimuli is reflected in diverse post-translational modifications, stability, catalytic activity of HIPK2, and the consequent determination of cell fates (Fig. 1). In this review we will focus on the determination of cell fate—cell death or survival—by differential states of diverse HIPK2 post-translational modifications (termed the “HIPK2 modification code”), in particular as determined by different levels of ROS (Fig. 2) or DNA damage stimuli (Fig. 3).

Figure 1. Regulation of HIPK2 depending on the level of DNA damage. HIPK2 regulation was initially understood simply as turning ON or OFF the activity of HIPK2 and phosphorylation of downstream targets (left panel). However, recent studies suggest that HIPK2 responds differentially to different dosages of environmental stimuli and by integrating different signaling cues (right panel), and thus determines the fate of cell death or survival depending on cellular context and HIPK2 modification patterns. Blue circles, yellow rectangles, and green triangles indicate phosphorylation, SUMOylation, and acetylation of HIPK2, respectively.

Role of Hipk2 in Unstressed Conditions

Proteasomal degradation of HIPK2 in unstressed conditions

Under normal growth conditions HIPK2 is maintained at low levels and this HIPK2 stabilization results in restriction of cell growth and proliferation. Tight regulation of HIPK2 levels is achieved by ubiquitination-dependent proteasomal degradation mediated by several E3 ubiquitin ligases. Three E3 ligases are engaged in the regulation of HIPK2 ubiquitination in unstressed conditions (Fig. 4A). WD repeat and SOCS box-containing protein 1 (WSB-1) ubiquitinates the C-terminus of HIPK2 and promotes proteasomal degradation. HIPK2 degradation by WSB-1 is terminated in DNA damage conditions, and autophosphorylation of HIPK2 is markedly increased. Phosphorylated HIPK2 dissociates from WSB-1, thus relieving HIPK2 from proteasomal degradation. Consistent with these findings, recent papers have reported that autophosphorylation is essential for the catalytic activity of HIPK2 in unstressed cells and for activation of HIPK2 in DNA damage conditions. Further studies on the correlation between HIPK2 autophosphorylation and its escape from degradation by WSB-1 would enhance our understanding of HIPK2 regulation. WSB-1 is also involved in hypoxia-driven HIPK2 degradation in combination with Siah1.

The SCF E3 ligase complex contains Fbx3 together with Cullin1 and Skp1, which were originally isolated as components of the promyelocytic leukemia nuclear body (PML-NB) complex. In unstressed conditions HIPK2, as well as p300, is constitutively degraded by Fbx3-mediated polyubiquitination. HIPK2 is degraded outside of the PML-NB because PML-IV prevents HIPK2 destabilization by Fbx3-mediated degradation in the PML-NB. Polyubiquitinated HIPK2 inside the PML-NB prevents HIPK2 destabilization by Fbx3-mediated degradation in the PML-NB. Polyubiquitination of HIPK2 inside the PML-NB is not degraded but instead potentiates p53-mediated transactivation. As recruitment of HIPK2 to PML-NB is a crucial step for the apoptotic response, degradation of HIPK2 by Fbx3 might be relieved by SUMOylation-mediated recruitment of HIPK2 to the PML-NB in response to lethal doses of DNA damage stimuli.

Siah1 is another E3 ligase responsible for HIPK2 degradation. Siah1 degrades HIPK2 constitutively under normal conditions. However, in response to lethal DNA damage ataxia-telangiectasia mutated (ATM) is activated and phosphorylates Siah1 at Ser19. HIPK2 is stabilized by escape from...
HIPK2 controls cytokinesis and expression of redox-regulating genes

Although HIPK2 is maintained at low levels under normal growth conditions, it has significant roles in the proper progression of the cell cycle and maintaining cellular homeostasis by preparing cells for potential DNA damage. Reactive oxygen species play versatile roles in cells as a second messenger in signaling pathways. At higher concentrations, ROS induce cell death, thus preventing the genomic instability caused by ROS-mediated DNA damage. HIPK2 functions differentially in response to ROS in a dose-dependent manner. In unstressed normal growth conditions (physiological low levels of ROS), HIPK2 is SUMOylated and associated with HDAC3, which maintains HIPK2 in a deacetylated state. In response to an endurable dose of ROS, HIPK2 SUMOylation is replaced by PCAF-mediated HIPK2 acetylation at multiple sites (10K), promoting cell survival. In the presence of a high dose of ROS that is above the threshold for apoptosis induction, HIPK2 acetylation is blocked and unmodified HIPK2 elicits p53-dependent and p53-independent apoptosis. Green triangles and yellow rectangles indicate acetylation and SUMOylation, respectively. HDAC, histone deacetylase; PCAF, P300/CBP-associated factor; PML, promyelocytic leukemia.

