

# Cell Fate Regulation upon DNA Damage: p53 Serine 46 Kinases Pave the Cell Death Road

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Mild and massive DNA damage are differentially integrated into the cellular signaling networks and, in consequence, provoke different cell fate decisions. After mild damage, the tumor suppressor p53 directs the cellular response to cell cycle arrest, DNA repair, and cell survival, whereas upon severe damage, p53 drives the cell death response. One posttranslational modification of p53, phosphorylation at Serine 46, selectively occurs after severe DNA damage and is envisioned as a marker of the cell death response. However, the molecular mechanism of action of the p53 Ser46 phospho-isomer, the molecular timing of this phosphorylation event, and its activating effects on apoptosis and ferroptosis still await exploration. In this essay, the current body of evidence on the molecular function of this deadly p53 mark, its evolutionary conservation, and the regulation of the key players of this response, the p53 Serine 46 kinases, are reviewed and dissected.

dangerous genetic alterations including mutations that may initiate or promote carcinogenesis.

At the molecular level, the cellular decision-making process between these different cell fate options needs to be guided based on specific signaling events, which favor the commitment to a specific cell fate option. The transcription factor and tumor suppressor p53 plays a delicate role in organizing the cell fate decision-making process in response to DNA damage, and it has been found to potentiate DNA repair, cellular senescence, and cell death. These obviously opposing functions of p53 appear to be channeled at the level of posttranslational modification marks including site-specific phosphorylation. Although it is


## 1. Introduction

DNA damage is evoked by numerous sources including spontaneous genomic lesions and genotoxic stress. Cells confronted with DNA damage have different options to react to this hazard. Possible cellular responses are frequently mutually exclusive ones, such as activation of DNA repair and subsequent cell survival or elimination of the severely damaged cells through programmed cell death. The cellular options for cell fate decision-making are largely determined by the strength and extent of DNA damage as well as by the cellular origin of the damaged cell. Accordingly, upon mild DNA damage different signal transduction pathways are activated than in response to massive damage (Figure 1). Both for living organisms and for the individual cell, it is of fundamental importance to find an adequate balance between these different cell fate options - on the one hand, to secure cellular homeostasis and tissue functions and, on the other hand, to avoid survival of damaged cells bearing potentially

still not understood how p53 exactly shapes the different cell fate decisions, transcriptional activation of different target gene sets as well as non-transcriptional effects in the cytoplasm on mitochondrial outer-membrane permeabilization (MOMP) are involved. The plasticity of the p53 response is regulated by the temporal expression patterns of p53, its interaction with other proteins as well as post-translational modifications of p53<sup>[1]</sup> (for more detailed information we recommend some recent reviews<sup>[2,3]</sup>). Whereas the p53 phosphorylation marks at Ser15 and Ser20 occur both upon mild and massive DNA damage and are critical to stabilize p53 by breaking the interaction with the negative regulatory ubiquitin ligase MDM2,<sup>[4]</sup> there is one particular phosphorylation mark on p53 reported, phosphorylation at Serine residue 46, which is selectively linked to its cell killing activity upon severe genotoxic stress (Figure 1).

In this review, we aim at highlighting the function and regulation of the deadly p53 Ser46 phosphorylation mark. We will focus on the regulation of the key players in p53 Ser46 phosphorylation, the p53 Ser46 kinases. In addition, we will discuss the conservation of the p53 Ser46 phosphorylation-site in different species and why this could be important for our understanding of the function of this phospho-mark. Finally, since activation of the cell death response is a major goal in cancer therapy, we will also explore potential deregulation of p53 Ser46 kinases in cancer as a mechanism of resistance.

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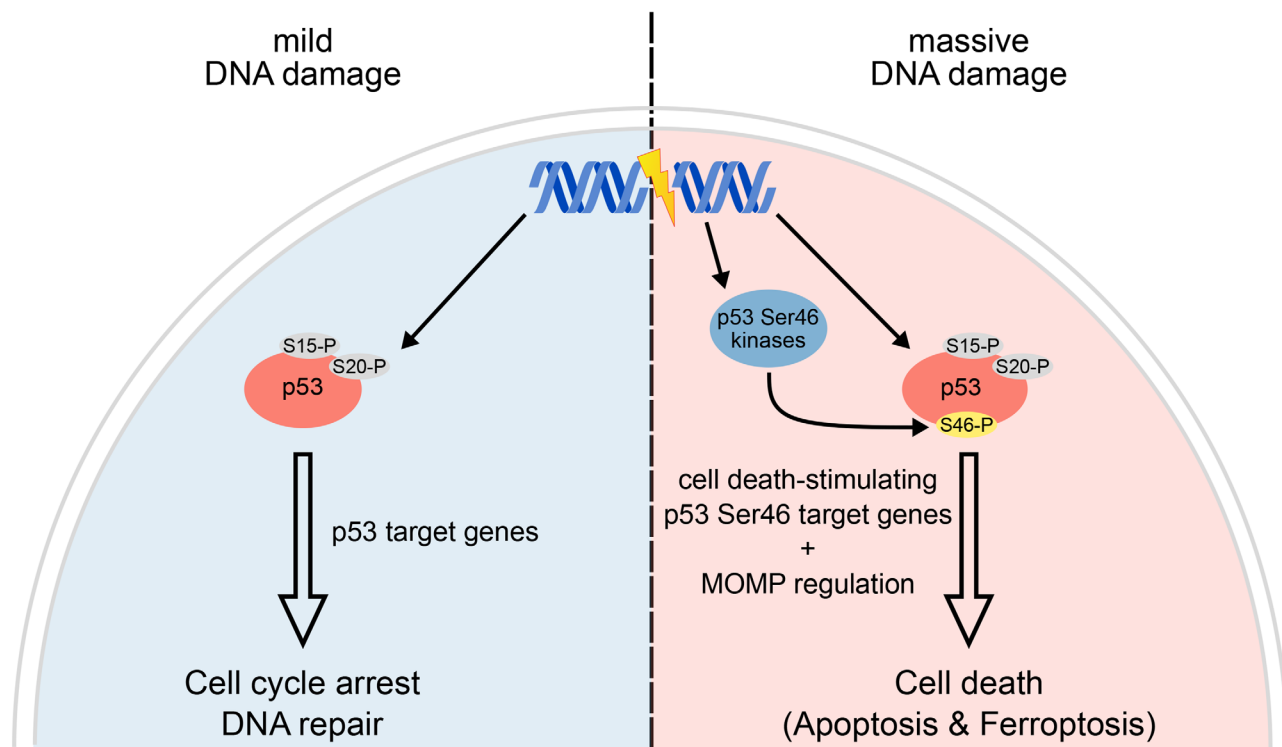
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## 2. The p53 Ser46 Phosphorylation Mark: A Molecular License to Kill?

The p53 Ser46 phosphorylation was initially reported along with the Ser33 phospho-mark in 1999 as novel p53 sites



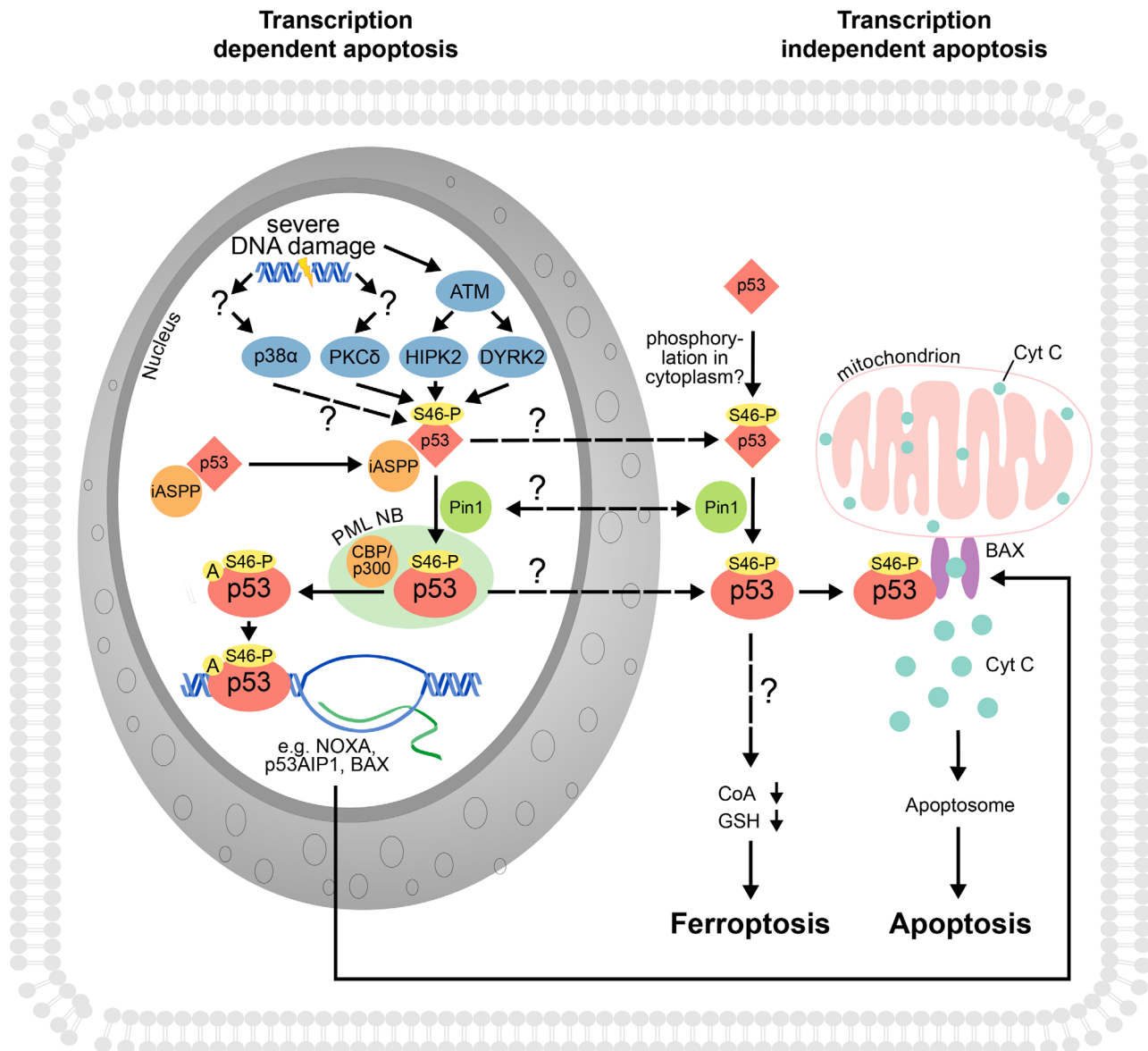
**Figure 1.** p53 differentially regulates cell fate decisions in response to mild or severe DNA damage. Upon mild damage, p53 is phosphorylated at Ser15 and Ser20 disrupting p53 degradation and thus leading to p53 stabilization and subsequently transactivation of p53 target genes. In response to severe DNA damage, the p53 Ser46 kinases additionally phosphorylate p53 at Ser46 resulting in preferential transactivation of cell death-stimulating p53 target genes and p53-dependent induction of mitochondrial outer-membrane permeabilization (MOMP).

