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MINI-REVIEW

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Multifaceted behavior of PEST sequence enriched nuclear proteins in cancer biology and role in gene therapy

Muhammad Sarfraz[1,2,3] | Attia Afzal[1,2] | Saadullah Khattak[1] | Umair A. K. Saddozai[1] | Hui-Min Li[1,4] | Qian-Qian Zhang[1] | Asadullah Madni[5] | Kashif S. Haleem[6] | Shao-Feng Duan[1,7] | Dong-Dong Wu[1,8] | Shao-Ping Ji[3] | Xin-Ying Ji[1]

[1]Henan International Joint Laboratory for Nuclear Protein Regulation & Kaifeng Key Laboratory of Infectious Diseases and Bio-safety, School of Basic Medical Sciences, Henan University College of Medicine, Kaifeng, Henan, China

[2]Faculty of Pharmacy, The University of Lahore, Lahore, Punjab, Pakistan

[3]Kaifeng Municipal Key Laboratory of Cell Signal Transduction, Henan Provincial Engineering Centre for Tumor Molecular Medicine, Henan University, Kaifeng, Henan, China

[4]Department of Histology and Embryology, Cell Signal Transduction Laboratory, School of Basic Medical Sciences, Bioinformatics Centre, Institute of Biomedical Informatics, Henan University, Kaifeng, Henan, China

[5]Faculty of Pharmacy and Alternative Medicine, The Islamia University of Bahawalpur, Bahawalpur, Punjab, Pakistan

[6]Department of Microbiology, Hazara University, Mansehra, Pakistan

[7]School of Pharmacy, Institute for Innovative Drug Design and Evaluation, Henan University, Kaifeng, Henan, China

[8]School of Stomatology, Henan University, Kaifeng, Henan, China

Correspondence
Dong-Dong Wu, Xin-Ying Ji, and Shao-Feng Duan, Henan International Joint Laboratory for Nuclear Protein Regulation & Kaifeng Key Laboratory of Infectious Diseases and Bio-safety, School of Basic Medical Sciences, Henan University College of Medicine, Kaifeng, 475004 Henan, China.
Email: ddwubiomed2010@163.com (D.-D. W.); 10190096@vip.henu.edu.cn (X.-Y. J.) and dsf_2007@163.com (S.-F. D.)

Shao-Ping Ji, Municipal Key Laboratory of Cell Signal Transduction, Henan Provincial Engineering Centre for Tumor Molecular Medicine, Henan University, Kaifeng, 475004 Henan, China.
Email: shaopingji@henu.edu.cn

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Abstract

The amino acid sequence enriched with proline (P), glutamic acid (E), serine (S), and threonine (T) (PEST) is a signal-transducing agent providing unique features to its substrate nuclear proteins (PEST-NPs). The PEST motif is responsible for particular posttranslational modifications (PTMs). These PTMs impart distinct properties to PEST-NPs that are responsible for their activation/inhibition, intracellular localization, and stability/degradation. PEST-NPs participate in cancer metabolism, immunity, and protein transcription as oncogenes or as tumor suppressors. Gene-based therapeutics are getting the attention of researchers because of their cell specificity. PEST-NPs are good targets to explore as cancer therapeutics. Insights into PTMs of PEST-NPs demonstrate that these proteins not only interact with each other but also recruit other proteins to/from their active site to promote/inhibit tumors. Thus, the role of PEST-NPs in cancer biology is multivariate. It is hard to obtain therapeutic objectives with single gene therapy. An especially designed combination gene therapy might be a promising strategy in cancer treatment. This

Abbreviations: ARF, ADP-ribosylation factor; BDNF, brain-derived neurotrophic factor; FBW7, F-box and WD repeat domain-containing 7; FOXF1, forkhead box protein F1; GLTSCR2, glioma tumor suppressor candidate region gene 2; HBP, hexosamine biosynthetic pathway; HDAC, histone deacetylase; HDM2, human double minute 2; HTH, helix-turn-helix; JNK, c-Jun NH2-terminal kinase; MAPK, mitogen-activated protein kinase; MBD, methyl-CpG-binding domain; MDM2, mouse double minute 2; MYOD1, myogenic differentiation 1; NES, nuclear export sequences; NF-κB, nuclear factor-κB; NLS, nuclear localization sequences; NP, nuclear protein; NSC, neural stem cell; NSCLC, non-small-cell lung cancer; PCNP, PEST-containing nuclear protein; PEST, proline (P), glutamic acid (E), serine (S) and threonine (T); PEST-NP, PEST sequence enriched nuclear protein; PICT-1, protein interacting with carboxy terminus 1; PTMs, posttranslational modifications; PUMA, p53 upregulated modulator of apoptosis; RNF144A, ring finger protein 144A; SCF, Skp1, cullin, and F-box protein; TAD, transactivation domain; VEGFR2, vascular endothelial growth factor receptor-2.

