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REVIEW ARTICLE

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Tumor suppressive role for kinases phosphorylating p53 in DNA damage-induced apoptosis

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Tumor suppressor p53 plays an important role in cancer prevention. Under normal conditions, p53 is maintained at a low level. However, in response to various cellular stresses, p53 is stabilized and activated, which, in turn, initiates DNA repair, cell-cycle arrest, senescence and apoptosis. Post-translational modifications of p53 including phosphorylation, ubiquitination, and acetylation at multiple sites are important to regulate its activation and subsequent transcriptional gene expression. Particularly, phosphorylation of p53 plays a critical role in modulating its activation to induce apoptosis in cancer cells. In this context, previous studies show that several serine/threonine kinases regulate p53 phosphorylation and downstream gene expression. The molecular basis by which p53 and its kinases induce apoptosis for cancer prevention has been extensively studied. However, the relationship between p53 phosphorylation and its kinases and how the activity of kinases is controlled are still largely unclear; hence, they need to be investigated. In this review, we discuss various roles for p53 phosphorylation and its responsible kinases to induce apoptosis and a new therapeutic approach in a broad range of cancers.

KEYWORDS
apoptosis, cancer, kinase, p53, phosphorylation

1 | INTRODUCTION

p53 is the most important tumor suppressor protein that transcriptionally regulates various genes involved in cell cycle, growth, survival, DNA repair, senescence, autophagy, and apoptosis.[1] The p53-mediated responses, especially p53-mediated apoptosis, have been implicated in an ability to suppress tumor development and to respond to cancer therapy. The present review will focus on the functional significance of p53 phosphorylation and its kinases to induce apoptosis for cancer prevention.

2 | p53 SIGNALING FOR APOPTOSIS IN RESPONSE TO DNA DAMAGE

Upon exposure to genotoxic stress, p53 is stabilized and activated by phosphorylation at Ser15 and Ser20 to regulate a cell cycle checkpoint and DNA repair. However, in response to severe DNA damage, p53 induces a large number of apoptotic genes that are associated with various steps of apoptosis by transcription-dependent and -independent mechanisms (Figure 1).[2] p53 can induce apoptosis by intrinsic and extrinsic pathways. It is important for cancer prevention to understand how p53 phosphorylation is controlled.

3 | KINASES RESPONSIBLE FOR p53 PHOSPHORYLATION TO INDUCE APOPTOSIS

3.1 | p53 phosphorylation sites related to apoptosis

Structurally, p53 comprises several domains that are crucial for mediating its various functions (Figure 2). Upon severe DNA damage, p53 is phosphorylated at specific amino acid residues, becomes stabilized

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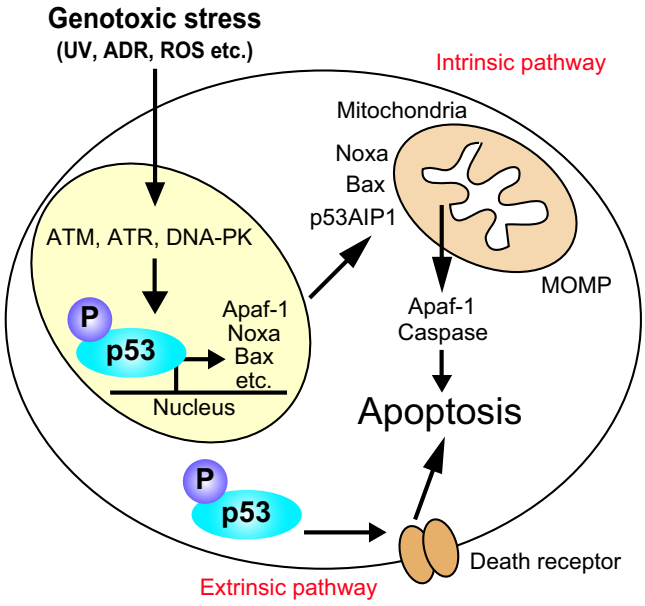