phosphorylated in response to ultraviolet (UV) damage.<sup>[5]</sup> Two years later, a seminal study based on the generation of a phospho-specific Ser46 antibody, clearly linked the activation of p53 Ser46 phosphorylation upon severe DNA damage after ionizing radiation (IR) or UV to cell death activation.<sup>[6]</sup> This study also revealed that, in contrast to the fast kinetics of Ser15 and Ser20 phosphorylation, Ser46 phosphorylation is a relatively late event and occurs hours post damage and on top of Ser15 and Ser20 phosphorylation. Of note, how the molecular timing of the Ser46 phosphorylation event is regulated still remains a conundrum, since ATM is an essential upstream regulator for most p53 Ser46 kinases that gets activated within seconds or minutes after mild and severe DNA damage, but the Ser46 phosphorylation mark only occurs hours selectively after severe genotoxic stress. By linking the transcriptional activation of the apoptotic p53 target gene *p53AIP1*, which encodes a regulator of MOMP, to the Ser46 phosphorylation event, this study established phosphorylation at Ser46 as a pro-apoptotic post-translational p53 modification specifically linked to severe genotoxic stress.<sup>[6]</sup>

Severe genotoxic damage can also lead to activation of the cellular senescence program, a permanent cell cycle arrest triggered by irreparable DNA damage and constitutive DNA damage checkpoint activation.<sup>[7]</sup> Interestingly, p53 Ser46 phosphorylation has also been detected in human keratinocytes, genetically altered mouse embryonic fibroblasts and in cancer cells undergoing cellular senescence in response to DNA damage.<sup>[8–11]</sup> Expression of a p53<sup>S46A</sup> mutant protein resulted in reduced expression of senescence associated (SA)-beta galactosidase upon UVB or

Adriamycin treatment as well as replicative senescence and Ras-induced senescence, indicating a role for p53 Ser46 phosphorylation in the senescence responses induced by DNA damage and oncogenes. However, the exact function of p53 Ser46 phosphorylation within the cellular senescence response remains to be defined in the future.

Mechanistically, p53 Ser46 phosphorylation appears to potentiate the cell death response through nuclear, transcriptional effects and through non-transcriptional effects at the mitochondria (**Figure 2**). Various publications indicate that phosphorylation of p53 at Ser46 modulates p53 target gene transactivation. The transactivation of a distinct set of pro-apoptotic p53 target genes including BAX,<sup>[12]</sup> p53AIP1,<sup>[6]</sup> p53INP1,<sup>[13]</sup> NOXA,<sup>[14]</sup> and of PTEN,<sup>[15]</sup> an antagonist of the AKT kinase, are enhanced upon p53 Ser46 phosphorylation. What kind of mechanism may underlie this transcriptional effect? The p53 Ser46 site is situated within the second transactivation domain of p53 (TAD2).<sup>[16]</sup> Interestingly, phosphorylation of p53 at Ser46 facilitates the interaction of p53 with the prolyl-peptidyl *cis/trans* isomerase Pin1, which changes the conformation of its client protein through isomerization of the phospho-Ser46-Proline bond.<sup>[17,18]</sup> Besides this PTM, phosphorylation of p53 at Ser33, Thr81, and Ser315 have also been found to be binding sites of Pin1.<sup>[17–20]</sup> Isomerization of p53 induced by Pin1 regulates transactivation of p53 target genes by two different means: on the one hand, this conformational change of p53 dissociates the apoptosis inhibitor iASPP from p53 and on the other hand, Pin1 mediates interaction of p53 with the acetyltransferase CBP/p300, which acetylates p53 at K373



**Figure 2.** p53 phosphorylated at Ser46 regulates transcription-dependent and transcription-independent apoptosis. Upon severe DNA damage, p53 is phosphorylated at Ser46 by the p53 Ser46 kinases PKC $\delta$ , HIPK2, DYRK2, ATM and p38 $\alpha$ . This post-translational modification, on the one hand, leads to the disruption of p53 from the anti-apoptotic iASPP protein and, on the other hand, serves as a binding site for the prolyl-peptidyl *cis/trans* isomerase Pin1, which catalyzes isomerization of the phospho-Ser46-Pro47 bond. This conformational change allows interaction of p53 with the acetyltransferases CBP and p300 at PML nuclear bodies resulting in acetylation of p53, inducing efficient transactivation of cell-death stimulating p53 target genes. Additionally, p53 phosphorylated at Ser46 also stimulates apoptosis in a transcription-independent manner. Cytosolic p53, which has been isomerized by Pin1, induces a conformational change in the pro-apoptotic protein BAX, which enhances BAX-mediated mitochondrial outer membrane polarization, cytochrome C (Cyt C) release and thus apoptosis. p53 Ser46 phosphorylation and isomerization have been shown to take place in the nucleus, which suggests that the phosphorylated and/or isomerized p53 shuttles from the nucleus to the cytoplasm. Furthermore, p53 phospho-Ser46 has also been implicated in regulating ferroptosis by depleting the levels of Coenzyme A (CoA) and glutathione (GSH).

and K382 potentiating p53 transcriptional activity<sup>[21]</sup> (Figure 2). The interesting question, whether the isomerized p53 isomer can make some unique, new contacts with different coactivators or regulators of transcription remains to be answered. Notably, isomerization of p53 by Pin1 not only regulates transcription-dependent p53-induced apoptosis, but also transcription-independent cell death (Figure 2). Binding of Pin1 enhances the translocation of p53 to mitochondria and potentiates mito-

chondrial outer membrane depolarization via p53-dependent activation of the pro-apoptotic Bcl2 family member BAX and subsequently apoptosis.<sup>[22,23]</sup>

Consistent with its role in regulating apoptosis, phosphorylation of p53 at Ser46 has been implicated in pathophysiology. Mutant Huntingtin, which causes the neurodegenerative Huntington's disease, was found to enhance p53 Ser46 phosphorylation and thus triggers apoptosis in neurons.<sup>[24]</sup> A substitution

of the proline 47 that follows Ser46 with a serine, the so-called p53 Pro47 polymorphism, which is found in up to 6–8% of people of African origin,<sup>[25]</sup> has been shown to decrease p53 Ser46 phosphorylation and transactivation of pro-apoptotic p53 target genes.<sup>[25,26]</sup> This polymorphism not only affects transcription-dependent (nuclear) p53-induced apoptosis but also regulates cytosolic p53 function. Mechanistically, the p53 phospho-Ser46-Pro *cis*-isomer binds directly to BAX and upon subsequent *cis*–*trans* isomerization activates BAX through triggering a conformational change enhancing apoptosis.<sup>[23]</sup>

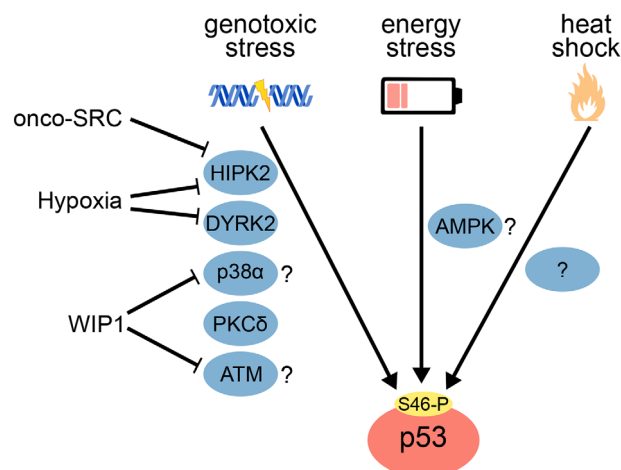
Since mice lack the p53 Ser46 site, a human p53 knock-in (Hupki) mouse model has been generated and used to investigate the effect of this site in the mouse.<sup>[27]</sup> This mouse model expressing human p53 has been successfully used to study the role of the human p53 and of the p53 Pro47 polymorphism in mice. Using human cell culture models, the p53 Pro47Ser polymorphism, which exhibits an increased cancer risk in humans, lacks p53 Ser46 phosphorylation and shows a blunted PUMA expression and cell death response upon genotoxic stress.<sup>[26]</sup> Furthermore, cells from the Hupki p53 Pro47Ser model show a defect in ferroptosis,<sup>[25]</sup> an iron-dependent cell death mechanism induced by depletion of anti-oxidative glutathione and loss of lipid hydroperoxide repair. In conclusion, p53 Ser46 phosphorylation occurs upon severe genotoxic stress and activates cell death by apoptosis and ferroptosis.