In summary, under normal growth conditions, HIPK2 is phosphorylated Siah1, and phosphorylates p53 for induction of proapoptotic genes. Downregulation of Siah1 and a reverse correlation between Siah1 and HIPK2 levels were also observed in HIV-expressing transgenic mice and in cells treated with hydrogen peroxide and adriamycin (ADR). Zyxin indirectly regulates HIPK2 degradation by inhibiting Siah1 activity. Ectopically expressed Zyxin inactivates Siah1 by interfering with Siah1 dimerization and consequently stabilizes HIPK2.

**Figure 2.** Regulation of cell fate by the HIPK2 modification code depending on different doses of reactive oxygen species. Under normal physiological conditions and basal reactive oxygen species (ROS) levels, HIPK2 is SUMOylated and associated with HDAC3, which maintains HIPK2 in a deacetylated state. In response to an endurable dose of ROS, HIPK2 SUMOylation is replaced by PCAF-mediated HIPK2 acetylation at multiple sites (10K), promoting cell survival. In the presence of a high dose of ROS that is above the threshold for apoptosis induction, HIPK2 acetylation is blocked and unmodified HIPK2 elicits p53-dependent and p53-independent apoptosis. Green triangles and yellow rectangles indicate acetylation and SUMOylation, respectively. HDAC, histone deacetylase; PCAF, P300/CBP-associated factor; PML, promyelocytic leukemia.

**Figure 3.** HIPK2 controls WIP1 homeostasis by phosphorylation-mediated proteasomal degradation. Recent studies revealed another layer of HIPK2 function in the maintenance of cellular homeostasis by preparing cells for potential DNA damage. DNA double-strand breaks (DSB) are induced by ionizing radiation (IR) or other DNA damaging reagents and are repaired by homologous recombination (HR) or nonhomologous end joining (NHEJ) depending on the cell cycle. DSB-driven recruitment of the Mre11-Rad50-Nbs1 (MRN) complex and activation of the apical protein kinase ATM promotes rapid phosphorylation cascades to induce cell cycle arrest and recruitment of repair machinery to the sites of DNA damage. DSB signaling is initiated by ATM, which is maintained in an inert state in unstressed conditions. Wild-type p53-inducible phosphatase 1 (WIP1) is a negative regulator that reverses the phosphorylation of ATM and its downstream targets. WIP1 must be maintained at low levels for the rapid activation of ATM and regulators of DSB signaling. Under normal growth conditions, HIPK2 controls WIP1 homeostasis by phosphorylation-mediated proteasomal degradation (Fig. 3). HIPK2 phosphorylates WIP1 at Ser54 and Ser85, and phosphorylated WIP1 is subject to proteasomal degradation so that WIP1 is maintained at low levels. This silencing of WIP1 function is required for rapid and full activation of DSB signaling regulators in the early phase of the DNA damage response. Depletion of HIPK2 induces WIP1 stabilization, which suppresses DSB signaling and in turn decreases the survival rate of cells exposed to IR. In response to IR, phosphorylation of WIP1 by HIPK2 is gradually decreased and the stabilized WIP1 terminates DSB signaling to allow cells to recover to normal conditions. Interestingly, this mechanism is reminiscent of Siah2 regulation by HIPK2, in which HIPK2 phosphorylates and induces degradation of Siah2 in unstressed conditions and this is reversed by Siah2-mediated HIPK2 degradation in response to hypoxia.

In summary, under normal growth conditions, HIPK2 is autophosphorylated at a basal level or exists in a...
hypophosphorylated state. HIPK2 is continuously polyubiquiti-
nated by 3 E3 ubiquitin ligases and subjected to proteasomal deg-
radation to maintain a low expression level. Nonetheless, in
normal conditions HIPK2 plays a significant role in the protec-
tion of cells from DNA damage. Conjugation of HIPK2 by
SUMO-1 antagonizes ROS-triggered HIPK2 acetylation, and
HIPK2-mediated WIP1 phosphorylation maintains WIP1 at low
levels to ensure a rapid response to ionizing radiation.