FIGURE 1 Model for p53-mediated apoptosis. Upon various genotoxic stresses, ataxia telangiectasia and Rad3 related (ATR), ataxia telangiectasia mutated (ATM) and DNA-PK kinases are activated. Subsequently, p53 transactivates numerous genes involved in the extrinsic and intrinsic apoptotic pathways. p53 upregulates proapoptotic proteins (Puma, Noxa, Bax, Bak, p53AIP1) and death receptors (Fas, Dr5), whereas anti-apoptotic proteins (Apaf1, caspase-6, Bnip3L) are repressed by p53

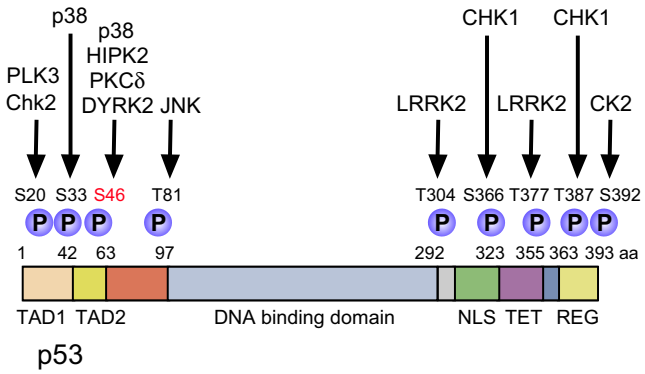

FIGURE 2 p53 phosphorylation sites and its kinases to induce apoptosis. The p53 protein is illustrated schematically, with important functional domains highlighted. TAD1 and TAD2 indicate the transcriptional activation domains. NLS is the nuclear localization sequence. TET indicates the tetramerization domain. REG indicates the C-terminal regulatory region. Phosphorylation sites related to apoptosis and their kinases are represented

and activated to induce apoptosis-related genes. To date, 10 kinases have been identified to phosphorylate p53 for apoptosis at specific serine/threonine residues in the N- and C-terminus domains. Nine phosphorylation sites (serine 20, 33, 46, 366, and 392 and threonine 81, 304, 377, and 387) for apoptosis have been detected in different types of cancer cell lines (Table 1).[3–12] Intriguingly, a kinase phosphorylates several sites on p53, whereas several kinases

TABLE 1 Phosphorylation sites and kinases responsible for apoptosis in cancer cell lines

Site	Kinase	Stimulus	Cell line (type of cancer)	Reference
S20	PLK3	Superoxide	MDAPanc-28 (pancreatic cancer)	3
S20	Chk2	Cisplatin	HCT116 (colon cancer)	4
S33	p38	UV	A549 (lung cancer)	5
S46	p38		*in vitro kinase assay only	5
S46	HIPK2	UV	MCF7 (breast cancer)	6
S46	PKCδ	Adriamycin	MCF7 (breast cancer) U2OS (osteosarcoma)	7
S46	DYRK2	Adriamycin	HCT116 (colon cancer) U2OS (osteosarcoma)	8
T81	JNK	UV	MCF7 (breast cancer)	9
T304	LRRK2		*in vitro kinase assay only	10
S366	CHK1	Camptothecin	LNCaP (prostate cancer)	11
T377	LRRK2		*in vitro kinase assay only	10
T387	CHK1	Camptothecin	LNCaP (prostate cancer)	11
S392	CK2	Nocodazole	HeLa (cervical cancer)	12

CHK1, checkpoint kinase 1; Chk2, checkpoint kinase 2; CK2, casein kinase 2; DYRK2, dual-specificity tyrosine-phosphorylation-regulated kinase 2; HIPK2, homeodomain-interacting protein kinase-2; LRRK2, leucine-rich repeat kinase 2; PLK3, Polo-like kinase 3. Asterisk indicates that each of the phosphorylation sites was determined by in vitro kinase assay.

phosphorylate the same site. In particular, a growing number of studies have indicated that Ser46 phosphorylation is mainly involved in the regulation of apoptosis after DNA damage. A thorough understanding of how p53 is phosphorylated by kinases to induce apoptosis will be extremely useful in the development of new strategies for preventing cancer.