### 3. The p53 Ser46 Kinases: Masters of Life and Death?

To date, five proline-directed Ser/Thr kinases have been proposed to catalyze p53 Ser46 phosphorylation in response to DNA-damaging such as IR,<sup>[6,28]</sup> UV,<sup>[6,29,30]</sup> and treatment with chemotherapeutic agents such as Adriamycin,<sup>[6,31,32]</sup> Cisplatin,<sup>[12]</sup> and Temozolomide,<sup>[33]</sup> namely HIPK2, ATM, DYRK2, p38 $\alpha$ , and PKC $\delta$  (Figure 3). Although p53 Ser46 phosphorylation has been most abundantly studied upon genotoxic stress,<sup>[6,28–30,34,35]</sup> the Ser46 phospho-mark has also been observed in response to heat shock and energy stress by glucose deprivation and AMPK $\alpha$  activation,<sup>[36,37]</sup> suggesting an even broader function in cytotoxic stress-mediated cell death responses (Figure 3). In the following paragraphs, we will summarize the current evidence linking these kinases to p53 Ser46 phosphorylation and we will highlight the mechanisms by which the Ser46 kinases are wired to the damage-induced signaling network.

#### 3.1. Homeodomain-Interacting Protein Kinase 2

Currently, the best studied kinase and the first one shown to phosphorylate the pro-apoptotic p53 Ser46 residue is HIPK2, a nuclear body localized Ser/Thr kinase.<sup>[38]</sup> Two independent studies identified HIPK2 as a novel p53-binding protein and showed that HIPK2 is capable of directly phosphorylating p53 at Ser46 in vitro and in vivo upon severe DNA damage in different cancer cell lines.<sup>[29,30]</sup> Subcellular localization studies using immunofluorescence microscopy demonstrated that HIPK2 forms a protein complex with p53 at promyelocytic leukemia

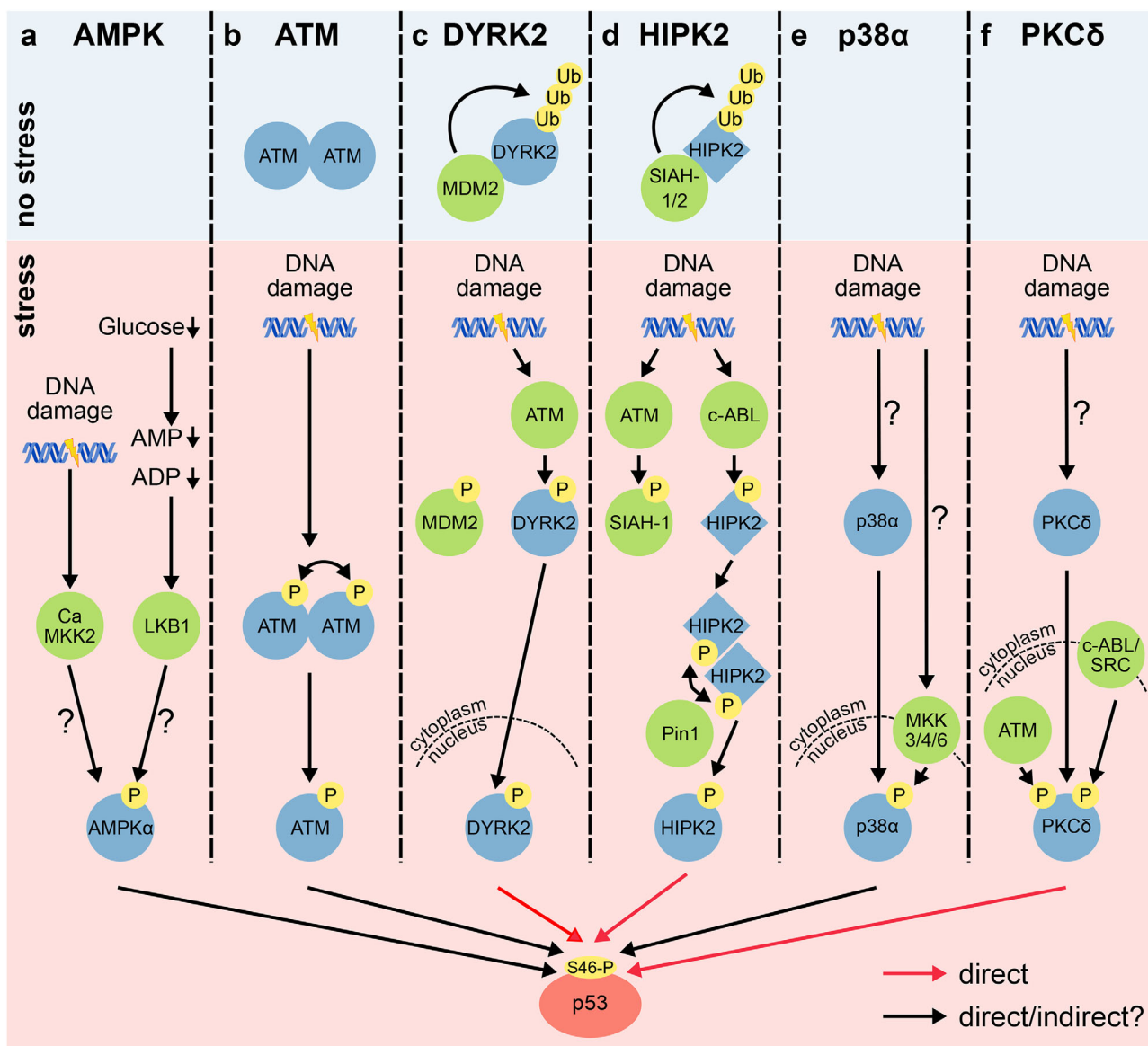


**Figure 3.** Different cellular stress conditions induce p53 Ser46 phosphorylation. In response to genotoxic stress, the p53 Ser46 kinases HIPK2, DYRK2, p38 $\alpha$ , PKC $\delta$ , and ATM catalyze phosphorylation of p53 at Ser46. It remains currently unclear if p38 $\alpha$  and ATM are direct p53 Ser46 kinases or regulate this post-translational modification by activating other kinases. The p53 Ser46 kinases are negatively regulated by other proteins, such as the kinase SRC or the phosphatase WIP1 as well as by hypoxia. p53 is also phosphorylated at Ser46 upon energy stress by the kinase AMPK $\alpha$  and upon heat shock by an unknown kinase.

(PML) bodies.<sup>[29,30,39]</sup> PML bodies are unique, stress-responsive nuclear domains regulating p53's posttranslational modification make-up and function.<sup>[40]</sup> Knock-down of PML or the use of *Pml* knock-out cells results in a dramatic drop of HIPK2-controlled p53 Ser46 phosphorylation, indicating a key role of this subcellular compartment in establishing a specific microenvironment facilitating p53 Ser46 phosphorylation.<sup>[39]</sup>

HIPK2 shares a major regulatory principle with p53, which is regulation by the ubiquitin-proteasome system (Figure 4d). Complex formation with the E3 ubiquitin ligases Siah-1 and Siah-2 triggers its poly-ubiquitination and degradation in unstressed cells.<sup>[35]</sup> Besides Siah-1 and Siah-2, additional E3 ligases have been shown to regulate HIPK2 stability.<sup>[41–43]</sup> Stabilization and activation of HIPK2 in response to genotoxic stress requires the checkpoint kinases ATM or ATR for phosphorylation-dependent disruption of the HIPK2-Siah-1 complex, and subsequent site-specific autophosphorylation at Thr880/Ser882 followed by a conformational change catalyzed by the prolyl-peptidyl *cis/trans*-isomerase Pin1.<sup>[28,32,35]</sup> This complex series of molecular events triggers HIPK2 stabilization and activation. HIPK2 stabilization is also supported by c-ABL-dependent HIPK2 phosphorylation at several tyrosine residues, which disrupt the HIPK2-Siah-1 complex.<sup>[44]</sup> In addition, HIPK2 stability and p53 Ser46 phosphorylation require expression of the Zyxin protein, which complexes with Siah-1 and Siah-2 ligases,<sup>[45]</sup> and HIPK2 activity is further boosted by caspase-dependent cleavage and release of its C-terminal negative regulatory domain upon genotoxic stress.<sup>[34]</sup> Interestingly, the p53 Ser46 kinase function of HIPK2 has also been shown to be negatively regulated by different mechanisms including rare mutations, reduced expression and oncogenic signaling, as specified in detail in Section 5. In conclusion, HIPK2 functions as a p53 Ser46 kinase in response to severe genotoxic stress and is activated by the ATM pathway to stimulate cell death.