Prosurvival Function of HIPK2 in Response
to Endurable DNA Damage

HIPK2 induces cell cycle arrest and expression of repair
enzymes
Mammalian cells have developed elaborate molecular net-
works to cope with endurable DNA damage. The DNA damage
checkpoint is activated to induce cell cycle arrest and expression
of regulatory proteins for DNA repair. HIPK2 is a component of
the molecular network that responds to repairable DNA damage.
To arrest the cell cycle, p21 expression is induced in multiple
ways. HIPK2 induces P300/CBP-associated factor (PCAF)-
mediated acetylation of p53 at the Lys320 residue and proteaso-
mal degradation of the p21 transcription repressor ZBTB4,43
both of which contribute to induction of p21 expression. Mem-
ers of the activating transcription factor/cAMP response element
binding protein (ATF/CREB) family are phosphorylation targets of HIPK2 that function to improve cell survival. HIPK2
induces expression of the BDNF growth factor by phosphorylat-
ing CREB at Ser271 in response to etoposide treatment. Under
conditions of repairable DNA damage, an antioxidant environ-
ment is also regulated by HIPK2, which phosphorylates ATF1 at
Ser198. Phosphorylated ATF1 potentiates the expression of a
series of antioxidant detoxification enzymes such as NADPH quin-
none oxidoreductase 1, glutathione S transferase, and heme oxy-
genase 1. HIPK2 can function at the transcriptional level as a
transcriptional corepressor and coactivator in a context-

Figure 3. Cell fate is differentially determined by HIPK2 modification and phosphorylation of downstream target proteins in response to ionizing radia-
tion. Under normal growth conditions, WIP1 is constitutively degraded by HIPK2-mediated phosphorylation, which is crucial to maintain an environment
favorable for rapid induction of the DNA damage response in response to potential DNA damage. In response to endurable ionizing radiation (IR), DSB
signaling is properly activated and DNA repair is completed. WIP1 is then stabilized by escape from HIPK2-mediated degradation and returns cells to a
normal state by dephosphorylating ATM and DSB signaling regulators. Upon a high dose of IR that is above the threshold for apoptosis induction, HIPK2
is stabilized and phosphorylates p53 at Ser46 for p53-mediated induction of apoptosis. Therefore, cell fate is differentially regulated by HIPK2 modifica-
tion and phosphorylation of downstream target proteins. Blue circles and irregular chains of circles indicate phosphorylation and polyubiquitination of
HIPK2, respectively. ATM, ataxia-telangiectasia mutated; AMPK, adenosine monophosphate-activated kinase; DSB, double-strand break; WIP-1, wild-type
p53-inducible phosphatase 1.
dependent manner. HIPK2-mediated expression of the repair enzyme p53R2 also contributes to DNA damage repair and the maintenance of genome integrity.

HIPK2 modification under conditions of repairable DNA damage

AMP-activated protein kinase (AMPK) is a versatile protein kinase responsible for numerous signaling pathways, in particular those involved in glucose sensing and autophagy induction. Upon the generation of DSBs caused by repairable doses of IR, ATM phosphorylates AMPKα, which in turn phosphorylates HIPK2 at 3 sites (T112, S114, and T1107). Phosphorylated HIPK2 dissociates from WIP1 (Fig. 3), preventing HIPK2-mediated phosphorylation and degradation of WIP1 and thus leading to WIP1 stabilization and termination of DSB repair signaling. In the late stage of the DSB response, transcriptional induction of WIP1 by p53 also assists in increasing the levels of WIP1.