3.2 | N-terminal phosphorylation sites

Polo-like kinase 3 (Plk3) and checkpoint kinase 2 (Chk2) phosphorylated p53 at Ser20 to induce its transcriptional activity in MDAPanc-28 and HCT116 cells.[3,4] Overexpression of WT Plk3 in HCT116 $p53^{+/+}$ cells induced rapid apoptosis, whereas overexpression of WT Plk3 in HCT116 $p53^{-/-}$ cells induced delayed onset of apoptosis. Additionally, several studies have shown that Plk3 is associated with cell cycle arrest and apoptosis by the p53 pathway by interacting directly with p53 and phosphorylating p53 on Ser20 in response to DNA damage.[13,14] Therefore, Plk3 plays a pivotal role not only in the regulation of microtubule dynamics and centrosomal function but also in cell cycle arrest and apoptosis.[15] Ataxia telangiectasia and Rad3 related (ATR) phosphorylated Chk2 and probably p53. Upon phosphorylation, Chk2 was activated to further phosphorylate and activate p53. Subsequently, p53 induced *PUMA*-α expression and

Bax activation, which leads to cytochrome c release followed by caspase activation and apoptosis.[4] Ser33 as well as Ser46 were phosphorylated by p38 in response to UV radiation in A549 cells.[5] Mutation of these sites decreased UV-induced apoptosis. p38 bound and stabilized p53 protein in H1299 cells. After UV irradiation, inhibition of p38 activation decreased phosphorylation of Ser33, Ser37 and Ser15 and markedly reduced apoptosis, indicating that p38 plays a prominent role in N-terminal phosphorylation to regulate p53-mediated apoptosis. Thr81 was phosphorylated by JNK in response to DNA damage and stress-inducing agents in MCF7 cells.[9] Forced expression of MKP5, a JNK phosphatase, showed decreased Thr81 phosphorylation, p53 transcriptional activity and p53-mediated apoptosis. Importantly, Thr81 and the JNK binding site of p53 encompassed the DNA-binding domain that contains the major somatic cancer mutations, suggesting that Thr81 phosphorylation by JNK may be essential in the stability and activity of p53.

3.3 | C-terminal phosphorylation sites

Thr304 and 377 were phosphorylated by leucine-rich repeat kinase 2 (LRRK2), one of the Parkinson's disease-causative genes shown by an in vitro kinase assay.[10] LRRK2-mediated phosphorylation enabled p53 to translocalize predominantly into the nucleus in SH-SY5Y cells. Activation of LRRK2 kinase was associated with increase of *p21* expression and cytotoxicity/apoptosis in differentiated SH-SY5Y cells and rat primary neurons. In contrast, no *p21* expression by LRRK2 was observed in HCT116 cells, suggesting that this effect may be neuron-specific. Ser366 and Thr387 were phosphorylated by checkpoint kinase 1 (CHK1) in response to camptothecin treatment in LNCaP cells.[11] Importantly, p53 phosphorylation at Ser366 and Thr387 and apoptosis-related *bax* mRNA were markedly reduced in LNCaP cells silenced for CHK1. Ser392 was reported to be phosphorylated by casein kinase 2 (CK2) in Hela cells.[12] CK2-depleted, nocodazole-treated cells showed a significant reduction in the G2 arrest and apoptotic fraction in Hela and HCT116 $p53^{+/+}$ cells. This effect was dependent on the presence of WT p53, as it was not apparent in HCT116 $p53^{-/-}$ cells.