**Figure 4.** Upstream regulation of the p53 Ser46 kinases. Activation of the p53 Ser46 kinases involves site-specific phosphorylation. a) AMPK activation involves phosphorylation at Thr172 of the catalytic alpha subunit, which is mediated by the kinases CaMKK2 and LKB1 in response to genotoxic and energy stress, respectively. b) ATM activation upon DNA damage involves autophosphorylation in *trans*. c) In unstressed cells, DYRK2 is ubiquitinated by the ubiquitin ligase MDM2 and subsequently degraded by the proteasome. In response to DNA damage, ATM-dependent phosphorylation of DYRK2 disrupts MDM2-mediated proteasomal degradation of DYRK2 and mediates accumulation of DYRK2 in the nucleus. d) HIPK2 levels are kept low due to polyubiquitination by the ubiquitin ligases Siah-1 and -2 in unstressed cells. Upon genotoxic stress, Siah-1 and HIPK2 are phosphorylated by the kinases ATM and c-ABL, respectively, resulting in dissociation of the HIPK2-Siah-1 protein complex and HIPK2 stabilization. This allows HIPK2 activation which involves autophosphorylation at Thr880/Ser882, which is followed by isomerization of HIPK2 catalyzed by the prolyl-peptidyl *cis/trans*-isomerase Pin1 leading to fully activated HIPK2. e) Activation of p38 $\alpha$  in response to DNA damage involves nuclear accumulation and phosphorylation by the upstream kinases MKK3/4/6. f) PKC $\delta$  translocates to the nucleus upon genotoxic stress, which is regulated by phosphorylation of PKC $\delta$  by the kinases ATM, c-ABL, and c-SRC.

### 3.2. Dual-Specificity Tyrosine Phosphorylation-Regulated Kinase 2

The mammalian dual-specificity tyrosine (Y) phosphorylation-regulated kinase (DYRK) family comprises five members: DYRK1A, DYRK1B, DYRK2, DYRK3, and DYRK4. These kinases catalyze autophosphorylation at tyrosine residues and

phosphorylation at serine/threonine residues of exogenous substrates.<sup>[46]</sup> Two members of the DYRK family have been shown to phosphorylate p53: DYRK1A serves as a p53 Ser15 kinase,<sup>[47]</sup> whereas DYRK2 was identified as a p53 Ser46 kinase by an expression-screening technique using a p53 phospho-Ser46 specific antibody. In vitro kinase assays confirmed that DYRK2 directly phosphorylates p53 at Ser46.<sup>[31]</sup> In vivo, DYRK2-mediated

p53 Ser46 phosphorylation was detected in response to UV or Adriamycin exposure and found to induce the expression of the p53 Ser46-responsive target gene p53AIP1 and apoptosis.<sup>[31]</sup>

Upon genotoxic stress, DYRK2 has been found to translocate from the cytoplasm to the cell nucleus<sup>[31]</sup> (Figure 4c). This nuclear shuttling was shown to be mediated by ATM-dependent phosphorylation of DYRK2 at Ser33 and Ser369, which are part of bona fide SQ motifs that serve as preferred ATM target sites. ATM-mediated phosphorylation protects DYRK2 from MDM2-regulated poly-ubiquitination and subsequent proteasomal degradation.<sup>[48]</sup> Furthermore, these phospho-marks of DYRK2 have also been shown to potentiate phosphorylation of p53 at Ser46 and apoptosis induction after DNA damage.<sup>[48]</sup> In addition to MDM2-mediated poly-ubiquitination, another E3 ubiquitin ligase, Siah-2, has been reported to regulate p53 Ser46 phosphorylation. Upon hypoxia, DYRK2 is poly-ubiquitinated by Siah-2 leading to reduced levels of DYRK2 and decreased p53 Ser46 phosphorylation.<sup>[49]</sup> Thus, the p53 Ser46 kinases DYRK2 and HIPK2 share some of their regulators including ATM for stabilization/activation and the ubiquitin ligase Siah-2 as a negative regulator. Whether HIPK2 and DYRK2 may cooperate for efficient p53 Ser46 phosphorylation upon genotoxic stress in the cell nucleus remains an interesting question to be addressed in the future. Moreover, since there appears to be no obvious functional differences between p53 Ser46 phosphorylation by DYRK2 and HIPK2 (both kinases can deliver this phospho-mark in response to UV damage or Adriamycin/Doxorubicin treatment in identical cellular model systems,<sup>[29,31,32,50]</sup> it is tempting to speculate that DYRK2 may even activate HIPK2 (and vice versa) upon DNA damage triggering efficient p53 Ser46 phosphorylation. In conclusion, DYRK2 acts as a p53 Ser46 kinase and stimulates cell death upon genotoxic stress downstream of ATM.

### 3.3. Ataxia Telangiectasia Mutated

The Ataxia Telangiectasia Mutated (ATM) kinase is a central mediator of the DNA damage response and is activated upon DNA damage, especially DNA double-strand breaks (DSBs). These DNA lesions are sensed by the MRE11-RAD50-NBS1 complex, which recruits and initiates ATM activation. ATM activation requires dissociation of the inactive ATM homodimer to the active monomer form, for which acetylation by the acetyltransferase TIP60 and in *trans* autophosphorylation of ATM are prerequisites. Once activated, ATM can phosphorylate hundreds of substrates among them p53.<sup>[51]</sup>

Upon genotoxic stress, ATM can directly phosphorylate p53 at Ser15<sup>[52–54]</sup> and, in addition, mediates p53 Ser20 phosphorylation via activation of its direct substrate CHK2, which functions as a p53 Ser20 kinase.<sup>[55]</sup> Additionally, ATM was also reported as a p53 Ser46 kinase<sup>[50]</sup> (Figure 4b). Kodama et al. showed that an engineered ATM kinase that is specifically sensitive to ATP analogues (which are not used by wild-type kinases) catalyzes phosphorylation of p53 at Ser46 by using in vitro kinase assays. Furthermore, the authors demonstrated that depletion of ATM severely reduces p53 Ser46 phosphorylation upon IR and UV damage, and that ATM-dependent p53 Ser46 phosphorylation occurs preferentially in the early response after DNA damage.<sup>[50]</sup> Given the essential

role of ATM for activation of the p53 Ser46 kinases HIPK2 and DYRK2,<sup>[28,48]</sup> the finding that ATM depletion blunts p53 Ser46 phosphorylation upon genotoxic stress could in principle also be explained through the loss of down-stream activation of HIPK2 and DYRK2 upon ATM depletion.<sup>[28,35,48]</sup>

Interestingly, subcellular localization studies using a phospho-Ser46 specific p53 antibody showed that Ser46 phosphorylated p53 partially overlaps with  $\gamma$ -H2AX, another ATM substrate marking the sites of DNA lesions, strongly suggesting that phospho-Ser46-p53 is recruited to DNA lesions. However, the function and relevance of this specific subcellular localization remains yet unclear. In conclusion, although it is well-established that ATM plays a central role in the activation of the p53 Ser46 kinases HIPK2 and DYRK2, its role as a kinase that directly phosphorylates the Ser46 residue after genotoxic stress is still under debate.

### 3.4. Mitogen-Activated Protein Kinase p38 $\alpha$

The MAP kinase p38 $\alpha$  is activated by extracellular stimuli such as growth factors, cytokines, heat, osmotic shock, UV light, and ionizing radiation and regulates a plethora of cellular processes including cell growth, cell differentiation, cell cycle, and cell death. p38 $\alpha$  is mainly activated by the upstream MAP kinase kinases (MKKs) 3, 4, and 6.<sup>[56,57]</sup> In unstressed cells, p38 $\alpha$  is localized in both the cytoplasm and the nucleus. Depending on the stimuli, activated p38 $\alpha$  can either accumulate in the cytoplasm or in the nucleus.<sup>[58]</sup> For example, UV light and IR induce nuclear translocation of p38 $\alpha$ <sup>[59]</sup> (Figure 4e). However, how p38 $\alpha$  is exactly activated in response to DNA damage remains still unknown.

Two independent studies reported that p38 $\alpha$  phosphorylates p53 at two serine residues, that is, Ser33 and Ser46.<sup>[5,60]</sup> However, other studies could not reproduce the finding that p38 $\alpha$  is a p53 Ser46 kinase. Sanchez-Prieto et al. employed in vitro kinase assays and compared p38 $\alpha$ -mediated phosphorylation of GST-p53 (1-86 aa) WT versus Ser46A and did not find a difference in the phosphorylation levels of these two p53 proteins.<sup>[61]</sup> Moreover, Oda et al. reported that they did not find evidence that p38 $\alpha$  acts as a p53 Ser46 kinase by using in vitro kinase assays and p38 inhibitors.<sup>[6]</sup> In conclusion, the exact function of p38 $\alpha$  in p53 Ser46 phosphorylation still remains unclear and further experiments are required to define its role.

### 3.5. Protein Kinase C Delta

The phosphorylation of p53 Ser46 was shown to be enhanced by the p53 target gene product TP53INP1.<sup>[13]</sup> Since TP53INP1 itself does not possess an intrinsic kinase activity, it has been postulated to recruit a kinase to regulate this PTM of p53. Yoshida et al. found that TP53INP1 interacts with the protein kinase C delta (PKC $\delta$ ) after Adriamycin exposure.<sup>[62]</sup> PKC $\delta$  belongs to the protein kinase C family and catalyzes the phosphorylation of serine and threonine residues. The authors demonstrated that PKC $\delta$  interacts with p53, directly phosphorylates p53 at Ser46 in vitro and in response to Adriamycin exposure, and enhances etoposide-induced apoptosis in a p53 phospho-Ser46-dependent manner.<sup>[62]</sup>

Interestingly, p53 and PKC $\delta$  regulate their expression by means of a positive feedback loop. On the one hand, the transcription factor p53 binds to the PKC $\delta$  promoter thereby enhancing PKC $\delta$  expression.<sup>[63]</sup> On the other hand, PKC $\delta$  regulates p53 transcription.<sup>[64]</sup> Liu et al. demonstrated that PKC $\delta$  forms a complex with the death-promoting transcriptional repressor BCLAF1. This complex binds to the p53 promoter and increases p53 expression.<sup>[65]</sup> In contrast, Vohhodina et al. failed to validate decreased p53 levels upon BCLAF1 knockdown,<sup>[66]</sup> raising the need for further experiments to clarify whether PKC $\delta$  regulates p53 expression via BCLAF1.