HIPK2 also shows altered modification patterns in response to endurable ROS levels (Fig. 2). At a physiological level of ROS under normal growth conditions, SUMOylated HIPK2 recruits HDAC3 to maintain HIPK2 in a deacetylated state. In response to elevated ROS, however, HIPK2 is deSUMOylated and acetylated at 10 lysine residues by CBP acetyl-transferase. Alteration of the HIPK2 modification pattern from SUMOylation to acetylation results in a shift in HIPK2 localization from nuclear speckles to the nucleoplasm and the cytoplasm and release of HIPK2-mediated transcriptional repression of several redox-regulating genes. Proper acetylation of HIPK2 at endurable ROS doses is essential for tolerance against ROS-induced cell death. HIPK2 SUMOylation performs additional roles in response to genotoxic stress. HIPK2 phosphorylates the Pc2 E3 SUMO ligase and enhances its enzymatic activity. Pc2 in turn SUMOylates HIPK2, which then participates in the transcriptional repression of proapoptotic genes such as bax to inhibit apoptosis and promote cell survival.
HIPK2 regulation by the p53-Mdm2 axis

At sublethal doses of ADR, Mdm2-mediated ubiquitination of HIPK2 provides an additional layer of HIPK2 modification. Mdm2 is well known as an E3 ligase responsible for p53 degradation and as a transcriptional target of p53. The p53 and Mdm2 regulatory loop affects numerous cellular events including tumorigenesis, the DNA damage response, and cell cycle regulation. Since HIPK2 and Mdm2 induce degradation of each other in a reciprocal manner depending on cellular context and are regulators of p53 acting in opposite directions, the mechanism underlying the mutual regulation between HIPK2 and Mdm2 is not simple. At endurable doses of ADR, the p53-Mdm2 equilibrium is shifted toward Mdm2, which inhibits p53-mediated apoptosis and also downregulates HIPK2 through Mdm2-mediated ubiquitination on the HIPK2 Lys1182 residue. In this case, Mdm2 also disrupts the Axin–p53 interaction by competitive binding to p53 independent of its E3 ligase activity. Artificial modulation of Mdm2 levels through treatment with RITA and nutlin-1 results in alterations of HIPK2 stability, p53 phosphorylation, and the apoptotic efficiency of tumor cells. Consequently, dual regulation of p53 and HIPK2 by Mdm2 in response to repairable doses of DNA damaging agents allows damaged cells to protect themselves from apoptosis and to accelerate a repair program.

Role of HIPK2 in the Apoptotic Response

p53-dependent and -independent induction of apoptosis by HIPK2

Apoptosis must be tightly controlled because deregulated apoptosis can cause developmental defects and diverse human diseases. Alteration of HIPK2 modifications in response to apoptotic stimuli leads to changes in both the amount and biochemical properties of HIPK2. HIPK2 is stabilized by escape from E3 ligase-mediated proteasomal degradation. During HIPK2 stabilization, HIPK2 autophosphorylation and phosphorylation of E3 ubiquitin ligases by ATM allow HIPK2 to dissociate from E3 ubiquitin ligases. Upon DNA damage, HIPK2 phosphorylation is induced by protein kinases such as AMPK, Src, and TAK1. HIPK2 induces apoptosis in both a p53-dependent and p53-independent manner. The HIPK2–p53 axis constitutes the central regulatory axis for p53-mediated induction of apoptosis. p53 protein that is phosphorylated at Ser46 is specifically recruited to the promoters of proapoptotic genes to induce apoptosis at the transcripational level. Several negative and positive regulators of the HIPK2–p53 axis have been shown to modulate and fine-tune HIPK2–p53-dependent apoptosis.

It is well established that Ser 46 phosphorylation and stabilization of p53 in the PML-NB are the predominant roles of HIPK2 in apoptotic conditions. A macromolecular complex including Axin and Daxx is involved in this mechanism. Axin is an adaptor for formation of the Axin–HIPK2–p53 complex that is important for HIPK2-mediated p53 phosphorylation in the PML-NB. The formation of this complex is inhibited by the negative regulator Pirh2 in sublethal damage conditions and potentiated by the positive regulator Tip60 at lethal levels of DNA damage. At sublethal conditions, Pirh2 competes with HIPK2 for Axin binding and inhibits p53 Ser46 phosphorylation. At lethal doses of DNA damage, however, Tip60-mediated inhibition of the Pirh2-Axin interaction promotes HIPK2–Axin binding and induces p53 Ser46 phosphorylation in an ATM/ ATR-dependent manner. Truncation of the Axin HIPK2-interacting domain impairs HIPK2-mediated p53 phosphorylation, indicating that Axin and HIPK2 are critical determinants of cell fate depending on the severity of genotoxic stresses. Daxx is also important for HIPK2-mediated phosphorylation of p53 by serving as a bridge between p53 and Axin. In addition, the integrity of PML-NB is also regulated by HIPK2-dependent PML phosphorylation. During early stages of DNA damage, HIPK2 phosphorylates PML at Ser8 and Ser38 to induce PML stabilization and SUMOylation for the induction of apoptosis.