3.4 | Ser46, a major phosphorylation site for apoptosis

Importantly, Ser46 phosphorylation of p53 by several kinases is required for apoptosis in response to DNA damage. Increased Ser46 phosphorylation of p53 induces apoptotic target gene transcription.[8,16,17] For example, p38 kinase phosphorylated p53 at Ser33 and 46 to induce its stabilization and activation.[5] Inhibition of p38 after UV irradiation decreased p53-mediated apoptosis. These effects are probably mediated by both JNK and p38 themselves. Furthermore, homeodomain-interacting protein kinase-2 (HIPK2) bound to and phosphorylated p53 at Ser46 after UV irradiation in MCF7 cells.[6] HIPK2 and p53 cooperated in the activation of p53-dependent transcription, nuclear localization, and apoptotic pathways. Ectopic expression of p300 in HIPK2-knockdown RKO cells rescued *Puma* and *Noxa* expression.[18] HIPK2 was regulated by MDM2-mediated degradation.[17]

Previously, we have indicated that dual-specificity tyrosine-phosphorylation-regulated kinase 2 (DYRK2) directly phosphorylates p53 at Ser46 in HCT116 and U2OS cells (Figure 3).[8] Our data showed that DYRK2 had the characteristics of an in vitro direct Ser46 kinase. Upon DNA damage, ataxia telangiectasia mutated (ATM) phosphorylated and stabilized DYRK2 in the nucleus, and then DYRK2 phosphorylated p53 at Ser46. We also confirmed the cytoplasmic localization of DYRK2 in unstimulated cells. Significantly, upon exposure to genotoxic stress, DYRK2 translocated into the nucleus to induce *p53AIP1* expression and apoptosis. The mechanism for nuclear targeting of DYRK2 remains obscure, whereas nuclear translocation may be important for efficient p53 phosphorylation at Ser46. These findings support a novel signaling mechanism in which

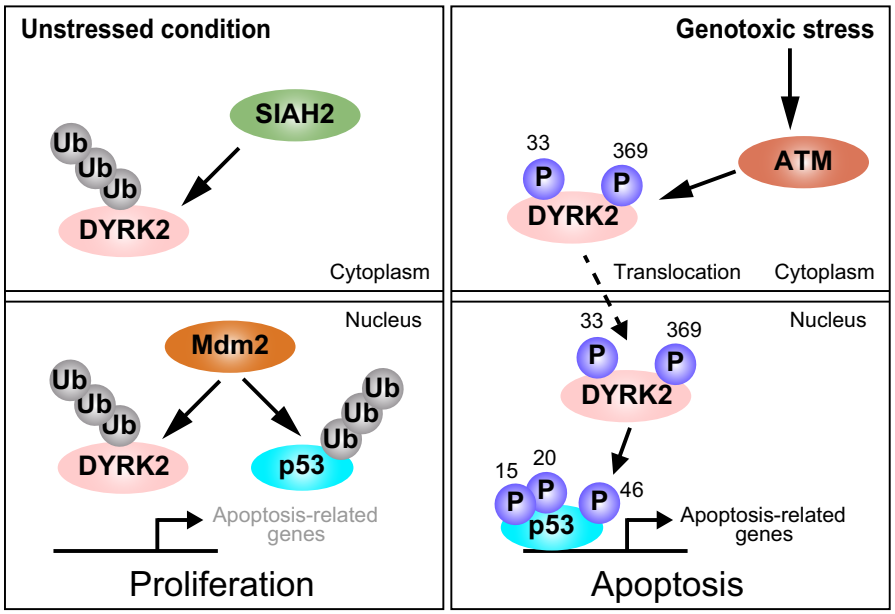

FIGURE 3 Proposed model for dual-specificity tyrosine-phosphorylation-regulated kinase 2 (DYRK2)-mediated p53 phosphorylation and apoptosis in response to DNA damage. In the unstressed condition, DYRK2 is ubiquitinated by MDM2 and SIAH2 to elicit its constitutive degradation. p53 is also ubiquitinated by MDM2 and maintained at a low level. In response to various genotoxic stresses, p53 is stabilized and activated by phosphorylation at Ser15 and Ser20. Cytoplasmic DYRK2 is activated and targeted to the nucleus and is phosphorylated by ataxia telangiectasia mutated (ATM). DYRK2 then phosphorylates p53 at Ser46. Ser46 phosphorylation triggers the induction of apoptosis-related genes, as shown in Figure 1