How is PKC $\delta$  linked to the DNA damage signaling network? Activation of PKC $\delta$  upon exposure to DNA damaging agents involves phosphorylation at tyrosine residues and nuclear localization<sup>[67–69]</sup> (Figure 4f). In response to apoptotic stimuli, PKC $\delta$  is phosphorylated at Tyr64 and Tyr155 by the kinases c-Src and c-Abl, respectively. These PTMs mediate the nuclear import of PKC $\delta$  by regulating the interaction of PKC $\delta$  with Importin- $\alpha$ .<sup>[70–73]</sup> The DNA damage mediator kinase ATM is another kinase, which has been identified to regulate the subcellular localization of PKC $\delta$ . Depletion of ATM attenuates nuclear accumulation of PKC $\delta$  in response to irradiation or cytarabine exposure.<sup>[74]</sup> However, it is unclear whether ATM directly phosphorylates PKC $\delta$  or ATM activates other kinases, which in turn phosphorylate PKC $\delta$ . In addition to Tyr64 and Tyr155, Tyr187 was identified as another PKC $\delta$  tyrosine residue which is phosphorylated in response to genotoxic stress. Blass et al. demonstrated that phosphorylation of PKC $\delta$  at Tyr64 and Tyr187 potentiates etoposide-induced apoptosis.<sup>[75]</sup> In conclusion, PKC $\delta$  functions as a Ser46 kinase and is presumably activated by the ATM-c-Abl kinase axis upon DNA damage.

### 3.6. AMP-Activated Protein Kinase

AMP-activated protein kinase (AMPK), which is a key regulator of cellular energy homeostasis, has also been linked to p53 Ser46 phosphorylation (Figure 4a). In response to glucose deprivation, both total p53 and p53 phospho-Ser46 levels are increased.<sup>[37]</sup> This rise can be repressed by depletion of AMPK.<sup>[37]</sup> In addition to Ser46 phosphorylation, AMPK has been identified to phosphorylate p53 at Ser15 in response to pharmacological AMPK activation, glucose deprivation, and MYC-induced AMPK activation.<sup>[76–78]</sup> AMPK-dependent p53 Ser15 phosphorylation potentiates its stabilization and leads to cell cycle arrest and p53 accumulation at mitochondria.<sup>[77,78]</sup> Whereas AMPK acts as a direct kinase for p53 Ser15,<sup>[76]</sup> its role in p53 Ser46 phosphorylation remains to be determined in detail, since no in vitro kinase assays have been performed and it thus remains to be demonstrated, if AMPK may directly phosphorylate this p53 residue. Instead, AMPK could act as an upstream activator of other p53 Ser46 kinases, which in turn directly catalyze p53 Ser46 phosphorylation.

How is AMPK activity regulated? The full activation of AMPK requires phosphorylation of AMPK Thr172, which is performed by two major upstream kinases, namely LKB1 and CaMKK2. LKB1 is responsible for AMPK phosphorylation and activation upon energy stress, whereas CaMKK2 phosphorylates AMPK

in response to an increase in intracellular Ca<sup>2+</sup> levels.<sup>[79]</sup> Interestingly, AMPK has also been found to be activated in response to genotoxic stress induced by the topoisomerase inhibitor etoposide. This activation is mediated by CaMKK2<sup>[80]</sup> and raises the possibility that AMPK not only phosphorylates p53 Ser46 in response to low glucose levels, but also contributes to this phosphorylation event in response to DNA damage. In conclusion, although AMPK is involved in p53 Ser46 phosphorylation, experimental evidence for direct phosphorylation is still to be awaited.

### 4. The p53 Ser46 Site During Evolution: Time Will Tell?

p53 function and regulation are known to substantially differ between different species and obviously have evolved in response to specific requirements depending on the lifestyle and specific environmental settings of a given species. For instance, the rapid and instant responsiveness of p53 activation by means of protein stabilization and its complex posttranslational modifications are not found in invertebrates (such as the round worm *Caenorhabditis elegans* and the fruitfly *Drosophila melanogaster*) or in vertebrates such as the zebrafish *Danio rerio*, where p53 appears to be largely transcriptionally regulated upon stress.<sup>[81,82]</sup>

To obtain insight into the evolutionary conservation of the p53 Ser46 phosphorylation site (which is flanked by a C-terminal Pro residue), we aligned the N-terminal p53 amino acid sequences surrounding the region around Ser46 of different species (Figure 5). This alignment indicated that the Ser46 phospho-site is absent in numerous species including virtually all important animal model systems used in basic and biomedical research, including rats, dogs, cats, and mice. In contrast, the Ser46 phospho-site appears to be fully conserved in primates, such as gorillas, chimpanzees, bonobos, orang-utans, macaques, and humans. Remarkably, outside this group, there is an additional small set of mammals showing also conservation of the Ser46 site. This group involves whales, wild boars, treeshrews, the naked mole rat and the African soft-furred rat *Mastomys coucha* (Figure 5), which is a multi-mammate rat and forms a separate genus that differs from rat or mouse. Strikingly, although the p53 Ser46 phosphorylation site is conserved in a number of species beyond human, any experimental evidence indicating phosphorylation at this site in cells derived of those species is currently lacking.

Although the Hupki mouse model has revealed important insights into the function of human p53 and p53 Ser46 phosphorylation in mice (see Section 2 of our review), it is worth reflecting the biological meaning of these observations from an evolutionary perspective since murine p53 lacks the Ser46 phosphorylation site. Thus, mice have successfully adapted to exist without the Ser46 residue, and therefore the molecular machineries reading and interpreting the Ser46 phosphorylation site did not evolve. Accordingly, it appears likely that murine cells are not equipped with the molecular mechanisms to handle and interpret the Ser46 phospho-mark. Thus, translating findings on the Ser46 site obtained from the mouse model to human should be handled with caution, since important functions of the Ser46 phosphorylation mark might simply be overseen in the murine context. In conclusion, the biology and pathobiology of the p53

Species	aa Sequence	aa
Homo sapiens (Human)	31 VLSPLP--SQA-MDDLMLSPDDIEQWFTEDPGPD 61	
Pan paniscus (Bonobo)	31 VLSPLP--SQA-MDDLMLSPDDIEQWFTEDPGPD 61	
Pan troglodites (Chimpanzee)	31 VLSPLP--SQA-MDDLMLSPDDIEQWFTEDPGPD 61	
Gorilla gorilla (Gorilla)	31 VLSPLP--SQA-MDDLMLSPDDIEQWFTEDPGPD 61	
Pongo abelii (Orangutan)	31 VLSPLP--SQA-VDDLMLSPDDIAQWFIEDPGPD 61	
Macaca mulatta (Rhesus macaque)	31 VLSPLP--SQA-VDDLMLSPDDLAQWLTEDEPGPD 61	
Heterocephalus glaber (Naked mole-rat)	31 VLSSSLS-SP--MDDLMLSPEDVNVNWLGGNP--D 59	
Tupaia belangeri (Northern treeshrew)	31 VLSPLP--SQA-MDDLMLSPDDIEQWFTEDPGPD 61	
Sorex araneus (Common shrew)	31 NVLY-----PE-VDDLMLSP-DVENWLDKSS--E 55	
Oryctolagus cuniculus (Rabbit)	31 LLTTSLN-PP--VDDL-LSAEDVANWLNEDP--E 58	
Cricetulus griseus (Chinese hamster)	31 VLSTLP-SSDSI-EELFLSP-ENVTGWLEDSGGAL 61	
Rattus norvegicus (Rat)	31 ILPTTATGSPNSMEDLFL-PQDVAELLEGPEEAL 60	
Mus musculus (Mouse)	34 ILPS-----PHCMDDLMLSPQDVEEFEGPSEAL 58	
Mastomys coucha (Southern multimammate mouse)	28 VLSE---ASPNSMDNMFLSP-DVNVNLEGPEEAL 54	
Sus scrofa (Wild boar)	31 LLSSELS-LAAV-NDLMLSP--VTNWLDENP--D 58	
Delphinapterus leucas (Beluga whale)	31 LLSSELS-PA-V-DLMLSPEDVANWLDERP--D 59	
Canis lupus familiaris (Dog)	31 VLSSELC-PAV--DELL-SPESVNVNWLDEDS--D 58	
Felis catus (Cat)	31 VLSSELS-SAM--NELPLSP-EDVANWLDEAP--D 58	

**Figure 5.** Evolutionary conservation of the p53 Ser46 phosphorylation site. Alignment of p53 amino acid sequences surrounding the human p53 Ser46 residue from different species is shown. Protein sequences were downloaded from the NCBI protein database, the amino acid sequences were aligned with Clustal Omega at EBI using default settings and manually curated. Highlighted are the human p53 transactivation domain 2 (TAD2, amino acids 40-60<sup>[16]</sup>), the Serine corresponding to human Ser46 in green and the Proline residue corresponding to human Pro47 in blue.

Ser46 residue in vivo still remain to be clarified. To obtain insight into the function of p53 Ser46 phosphorylation in vivo, it will be important to consider using animal models different from mice or rats—model systems that show clear conservation of the p53 Ser46 phosphorylation site.

## 5. Are p53 Ser46 Kinases Deregulated in Human Cancers?

Due to the essential role of p53 Ser46 phosphorylation in guiding cell fate decisions after genotoxic stress toward cell death, it is tempting to speculate that cancer cell resistance to radiation therapy or chemotherapy, at least in part may involve deregulation of the p53 Ser46 kinases.