Muhammad Sarfraz, Attia Afzal, and Saadullah Khattak contributed equally to this study.

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review highlights the multifaceted behavior of PEST-NPs in cancer biology. We have summarized a number of studies to address the influence of structure and PEST-mediated PTMs on activation, localization, stability, and protein–protein interactions of PEST-NPs. We also recommend researchers to adopt a pragmatic approach in gene-based cancer therapy.

KEYWORDS

cancer biology, gene therapy, nuclear proteins, PEST sequence, posttranslational modifications, protein–protein interactions

1 | INTRODUCTION

Nuclear proteins (NPs) are involved in cancer proliferation and inhibition. They play a vital role by regulating the cell cycle (Hydbring, Castell, & Larsson, 2017), stem cell generation (Ammirante et al., 2013), exercise metabolism (Park et al., 2009), immune response (S. R. Ho, Mahanic, Lee, & Lin, 2014) and DNA damage and repair (Bakhanashvili et al., 2008; Nithipongvanitch et al., 2007). These different cellular activities are regulated by posttranslational modifications (PTMs) of proteins. The NPs are modified by phosphorylation, ubiquitin conjugation (S. R. Ho et al., 2014), SUMOylation (Nie, Moser, Nakamura, & Boddy, 2017), PARsylation (Zhou, Chan, Xiao, & Tan, 2011) and so on. The NPs exhibit various molecular and biological effects through different modifications based on different expression levels at target sites. For example, nuclear protein p53 is a tumor suppressor in normal conditions but at increasing concentrations, mutant p53 acts as a tumor promoter. Similarly, a ring finger protein 144A (RNF144A; Ho et al., 2014), with a wide cellular distribution mainly around the plasma membrane and the perinuclear area, is produced as a result of DNA damage in response to p53 activation. It interacts with DNA dependent protein kinases and performs as a ubiquitin E3 ligase to degrade p53 while it undergoes auto-ubiquitination when overexpressed. Some NPs such as XRCC1, KU70, DNA ligase III, and PARP1 degrade through PAR-dependent modification (Kang et al., 2011). The PTMs mediate several other physiological processes of NPs; for example, ubiquitination governs the stability of PCNP, translocation of p53, and stimulation/inhibition of MeCP2.

1.1 | Proline (P), glutamic acid (E), serine (S), and threonine (T) sequence enriched nuclear proteins (PEST-NPs)

Among the NPs, PEST sequence enriched nuclear proteins (PEST-NPs) are abundant, widely distributed, and involved in various cell biological and physiological functions. The PEST-NPs are known as guardians of the cell and interfere in several pathways such as the ubiquitin proteasome pathway, glycosylation of nuclear pores, and the hexosamine biosynthetic pathway (Afzal et al., 2019). These proteins are involved in nutrient regulation of cellular metabolism and physiology, nucleocytoplasmic transport, cell-cycle regulation, and cyclic nucleotide signaling pathways (Rogers, Wells, & Rechsteiner, 1986). In cancer cell biology, some PEST-NPs regulate the tumor cell cycle either directly by binding with DNA or indirectly by ubiquitinating the cyclins D1 and E1 hence inducing G1 arrest in cancer cells. The involvement of PEST-NPs in cell cycle arrest and cell apoptosis is illustrated in Figure 1. The PEST-NPs primarily regulate cancer metabolism via the PI3K pathway, mTOR pathway, and mitogen-activated protein kinase (MAPK) pathway and the cancer-immune mechanism via apoptosis and autophagy, however; intracellular localization and the protein level at the target site also influence the type of biological function. The PTMs of PEST-NP like phosphorylation and ubiquitination regulate the intracellular localization, activation, and expression level of substrate proteins.

1.2 | PTMs are modulated by PEST motif

The PEST motif is a signal-transducing agent mainly responsible for degradation of its substrate either by proteasomal degradation of proteins (Chakraborty et al., 2011), endocytosis of yeast α-factor receptor STE3 (Roth, Sullivan, & Davis, 1998), or lysosomal degradation of the cell surface human calcium receptor (Zhuang, Northup, & Ray, 2012). The carboxyl-terminus of the PEST domain undergoes phosphorylation and degrades the substrate proteins; like IkBα and IKKβ are degraded by Ck2 phosphorylation (Perkins, 2006) and proteins involved in cyclic nucleotide action and metabolism are degraded by cAMP-dependent protein kinase (Sekhar & Freeman, 1998). Other than phosphorylation, the proteins with the PEST motif such as PCNP and MeCP2 are highly subjected to ubiquitination (Bellini et al., 2014), Bcl6 modifies by P300 acetylation (Bereshchenko, Gu, & Dalla-Favera, 2002) and IKKβ degrades by non-lysosomal cysteine protease (μ-calpain)–mediated degradation (J. F. Zhao, Shyue, & Lee, 2016). The modification of serine 1188 of the PEST sequence of vascular endothelial growth factor receptor-2 (VEGFR-2) mediates both stability and MAPK mediated activation of this receptor (R. Liu et al., 2017). Owing to PEST-regulated proteasomal degradation, PEST-NPs are short-lived and maintain their cellular levels with the help of ubiquitin-conjugating and ligase enzymes in normal conditions as shown in Figure 2.