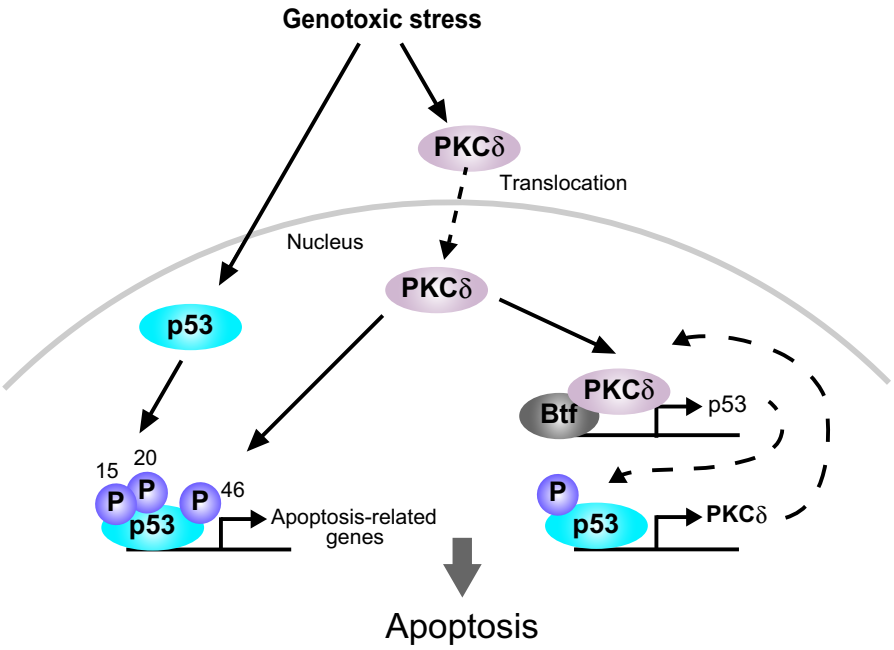

FIGURE 4 Proposed model for PKCδ-mediated p53 phosphorylation and apoptosis in response to DNA damage. In response to various genotoxic stresses, PKCδ translocates to the nucleus and phosphorylates p53 at Ser46. PKCδ is also implicated in the transcriptional regulation of the *p53* gene following DNA damage. In turn, the *PKC*δ gene is transcriptionally regulated by p53. Therefore, p53 and PKCδ regulate apoptosis in a positive feedback mechanism

phosphorylation of p53 at Ser46 by DYRK2 regulates apoptotic cell death in response to DNA damage.[1,19]

Previous findings also showed that p53 was phosphorylated by PKCδ in MCF7 and U2OS cells upon exposure to genotoxic agents (Figure 4).[7] PKCδ-mediated phosphorylation was required for the interaction of PKCδ with p53. PKCδ also induced the promoter activity of p53 through the p53 core promoter element and that the induction was enhanced following DNA damage.[20] Nuclear import of PKCδ was required for initiation of the apoptotic pathway.[21] In this context, PKCδ also induces apoptosis through various nuclear substrates, such as DNA-dependent protein kinase,[22] phospholipid scramblase 3,[23] lamin B,[24] Rad9,[25] or Evi-1 and PLZF.[26] Intriguingly, our recent report demonstrated that PKCδ was transcriptionally regulated by p53 upon genotoxic stress. This regulation was tightly controlled in a positive feedback mechanism to induce apoptosis.[27,28] Taken together, uncovering the relationship between Ser46 phosphorylation and the crosstalk with its kinases provides a novel insight into apoptosis signaling for cancer treatment and prevention.[29] Further studies are required for a better understanding of the molecular basis of Ser46 phosphorylation of p53 to induce apoptosis.