In principle, the function of p53 Ser46 kinases in cancer cells could be disturbed by various means, including mutations, low expression, increased proteasomal degradation, subcellular mislocalization, and deregulated activation of their kinase activity. Indeed, our analysis of the TCGA PanCancer Atlas Studies revealed that the p53 Ser46 kinases have overall mutations rates between 1.4% and 6% in human cancer samples (including the five most common cancer entities worldwide: lung, breast, colorectal, prostate, and stomach cancers). AMPK shows an

overall mutation rate of 2.1–3% (for the two isoforms of the catalytic subunit, respectively), ATM of 6%, DYRK2 of 2.7%, HIPK2 of 2.9%, p38 $\alpha$  of 1.4%, and PKC $\delta$  of 1.7% (assessed via <http://cbiportal.org>). Reduced expression of the p53 Ser46 kinases in human tumors has been reported for four of the kinases: ATM in hormone-negative breast cancer<sup>[83]</sup> and non-small cell lung cancer;<sup>[84]</sup> DYRK2 in colorectal cancer,<sup>[85]</sup> hepatocellular carcinoma,<sup>[86]</sup> and breast cancer;<sup>[87]</sup> HIPK2 in oesophageal squamous cell carcinoma;<sup>[88]</sup> and PKC $\delta$  in colon cancer.<sup>[89]</sup> However, since the function of kinases depends on their specific activity and a number of Ser46 kinases, including DYRK2, HIPK2, PKC $\delta$ , and p38 $\alpha$ , are regulated by their subcellular localization, it will be a complex task to determine their potential deregulation in cancer. Activation of these kinases involves phosphorylation at specific residues, which could be assessed by mass spectrometry or by immunohistochemistry using phospho-specific antibodies. To our knowledge, so far exclusively the activation of AMPK has been conclusively investigated in cancer samples by immunohistochemistry. Low expression of phospho-AMPK has been detected in bladder cancer,<sup>[90]</sup> breast cancer,<sup>[91]</sup> hepatocellular carcinoma,<sup>[92]</sup> and in pancreatic ductal adenocarcinoma.<sup>[93]</sup> In addition, in pancreatic ductal adenocarcinoma, low levels of phospho-AMPK correlated with reduced survival.<sup>[93]</sup> In line with these findings, high levels of phospho-AMPK were associated



with better overall survival in lung adenocarcinoma,<sup>[94]</sup> whereas no clear correlation between survival and phospho-AMPK levels was detected in patients with colorectal cancer.<sup>[95]</sup> Since AMPK can context dependently both suppress and promote carcinogenesis, pharmacological activation of AMPK activity has to be critically evaluated on a case-to-case basis.<sup>[96]</sup>

Both HIPK2 and DYRK2 are negatively regulated by the E3 ubiquitin ligase Siah-2, which is an important regulator of the hypoxia response of tumors.<sup>[97]</sup> Accordingly, on bases of cell culture studies, it has been demonstrated that both HIPK2 and DYRK2 are degraded under hypoxic conditions, which leads to reduced p53 Ser46 phosphorylation upon chemotherapeutic treatment using Adriamycin/Doxorubicin.<sup>[49,98,99]</sup> Since this was the only chemotherapeutic drug tested in this context, it remains currently unknown whether this effect is drug-specific or may represent a more general phenomenon.

In addition, Siah-1 and Siah-2 are overexpressed in various cancer entities including prostate, lung, and breast cancer.<sup>[100–102]</sup> In principle, this may also result in reduced HIPK2 and DYRK2 protein levels. However, formal proof of this assumption is currently lacking and it remains to be determined in the future.

A few reports indicate that the function of HIPK2 as p53 Ser46 kinase is negatively regulated by oncogenic signaling. For instance, the cutaneous Human Papilloma Virus (HPV) type 23 expressed E6 protein binds to HIPK2 and interferes with p53 Ser46 phosphorylation.<sup>[103]</sup> An increased load of HPV23 and HPV36 is significantly associated with development of cutaneous squamous cell carcinoma, suggesting that this interference might be of relevance.<sup>[104]</sup> In addition, oncogenic Src kinase has been found to phosphorylate HIPK2 at numerous Tyr residues, which leads to a mislocalization of the kinase to the cytoplasm.<sup>[105]</sup> Accordingly, Src expression inhibited HIPK2-mediated p53 Ser46 phosphorylation and cell death induction in response to Adriamycin treatment.<sup>[105]</sup> However, it is currently unknown whether cancer entities showing oncogenic Src activation also escape chemotherapy through this mechanism. In conclusion, although there is first evidence for a potential deregulation of selected p53 Ser46 kinases in some cancer entities, clear cut evidence for a functional deregulation of p53 Ser46 kinases in cancer still awaits to be provided.

Last but not least, many of the p53 Ser46 kinases show context specific tumor-suppressive as well as tumor-promoting functions,<sup>[67,106,107]</sup> which is not surprising given the plethora of substrates of each kinase. This highlights the need for further research into the function of the p53 Ser46 kinases on carcinogenesis with a focus on the tumor entity and cancer therapy.

## 6. Conclusions and Perspectives

During the past years, p53 Ser46 phosphorylation has been defined as a p53 posttranslational modification that is specifically linked to severe, cell death-promoting DNA damage and is absent in response to mild, repairable genotoxic stress.<sup>[6,31,35,108]</sup> Although the mechanism of action of Ser46 phosphorylation has been linked to p53-dependent transcriptional regulation and non-transcriptional effects regulating MOMP at mitochondria, its detailed mechanism of action still needs to be defined and numerous important questions need to be addressed to

understand the function and regulation of this fascinating p53 mark. For instance, how is the molecular timing of this late-occurring phosphorylation event controlled, as ATM, which is a major upstream regulator of Ser46 kinases, is instantly activated after damage? Moreover, do the *cis* and *trans* isomers of p53-Ser46-Pro show different DNA binding properties due to isomerization within the transactivation domain 2 of p53? Furthermore, does isomerization affect the protein–protein interaction properties of p53 in a broader range? What is the genome-wide set of p53 target genes that are specifically bound and/or transactivated by phospho-Ser46 p53? In addition, the exact role of p53 Ser46 in ferroptosis regulation still needs to be defined. Finally, it will be of particular interest to develop strategies to pharmacologically manipulate p53 Ser46 phosphorylation in order to trigger efficiently the cell death road in cancer cells or to prevent disease-associated cell death in neurodegenerative diseases.

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## Conflict of Interest

The authors declare no conflict of interest.

## Keywords

apoptosis, cancer, DNA damage, ferroptosis, HIPK2, kinase, p53 Serine 46

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- [1] A. Hafner, M. L. Bulyk, A. Jambhekar, G. Lahav, *Nat. Rev. Mol. Cell Biol.* **2019**, *20*, 199.
- [2] Y. Liu, O. Tavana, W. Gu, *J. Mol. Cell Biol.* **2019**, *11*, 564.
- [3] S. Matt, T. G. Hofmann, *Cell. Mol. Life Sci.* **2016**, *73*, 2829.
- [4] J. P. Kruse, W. Gu, *Cell* **2009**, *137*, 609.
- [5] D. V. Bulavin, S. Saito, M. C. Hollander, K. Sakaguchi, C. W. Anderson, E. Appella, A. J. Fornace, *EMBO J.* **1999**, *18*, 6845.
- [6] K. Oda, H. Arakawa, T. Tanaka, K. Matsuda, C. Tanikawa, T. Mori, H. Nishimori, K. Tamai, T. Tokino, Y. Nakamura, Y. Taya, *Cell* **2000**, *102*, 849.
- [7] F. Rossiello, U. Herbig, M. P. Longhese, M. Fumagalli, F. d'Adda di Fagagna, *Curr. Opin. Genet. Dev.* **2014**, *26*, 89.
- [8] L. Feng, M. Hollstein, Y. Xu, *Cell Cycle* **2006**, *5*, 2812.
- [9] A. Kurihara, H. Nagoshi, M. Yabuki, R. Okuyama, M. Obinata, S. Ikawa, *Genes Cells* **2007**, *12*, 853.
- [10] D. A. Lewis, Q. Yi, J. B. Travers, D. F. Spandau, *Mol. Biol. Cell* **2008**, *19*, 1346.
- [11] S.-J. Lee, S.-H. Lee, M.-H. Yoon, B.-J. Park, *Neoplasia* **2013**, *15*, 727.
- [12] V. Di Stefano, C. Rinaldo, A. Sacchi, S. Soddu, G. D'Orazi, *Exp. Cell Res.* **2004**, *293*, 311.