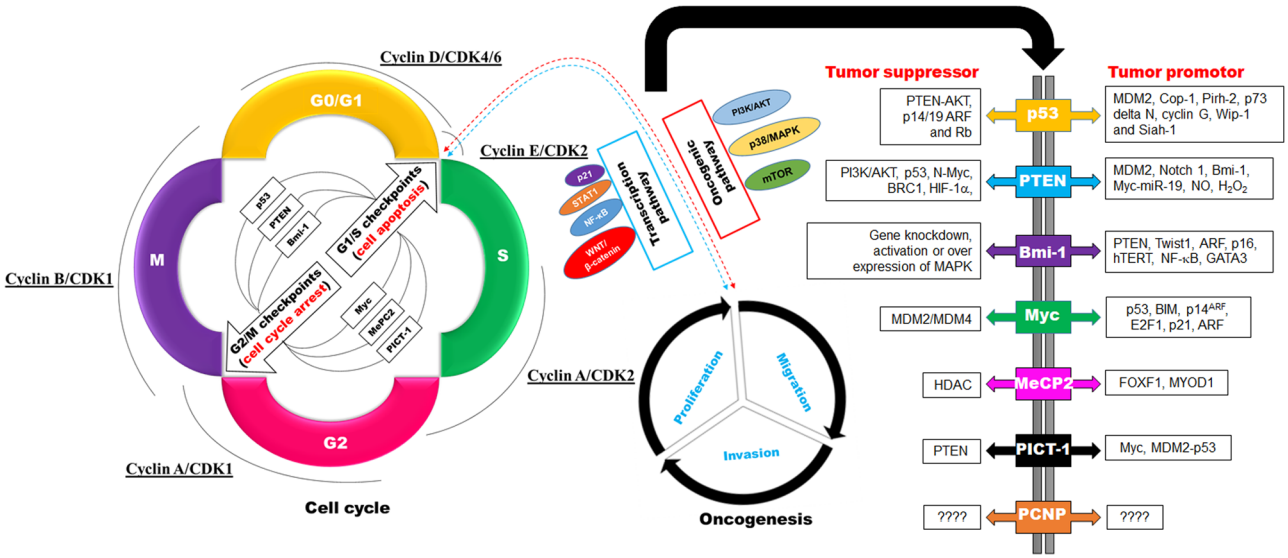

FIGURE 1 Multifaceted behavior of PEST sequence enriched nuclear proteins (PEST-NPs): p53 and PTEN are "tumor suppressors" and Bmi and Myc are "tumor promotors" in the normal cancer microenvironment. MeCP2, PICT-1, and PCNP are heterogeneous in the normal cancer environment. Tumor suppressor activity of these nuclear proteins is regulated via CDK-complex to interrupt the G2/M phase (cell cycle arrest) or G1/the S phase (cell apoptosis). The cellular location (nucleus/cytoplasm), concentration, mutation, and stress conditions further define their dominant role (either tumor suppressor or tumor promoter) in the cancer microenvironment via interfering with other oncogenes (Oncogenic pathway) or transcription factors (Transcription pathway). PCNP (PEST-containing nuclear protein) is a novel protein in the PEST-NP class; however, the underlying mechanism of the tumor suppressor/tumor promotor in the cancer microenvironment is not yet defined. ARF, adipose-riobosylation factor; BIM, Bcl-2-interacting mediator of cell death; BRCA-1, breast cancer susceptibility gene 1; Cop-1, constitutive photomorphogenesis protein 1; E2F-1, early-region-2 transcription-factor-1; FOXF1, forkhead box protein F1; GATA3, GATA binding protein 3; HDAC, histone deacetylase; HIF-1α, hypoxia-inducible factor-1α; hTERT, human telomerase reverse transcriptase; MDM2, murine double minute 2; MYOD1, myogenic differentiation 1; NF-κB, nuclear factor-κB; Pirh-2, p53-induced protein with a RING-H2 domain; Siha-1, siah E3 ubiquitin-protein ligase 1; Wip-1, WASP-interacting protein 1

1.3 | PTMs of PEST-NPs are responsible for their activation/inhibition, stability/degradation, and/or intracellular localization