4 | p53 PHOSPHORYLATION IN TUMOR SUPPRESSIVE ROLES

Does p53 phosphorylation contribute to any tumor suppressive role? Several studies have shown that p53 phosphorylation (eg, Ser15, 20, 37, 46) induces apoptosis through multiple effectors, such as inositol pyrophosphates, selenocysteine, nutlin-3a, and palmdelphin in cancer cells.[30–33] Moreover, there has been considerable effort to understand the mechanism of p53 regulation by post-translational modification using genetically engineered mouse models.[34] A previous report showed partially impaired p53-dependent apoptosis in thymocytes from mice defective for p53 phosphorylation at Ser46 (S46A mutant) whereas its tumorigenic phonotype was not confirmed.[35] Other reports also showed that mice defective for p53 phosphorylation at several sites (S312A and S18/23A mutants) were more susceptible to tumorigenesis, although mice engineered to have *p53* gene knockout developed tumors at an increased rate.[36–38] In this way, p53 phosphorylation is important for p53-dependent suppression of tumorigenesis in mice.

In contrast, the *p53* gene is one of the most common sites for genetic alterations, leading to the expression of mutant p53 proteins, in human solid cancers as it is mutated in more than 50% of cancer cases worldwide.[39] Additionally, inherited *p53* gene mutation in patients with Li-Fraumeni syndrome carrying an R248Q mutation is also characterized by a strikingly increased risk of early-onset cancers including breast carcinomas, brain tumors, leukemias and sarcomas, among others.[40] Some mutant p53 proteins give rise to a more aggressive tumor profile, suggesting they have acquired gain-of-function activity.[41] Accordingly, does mutant p53 phosphorylation contribute to tumor progression or not? For example, mutant p53 protein in UV-induced murine primary skin tumors and cultured cell lines was constitutively phosphorylated at Ser15 residue and localized in the nuclei.[42] Conversely, Ser392 phosphorylation of mutant p53 could not confer cellular resistance to DNA-damaging agents.[43] Analysis of mutant p53 phosphorylation by phosphoantibodies showed a marked increase in the degree of p53 phosphorylation in tumor-derived cell lines as well as in freshly processed tumor tissues.[44] Therefore, phosphorylation of mutant p53 would be important for tumor progression, despite a small number of studies with conflicting findings.[45] It is thus necessary to carefully evaluate biological function of mutant p53 phosphorylation in various experimental conditions, such as type of genotoxic stress, type of cell, and growth status in the cell.

It is not surprising that kinases involved in p53 phosphorylation-inducible apoptosis are maintained at low level or mutated in cancer cells. For instance, recent reports showed that the expression of DYRK2 protein was reduced in cancer tissues compared with that in normal tissues and was correlated with patient survival in many types of cancer.[46–50] Recent genome-wide association studies also identified that somatic mutation of the *DYRK2* gene was correlated with breast cancer risk.[51] Moreover, DYRK2 was ubiquitinated by MDM2 and SIAH2, resulting in its constitutive degradation and impaired DYRK2-mediated phosphorylation of p53 at Ser46.[52,53] In response to genotoxic stress, DYRK2 was phosphorylated at Thr33 and Ser369 by ATM, stabilized by inhibiting MDM2-mediated degradation, to induce the kinase activity toward p53 at Ser46 in the nucleus.[52] Moreover, knockdown of DYRK2 increased cell proliferation in MCF7 cells and tumor progression in vivo through the escape of c-Jun and c-Myc from ubiquitination-mediated degradation.[54,55] These findings collectively indicate that DYRK2 is implicated in an antitumor effect. Further studies are required for cancer prevention to define how the activity and expression level of DYRK2 is controlled in each type of cancer.