- [13] S. Okamura, H. Arakawa, T. Tanaka, H. Nakanishi, C. C. Ng, Y. Taya, M. Monden, Y. Nakamura, *Mol. Cell* **2001**, *8*, 85.
- [14] S. J. A. Ichwan, S. Yamada, P. Sumrejkanchanakij, E. Ibrahim-Auerkari, K. Eto, M.-A. Ikeda, *Oncogene* **2006**, *25*, 1216.
- [15] L. D. Mayo, Y. R. Seo, M. W. Jackson, M. L. Smith, J. Rivera Guzman, C. K. Korgaonkar, D. B. Donner, *J. Biol. Chem.* **2005**, *280*, 25953.
- [16] K. H. Vousden, C. Prives, *Cell* **2009**, *137*, 413.
- [17] P. Zacchi, M. Gostissa, T. Uchida, C. Salvagno, F. Avolio, S. Volinia, Z. Ronai, G. Blandino, C. Schneider, G. Del Sal, *Nature* **2002**, *419*, 853.
- [18] T. Polonio-Vallon, D. Krüger, T. G. Hofmann, *Front. Oncol.* **2014**, *4*, 148.
- [19] H. Zheng, H. You, X. Z. Zhou, S. A. Murray, T. Uchida, G. Wulf, L. Gu, X. Tang, K. P. Lu, Z.-X. J. Xiao, *Nature* **2002**, *419*, 849.
- [20] G. M. Wulf, Y.-C. Liou, A. Ryo, S. W. Lee, K. P. Lu, *J. Biol. Chem.* **2002**, *277*, 47976.
- [21] F. Mantovani, F. Tocco, J. Girardini, P. Smith, M. Gasco, X. Lu, T. Crook, G. Del Sal, *Nat. Struct. Mol. Biol.* **2007**, *14*, 912.
- [22] G. Sorrentino, M. Mioni, C. Giorgi, N. Ruggeri, P. Pinton, U. Moll, F. Mantovani, G. Del Sal, *Cell Death Differ.* **2013**, *20*, 198.
- [23] A. V. Follis, F. Llambi, P. Merritt, J. E. Chipuk, D. R. Green, R. W. Kriwacki, *Mol. Cell* **2015**, *59*, 677.
- [24] A. Grison, F. Mantovani, A. Comel, E. Agostoni, S. Gustincich, F. Persichetti, G. Del Sal, *Proc. Natl. Acad. Sci. USA* **2011**, *108*, 17979.
- [25] M. Jennis, C.-P. Kung, S. Basu, A. Budina-Kolomets, J. I.-J. Leu, S. Khaku, J. P. Scott, K. Q. Cai, M. R. Campbell, D. K. Porter, X. Wang, D. A. Bell, X. Li, D. S. Garlick, Q. Liu, M. Hollstein, D. L. George, M. E. Murphy, *Genes Dev.* **2016**, *30*, 918.
- [26] X. Li, P. Dumont, A. Della Pietra, C. Shetler, M. E. Murphy, *J. Biol. Chem.* **2005**, *280*, 24245.
- [27] J.-L. Luo, Q. Yang, W.-M. Tong, M. Hergenbahn, Z.-Q. Wang, M. Hollstein, *Oncogene* **2001**, *20*, 320.
- [28] I. Dauth, J. Kruger, T. G. Hofmann, *Cancer Res.* **2007**, *67*, 2274.
- [29] T. G. Hofmann, A. Möller, H. Sirma, H. Zentgraf, Y. Taya, W. Dröge, H. Will, M. L. Schmitz, *Nat. Cell Biol.* **2002**, *4*, 1.
- [30] G. D'Orazi, B. Cecchinelli, T. Bruno, I. Manni, Y. Higashimoto, S. Saito, M. Gostissa, S. Coen, A. Marchetti, G. Del Sal, G. Piaggio, M. Fanciulli, E. Appella, S. Soddu, *Nat. Cell Biol.* **2002**, *4*, 11.
- [31] N. Taira, K. Nihira, T. Yamaguchi, Y. Miki, K. Yoshida, *Mol. Cell* **2007**, *25*, 725.
- [32] N. Bitomsky, E. Conrad, C. Moritz, T. Polonio-Vallon, D. Sombroek, K. Schultheiss, C. Glas, V. Greiner, C. Herbel, F. Mantovani, G. del Sal, F. Peri, T. G. Hofmann, *Proc. Natl. Acad. Sci. USA* **2013**, *110*, E4203.
- [33] Y. He, W. P. Roos, Q. Wu, T. G. Hofmann, B. Kaina, *Mol. Cancer Res.* **2019**, *17*, 1129.
- [34] E. Gresko, A. Roscic, S. Ritterhoff, A. Vichalkovski, G. del Sal, M. L. Schmitz, *EMBO J.* **2006**, *25*, 1883.
- [35] M. Winter, D. Sombroek, I. Dauth, J. Moehlenbrink, K. Scheuermann, J. Crone, T. G. Hofmann, *Nat. Cell Biol.* **2008**, *10*, 812.
- [36] L. Li, Z. Su, Z. Zou, H. Tan, D. Cai, L. Su, Z. Gu, *Cell Death Dis.* **2019**, *10*, 96.
- [37] R. Okoshi, T. Ozaki, H. Yamamoto, K. Ando, N. Koida, S. Ono, T. Koda, T. Kamijo, A. Nakagawara, H. Kizaki, *J. Biol. Chem.* **2008**, *283*, 3979.
- [38] T. G. Hofmann, C. Glas, N. Bitomsky, *BioEssays* **2013**, *35*, 55.
- [39] A. Möller, H. Sirma, T. G. Hofmann, S. Rueffer, E. Klimczak, W. Dröge, H. Will, M. L. Schmitz, *Cancer Res.* **2003**, *63*, 4310.
- [40] H. R. Chang, A. Munkhjargal, M.-J. Kim, S. Y. Park, E. Jung, J.-H. Ryu, Y. Yang, J.-S. Lim, Y. Kim, *Mutat. Res., Fundam. Mol. Mech. Mutagen.* **2018**, *809*, 99.
- [41] C. Rinaldo, A. Prodosmo, F. Mancini, S. Iacovelli, A. Sacchi, F. Moretti, S. Soddu, *Mol. Cell* **2007**, *25*, 739.
- [42] D. W. Choi, Y.-M. Seo, E.-A. Kim, K. S. Sung, J. W. Ahn, S.-J. Park, S.-R. Lee, C. Y. Choi, *J. Biol. Chem.* **2008**, *283*, 4682.
- [43] Y. Shima, T. Shima, T. Chiba, T. Irimura, P. P. Pandolfi, I. Kitabayashi, *Mol. Cell Biol.* **2008**, *28*, 7126.
- [44] N. Reuven, J. Adler, Z. Porat, T. Polonio-Vallon, T. G. Hofmann, Y. Shaul, *J. Biol. Chem.* **2015**, *290*, 16478.
- [45] J. Crone, C. Glas, K. Schultheiss, J. Moehlenbrink, E. Kriehoff-Henning, T. G. Hofmann, *Cancer Res.* **2011**, *71*, 2350.
- [46] U. Soppa, W. Becker, *Curr. Biol.* **2015**, *25*, R488.
- [47] J. Park, Y. Oh, L. Yoo, M.-S. Jung, W.-J. Song, S.-H. Lee, H. Seo, K. C. Chung, *J. Biol. Chem.* **2010**, *285*, 31895.
- [48] N. Taira, H. Yamamoto, T. Yamaguchi, Y. Miki, K. Yoshida, *J. Biol. Chem.* **2010**, *285*, 4909.
- [49] M. Pérez, C. García-Limones, I. Zapico, A. Marina, M. L. Schmitz, E. Muñoz, M. A. Calzado, *J. Mol. Cell Biol.* **2012**, *4*, 316.
- [50] M. Kodama, C. Otsubo, T. Hirota, J. Yokota, M. Enari, Y. Taya, *Mol. Cell Biol.* **2010**, *30*, 1620.
- [51] Y. Shiloh, Y. Ziv, *Nat. Rev. Mol. Cell Biol.* **2013**, *14*, 197.
- [52] J. D. Siliciano, C. E. Canman, Y. Taya, K. Sakaguchi, E. Appella, M. B. Kastan, *Genes Dev.* **1997**, *11*, 3471.
- [53] S. Banin, L. Moyal, S.-Y. Shieh, Y. Taya, C. W. Anderson, L. Chessa, N. I. Smorodinsky, C. Prives, Y. Reiss, Y. Shiloh, Y. Ziv, *Science* **1998**, *281*, 1674.
- [54] C. E. Canman, D. S. Lim, K. A. Cimprich, Y. Taya, K. Tamai, K. Sakaguchi, E. Appella, M. B. Kastan, J. D. Siliciano, *Science* **1998**, *281*, 1677.
- [55] A. M. Bode, Z. Dong, *Nat. Rev. Cancer* **2004**, *4*, 793.
- [56] T. Zarubin, J. Han, *Cell Res.* **2005**, *15*, 11.
- [57] D. Brancho, N. Tanaka, A. Jaeschke, J.-J. Ventura, N. Kelkar, Y. Tanaka, M. Kyuuma, T. Takeshita, R. A. Flavell, R. J. Davis, *Genes Dev.* **2003**, *17*, 1969.
- [58] A. Cuadrado, A. R. Nebreda, *Biochem. J.* **2010**, *429*, 403.
- [59] C. D. Wood, T. M. Thornton, G. Sabio, R. A. Davis, M. Rincon, *Int. J. Biol. Sci.* **2009**, *5*, 428.
- [60] J.-L. Perfettini, M. Castedo, R. Nardacci, F. Ciccocanti, P. Boya, T. Roumier, N. Larochette, M. Piacentini, G. Kroemer, *J. Exp. Med.* **2005**, *201*, 279.
- [61] R. Sanchez-Prieto, J. M. Rojas, Y. Taya, J. S. Gutkind, *Cancer Res.* **2000**, *60*, 2464.
- [62] K. Yoshida, H. Liu, Y. Miki, *J. Biol. Chem.* **2006**, *281*, 5734.
- [63] N. Dashzeveg, S. Yogosawa, K. Yoshida, *Cancer Lett.* **2016**, *374*, 167.
- [64] T. Abbas, D. White, L. Hui, K. Yoshida, D. A. Foster, J. Bargonetti, *J. Biol. Chem.* **2004**, *279*, 9970.
- [65] H. Liu, Z.-G. Lu, Y. Miki, K. Yoshida, *Mol. Cell Biol.* **2007**, *27*, 8480.
- [66] J. Vohhodina, E. M. Barros, A. L. Savage, F. G. Liberante, L. Manti, P. Bankhead, N. Cosgrove, A. F. Madden, D. P. Harkin, K. I. Savage, *Nucleic Acids Res.* **2017**, *45*, 12816.
- [67] M. E. Reyland, D. N. M. Jones, *Pharmacol. Ther.* **2016**, *165*, 1.
- [68] T. A. DeVries, M. C. Neville, M. E. Reyland, H. Kasaki, S. Kharbanda, M. Robertson, T. Ghayur, W. W. Wong, R. Kamen, R. Weichselbaum, *EMBO J.* **2002**, *21*, 6050.
- [69] T. A. DeVries-Seimon, A. M. Ohm, M. J. Humphries, M. E. Reyland, *J. Biol. Chem.* **2007**, *282*, 22307.
- [70] T. S. Adwan, A. M. Ohm, D. N. M. Jones, M. J. Humphries, M. E. Reyland, *J. Biol. Chem.* **2011**, *286*, 35716.
- [71] S. M. Wie, T. S. Adwan, J. DeGregori, S. M. Anderson, M. E. Reyland, *J. Biol. Chem.* **2014**, *289*, 10900.
- [72] M. J. Humphries, A. M. Ohm, J. Schaack, T. S. Adwan, M. E. Reyland, *Oncogene* **2008**, *27*, 3045.
- [73] Z.-M. Yuan, T. Utsugisawa, T. Ishiko, S. Nakada, Y. Huang, S. Kharbanda, R. Weichselbaum, D. Kufe, *Oncogene* **1998**, *16*, 1643.
- [74] K. Yoshida, H.-G. Wang, Y. Miki, D. Kufe, S. Kharbanda, M. Robertson, T. Ghayur, W. W. Wong, R. Kamen, R. Weichselbaum, *EMBO J.* **2003**, *22*, 1431.