In response to lethal doses of genotoxic stresses, HIPK2 is stabilized by escape from proteasomal degradation and induces apoptosis through dual regulation of p53 and its negative regulator Mdm2. HIPK2 phosphorylates Mdm2, resulting in its nuclear export and degradation. The accumulated HIPK2 also phosphorylates p53 at the Ser46 residue. The simultaneous regulation of p53 and Mdm2 by HIPK2 leads to efficient induction of apoptosis.

The various functions of p53 under DNA damage conditions are not limited to the Ser46 phosphoprotein. Acetylation of p53 at Lys382 is also important for cell cycle arrest through transcriptional activation of p21 and recruitment of p53 to the promoters of pro-apoptotic genes such as Noxa and p53AIP. HIPK2 affects p53 acetylation by modulating p300/CBP and PCAF, which acetylate p53 at different sites. HIPK2 also suppresses expression of Noxa1, a catalytic subunit of NADPH oxidase, and consequently inhibits SIRT1 for p53 deacetylation, indicating that HIPK2 potentiates p53 acetylation by activation of p53 acetyl-transferase and by inhibition of the p53 deacetylating enzyme SIRT1. In addition, HIPK2 also participates in termination of the p53 response to avoid prolonged and exaggerated p53 activity by indirectly modulating p53 acetylation.

Regulation of apoptosis by HIPK2 is not only dependent on p53. Expression of proapoptotic genes is suppressed by the transcriptional corepressor C-terminal binding protein (CtBP). Under DNA damage conditions, HIPK2 phosphorylates the anti-apoptotic CtBP protein. Phosphorylated CtBP is degraded in a proteasome-dependent manner which in turn relieves CtBP-mediated downregulation of several proapoptotic genes such as PERP, p21, and Noxa. Therefore, HIPK2 induces apoptosis in p53 null cells through CtBP degradation in response to apoptotic stimuli. Upon TGF-β treatment, HIPK phosphorylates Daxx and releases it from PML-NB. The released Daxx translocates to...
the cytoplasm and activates JNK, which plays an important role in TGF-β–induced apoptosis.72

HIPK2 cleavage under DNA damage conditions and cellular differentiation

Cleavage of the autoinhibitory domain of HIPK2 adds yet another layer of HIPK2 modification in response to lethal damage.73 Caspase-6 is a transcriptional target of p53,74 positive feedback amplification of the p53–HIPK2 loop increases the sensitivity of the apoptotic response. Caspase-mediated HIPK2 cleavage is also involved in myoblast differentiation as full-length HIPK2 is required for repression of myogenic genes and gradual induction of HIPK2 cleavage results in induction of myogenic gene expression as a result of defective corepressor function of the truncated HIPK2 protein (Fig. 4C).11

Several reports have proposed the concept that HIPK2 modifications affect HIPK2 function in a combinational and complicated manner, rather than independently or individually, and thus collectively determine cell fate after differential doses of DNA damage.56,75 In contrast to HIPK2-mediated induction of apoptosis under lethal DNA damage conditions, endurable levels of DNA damage alter HIPK2 kinetics to a prosurvival function through a combination of post-translational modifications.

HIPK2 Autophosphorylation and Transphosphorylation by Other Protein Kinases

Phosphorylations of HIPK2 are among the major modifications required for induction of apoptosis in lethal DNA damage conditions. It was recently reported that HIPK2 is regulated by both autophosphorylation and transphosphorylation by other protein kinases under normal and DNA damage conditions. HIPK2 phosphorylation at Thr880 and Ser882 is induced in response to genotoxic stress and is crucial for p53 Ser46 phosphorylation and induction of apoptosis.26 Treatment of cells with lethal doses of ADR induces oligomerization and autophosphorylation of HIPK2. HIPK2 phosphorylated at T880/S882 is recognized by Pin1, which stabilizes HIPK2 through the induction of isomerization and conformational changes in HIPK2 and dissociation of Siah1. At later stages of the DNA damage response HIPK2 phosphorylates p53 at the Ser46 residue, which acts as another target of Pin1 to synergistically activate the apoptotic process.