*PKC*δ gene is most commonly mutated in gastrointestinal cancers (pancreatic, stomach, and colorectal), with a lesser mutation burden in melanomas and lung cancers.[56] However, PKCδ shows conflicting evidence as to whether it acts as an oncogene or as a tumor suppressor. PKCδ appeared to be a tumor suppressor because of its proapoptotic functions.[57] Inhibiting PKCδ blocked both basal transcription of the human *p53* gene and initiation of transcription from the human *p53* promoter.[58] It is thus conceivable that the tumor-suppressing effects of PKCδ are mediated at least in part through activating *p53* transcription. Knockdown of PKCδ in colon cancer cells increased tumor growth, and overexpression of PKCδ in keratinocytes decreased tumorigenicity in immunodeficient mice.[59,60] Decreased PKCδ levels correlated with increased tumor grade in bladder and endometrial cancer and glioma.[61–63] In contrast, PKCδ promoted tumor progression of lung and pancreatic cancers in certain contexts.[64,65] Additional evidence is definitely required to determine each cancer-specific PKCδ function.

HIPK2 is activated by numerous genotoxic agents and can be deregulated in tumors by several conditions including hypoxia.[66] HIPK2 was required for the Fbw7-dependent proteasomal degradation of Notch1 by phosphorylating its intracellular domain, suggesting that HIPK2 regulates tumor progression.[67] Additionally, vimentin downregulation by HIPK2 correlated with inhibition of breast tumor cell invasion.[68] In heterozygous *p53*-deficient background, mice with heterozygous loss of *HIPK2* gene developed more lymphomas after irradiation than those with wild-type *HIPK2* gene.[69] These findings indicate that HIPK2 is a promising target for cancer treatment.[66,70]

5 | CONCLUDING REMARKS

The ability of p53 phosphorylation to induce apoptosis has significant antitumor potential that can be exploited for cancer treatment. p53 phosphorylation is a complex process that associates with various proteins and multiple layers of regulation. The molecular basis of how p53 and its kinases induce apoptosis for cancer prevention has been extensively studied. However, the relative contribution of each regulator and how stability and activity of kinases are controlled remains to be determined. As described earlier, the expression level of DYRK2 protein is controlled by ubiquitination-mediated proteasomal degradation and, more importantly, it is downregulated in various human cancer tissues. Therefore, inhibition of DYRK2 protein degradation by blockage of E3 ligase, such as MDM2 and SIAH2, would be efficient strategies for cancer prevention. Additionally, reactivation of DYRK2 protein including directly re-expressing DYRK2 in tumors by virus-mediated delivery systems, restoring mutant forms by CRISPR/Cas9-mediating gene editing, or DYRK2 reactivating compounds may be potentially used for cancer treatment.

For instance, a selective small-molecule activator of PKCδ, ingenol 3-angelate (PEP005) and the 7α-acetoxy-6β-benzoyloxy-12-O-benzoylroyleanone (Roy-Bz) are anticancer drug candidates.[71,72] PEP005 induced a rapid nuclear translocation of PKCδ and PKCδ-dependent phosphorylation of caspase-3 in myeloid leukemia cell lines and primary acute myeloid leukemia cells, suggesting that PEP005 has potent antileukemic activity.[71] Roy-Bz potently inhibited the proliferation of colon cancer cells by inducing a PKCδ-dependent mitochondrial apoptotic pathway with caspase-3 activation. It also exerted a PKCδ-dependent antitumor effect in xenograft mouse models.[72]

Consistently, reactivation of WT p53 and downregulation and/or restoration of mutant p53 may also be beneficial in many tumors. In fact, p53 restoration resulted in elevated apoptosis and decreased tumor growth in mice inheriting a *p53* null or R172H mutation.[73,74] Moreover, a mutant-p53-targeting compound, PRIMA-1MET (APR-246), could restore mutant p53 proteins to a WT p53 conformation and lead to enhanced expression of *Puma*, *Noxa* and *Bax* in p53 mutant cells. In 2012, APR-246 was tested in phase I/IIa clinical trials.[75]

Finally, once we understand the detailed mechanisms of p53 phosphorylation and its kinases for apoptosis, we will be able to develop highly effective and specific strategies for cancer prevention.

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CONFLICTS OF INTEREST

Authors declare no conflicts of interest for this article.

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