- [75] M. Blass, I. Kronfeld, G. Kazimirsky, P. M. Blumberg, C. Brodie, *Mol. Cell. Biol.* **2002**, *22*, 182.
- [76] K. Imamura, T. Ogura, A. Kishimoto, M. Kaminishi, H. Esumi, *Biochem. Biophys. Res. Commun.* **2001**, *287*, 562.
- [77] R. G. Jones, D. R. Plas, S. Kubek, M. Buzzai, J. Mu, Y. Xu, M. J. Birnbaum, C. B. Thompson, *Mol. Cell* **2005**, *18*, 283.
- [78] A. I. Nieminen, V. M. Eskelinen, H. M. Haikala, T. A. Tervonen, Y. Yan, J. I. Partanen, J. Klefström, *Proc. Natl. Acad. Sci. USA* **2013**, *110*, E1839.
- [79] D. Garcia, R. J. Shaw, *Mol. Cell* **2017**, *66*, 789.
- [80] D. Vara-Ciruelos, M. Dandapani, A. Gray, E. O. Egbani, A. M. Evans, D. G. Hardie, *Mol. Cancer Res.* **2018**, *16*, 345.
- [81] A. C. Joerger, A. R. Fersht, *Annu. Rev. Biochem.* **2016**, *85*, 375.
- [82] A. W. MacInnes, A. Amsterdam, C. A. Whittaker, N. Hopkins, J. A. Lees, *Proc. Natl. Acad. Sci. USA* **2008**, *105*, 10408.
- [83] X. Feng, H. Li, M. Dean, H. E. Wilson, E. Kornaga, E. K. Enwere, P. Tang, A. Paterson, S. P. Lees-Miller, A. M. Magliocco, G. Bebb, *Breast Cancer Res.* **2015**, *17*, 65.
- [84] L. F. Petersen, A. C. Klimowicz, S. Otsuka, A. A. Elegbede, S. K. Petrillo, T. Williamson, C. T. Williamson, M. Konno, S. P. Lees-Miller, D. Hao, D. Morris, A. M. Magliocco, D. G. Bebb, L. F. Petersen, A. C. Klimowicz, S. Otsuka, A. A. Elegbede, S. K. Petrillo, T. Williamson, C. T. Williamson, M. Konno, S. P. Lees-Miller, D. Hao, D. Morris, A. M. Magliocco, D. Gwyn Bebb, *Oncotarget* **2017**, *8*, 38326.
- [85] H. Yan, K. Hu, W. Wu, Y. Li, H. Tian, Z. Chu, H. P. Koeffler, D. Yin, *PLoS One* **2016**, *11*, e0159954.
- [86] X. Zhang, P. Xu, W. Ni, H. Fan, J. Xu, Y. Chen, W. Huang, S. Lu, L. Liang, J. Liu, B. Chen, W. Shi, *Pathol., Res. Pract.* **2016**, *212*, 162.
- [87] R. Mimoto, N. Taira, H. Takahashi, T. Yamaguchi, M. Okabe, K. Uchida, Y. Miki, K. Yoshida, *Cancer Lett.* **2013**, *339*, 214.
- [88] Z. Zhang, P. Wen, F. Li, C. Yao, T. Wang, B. Liang, Q. Yang, L. Ma, L. He, *Exp. Ther. Med.* **2018**, *15*, 1113.
- [89] P. Kahl-Rainer, J. Karner-Hanusch, W. Weiss, B. Marian, *Carcinogenesis* **1994**, *15*, 779.
- [90] S. Kopsiaftis, P. Hegde, J. A. Taylor, K. P. Claffey, *Transl. Oncol.* **2016**, *9*, 606.
- [91] S. M. Hadad, L. Baker, P. R. Quinlan, K. E. Robertson, S. E. Bray, G. Thomson, D. Kellock, L. B. Jordan, C. A. Purdie, D. G. Hardie, S. Fleming, A. M. Thompson, *BMC Cancer* **2009**, *9*, 307.
- [92] J. Cheng, T. Huang, Y. Li, Y. Guo, Y. Zhu, Q. Wang, X. Tan, W. Chen, Y. Zhang, W. Cheng, T. Yamamoto, X. Jing, J. Huang, *PLoS One* **2014**, *9*, e93256.
- [93] W. Duan, K. Chen, Z. Jiang, X. Chen, L. Sun, J. Li, J. Lei, Q. Xu, J. Ma, X. Li, L. Han, Z. Wang, Z. Wu, F. Wang, E. Wu, Q. Ma, Z. Ma, *Cancer Lett.* **2017**, *385*, 225.
- [94] W. N. William, J.-S. Kim, D. D. Liu, L. Solis, C. Behrens, J. J. Lee, S. M. Lippman, E. S. Kim, W. K. Hong, I. I. Wistuba, H.-Y. Lee, *Ann. Oncol.* **2012**, *23*, 78.
- [95] Y. Baba, K. Noshio, K. Shima, J. A. Meyerhardt, A. T. Chan, J. A. Engelman, L. C. Cantley, M. Loda, E. Giovannucci, C. S. Fuchs, S. Ogino, *Br. J. Cancer* **2010**, *103*, 1025.
- [96] D. G. Hardie, *Clin. Cancer Res.* **2015**, *21*, 3836.
- [97] K. Nakayama, I. J. Frew, M. Hagensen, M. Skals, H. Habelhah, A. Bhoumik, T. Kadoya, H. Erdjument-Bromage, P. Tempst, P. B. Frappell, D. D. Bowtell, Z. Ronai, *Cell* **2004**, *117*, 941.
- [98] J. Moehlenbrink, N. Bitomsky, T. G. Hofmann, *Cancer Lett.* **2010**, *292*, 119.
- [99] M. A. Calzado, L. de la Vega, A. Möller, D. D. L. Bowtell, M. L. Schmitz, *Nat. Cell Biol.* **2009**, *11*, 85.
- [100] A. U. Ahmed, R. L. Schmidt, C. H. Park, N. R. Reed, S. E. Hesse, C. F. Thomas, J. R. Molina, C. Deschamps, P. Yang, M. C. Aubry, A. H. Tang, *JNCI, J. Natl. Cancer Inst.* **2008**, *100*, 1606.
- [101] K. C. Behling, A. Tang, B. Freydin, I. Chervoneva, S. Kadakia, G. F. Schwartz, H. Rui, A. K. Witkiewicz, *Breast Cancer Res. Treat.* **2011**, *129*, 717.
- [102] J. Qi, K. Nakayama, R. D. Cardiff, A. D. Borowsky, K. Kaul, R. Williams, S. Krajewski, D. Mercola, P. M. Carpenter, D. Bowtell, Z. A. Ronai, *Cancer Cell* **2010**, *18*, 23.
- [103] D. Muschik, I. Braspenning-Wesch, E. Stockfleth, F. Rösl, T. G. Hofmann, I. Nindl, *PLoS One* **2011**, *6*, e27655.
- [104] R. E. Neale, S. Weissenborn, D. Abeni, J. N. B. Bavinck, S. Euvrard, M. C. W. Feltkamp, A. C. Green, C. Harwood, M. de Koning, L. Naldi, I. Nindl, M. Pawlita, C. Proby, W. G. Quint, T. Waterboer, U. Wieland, H. Pfister, *Cancer Epidemiol., Biomarkers Prev.* **2013**, *22*, 719.
- [105] T. Polonio-Vallon, J. Kirckpatrick, J. Krijgsveld, T. Hofmann, *Cell Cycle* **2014**, *13*, 115.
- [106] G. R. Steinberg, D. Carling, *Nat. Rev. Drug Discovery* **2019**, *18*, 527.
- [107] A. Igea, A. R. Nebreda, *Cancer Res.* **2015**, *75*, 3997.
- [108] E. Conrad, T. Polonio-Vallon, M. Meister, S. Matt, N. Bitomsky, C. Herbel, M. Liebl, V. Greiner, B. Kriznik, S. Schumacher, E. Krieghoff-Henning, T. G. Hofmann, *Cell Death Differ.* **2016**, *23*, 110.