Although the HIPK family shows amino acid similarity with the dual-specificity tyrosine phosphorylation-regulated kinase (DYRK) family,76 HIPK2 differs in the mode of action in its activation loop. Under normal conditions, cis-phosphorylation of Tyr354 in the activation loop is critical for HIPK2 kinase activity and subcellular localization.27,28 Substitution of the Tyr354 residue to phenylalanine results in cytoplasmic localization of HIPK2. Tyr354 is a target of TGF-β-induced TAK18 and Src kinase, pivotal enzymes for cell survival and development.57 Phosphorylation of Tyr354, as well as other tyrosine residues, by Src results in inactivation of HIPK2 through translocation from the nucleus to the cytosol. However, phosphorylation of Tyr354 by TGF-β-induced TAK1 is important in the transcriptional suppression of several potent angiogenic genes such as Mmp10 and Vegf. These discrepancies in the outcome of Tyr354 phosphorylation induced by different signaling cues might be explained by differential phosphorylation of HIPK2 at other sites in addition to Tyr354. In addition, phosphorylation of HIPK2 by AMPK in response to repairable doses of IR enables dissociation of WIP1 from HIPK2 to terminate DSB signaling after completion of DNA repair.56 Therefore, HIPK2 phosphorylation by cis-autophosphorylation or transphosphorylation at different sites may differentially affect HIPK2 activity, substrate recognition, and the function of HIPK2 in the determination of cell fate.

It should be noted that identification of sites of post-translational protein modification is usually conducted with over-expressed affinity-purified protein because of limited quantities of endogenous protein. However, depending on the experimental conditions overexpressed HIPK2 does not necessarily recapitulate endogenous HIPK2, especially regarding localization and post-translational modifications, because HIPK2 levels are critical for its function and post-translational modification. This might explain the discrepancy in the patterns of HIPK2 modification and tentative HIPK2 functions proposed by different researchers. Since HIPK2 is dynamically regulated at the protein level and by post-translational modification in response to various signaling cues, HIPK2 function should ideally be addressed under physiological conditions.

Concluding Remarks and Future Perspectives

Cellular responses to variable amounts of DNA damage are crucial to protect organisms from genomic instability.87 The balance between antagonistic enzymatic functions, such as protein kinases versus phosphatases or acetyl-transferases vs. deacetylating enzymes, is shifted to cope with damage stimuli and either protect cells from death or promote it. Such signal sensors integrate the severity of DNA damage or recognize the cellular threshold of the DNA damage response and determine whether the cell will die or live. HIPK2 is an integrator of several signaling pathways, especially the pathways involved in DNA damage caused by ROS or IR.22,78 Recent studies reveal that integration of DNA damage stimuli is mediated by various reversible modifications on HIPK2 (Fig. 4B). However, findings that support the existence of a “HIPK2 modification code” (Fig. 4C) raise further questions. The first concerns the regulatory factor responsible for the shift in the HIPK2 modification code. HIPK2 autophosphorylation and transphosphorylations by other protein kinases are associated with HIPK2 stability, cellular localization, and corepressor activity. Reversible conjugation of HIPK2 to the
SUMO moiety can be regulated by Pc2, SENP1, and HIPK2. 

SUMOylation determines HIPK2 acetylation, which is crucial for the protective function of HIPK2 depending on ROS concentration. Therefore, deciphering the molecular networks linking different post-translational modifications will further unveil the HIPK2 modification code and provide greater understanding of the basis for the fine-tuning of HIPK2 as a signaling hub. The second question concerns whether other modifications are involved in HIPK2 regulation. As HIPK2 is frequently regulated by other covalent modifications such as ISGylation, monoubiquitination, or acetylation, which is crucial for the protective function of HIPK2, the question will provide valuable clues to understanding the basis for the fine-tuning of HIPK2 as a signaling hub. The sophistication of human homodomain-interacting protein kinase 4 (HIPK4) as a unique member of the HIPK family. Mol Cell Pharmacol 2010; 26:1-8; PMID:20560833

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Funding

This work was supported by the Basic Science Research Program (2012R1A1A2008737 to Choi CY) and Nuclear Research & Development Program (2014M2B2A9030630 to Choi CY) through the National Research Foundation (NRF) of Korea funded by the Ministry of Education, Science and Technology. We apologize to all authors whose original papers could not be cited due to space limitations.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.
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