The bio-molecular alterations of proteins mediate transcription, intracellular localization and/or degradation following two types of genetic alterations that is, the oncogene activation or the loss of tumor suppressor activity leading to carcinogenesis. The PTMs are responsible for over-supply or over-activity of transcription factors like Myc and NF-κB followed by unrestrained growth and metastatic behavior of several types of human cancer (Afzal et al., 2019; Afzal, Sarfraz, Wu, Wang, & Sun, 2016). It is well understood that SUMOylation by SUMO-1 and SUMO-2/-3, mono- or poly-ubiquitination of PTEN regulate the activity, localization and/or stability of PTEN (Lang et al., 2015; N. Li et al., 2015). Similarly, in the case of NF-κB, phosphorylation of the PEST motif and subsequent

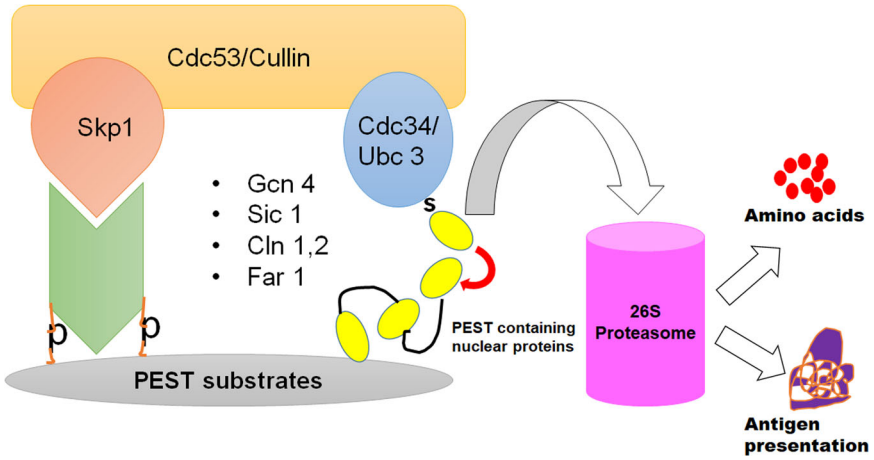

FIGURE 2 Degradation of phosphorylated substrates containing PEST sequences by a complex of Cdc34/Ubc3 and SCF (Skp1, Cullin, and F-box protein). Cdc, cell division cycle; PEST, proline (P), glutamic acid (E), serine (S), and threonine (T); SCF, Skp1, cullin, and F-box protein; Ub, ubiquitin; Ubc, Ub-conjugating enzyme

proteolytic degradation of the inhibitor of NF-κB (IκB) kinase initiates hetero-dimerization leading to transcriptional activation and nuclear localization of NF-κB followed by DNA damage. In the case of PEST-NPs, PTMs are responsible for activation/inhibition, stability/degradation and/or Intracellular localization.

1.3.1 | Activation/inhibition

Anticancer PEST-NP, p53 executes apoptosis through mitochondrial permeability. During stress conditions, the p53 upregulated modulator of apoptosis (PUMA) is released by nuclear p53 that interacts with the cytoplasmic Bcl-xl-p53 complex of tumor suppressor protein p53, setting it free from the complex to initiate mitochondria-dependent apoptosis in the cytoplasm. After executing apoptotic effects, p53 degrades rapidly both in the nucleus and in the cytoplasm. The nuclear p53 conjugates with ubiquitin (p53-Ub) and passes through the nuclear membrane into the cytoplasm where p53-Ub and cytoplasmic p53 degrade by ubiquitin ligase mouse double minute 2 (MDM2), a phenomenon observed in many tumor types (Chipuk, Bouchier-Hayes, Kuwana, Newmeyer, & Green, 2005; Lisachev, Pustylnyak, & Shtark, 2015).

1.3.2 | Stability/degradation

The modification of the PEST motif is not only responsible for the activation/deactivation but also for the stability of PEST-NPs. The enzyme O-GlcNAcylation transferase directly interacts with the PEST-containing transcription-factor Bmi-1 at serine 255 and increases the stability and oncogenic activity of Bmi-1 via inhibiting p53, PTEN and CDKN1A/CDKN2A (Y. Li, Wang, et al., 2017). Similarly, PTEN is ADP-ribosylated by tankyrases and E3 ligase RNF146 ubiquitinates the ribosylated-PTEN and degrades PTEN (N. Li et al., 2015).

1.3.3 | Intracellular localization

In addition to activation/inhibition and stability/degradation, the PTMs of the PEST motif also regulate intracellular localization of PEST-NPs. Intracellular localization is important to execute a pharmacological anticancer or carcinogenic response. These proteins are transported by importins and exportins that identify the nuclear localization sequences (NLSs) and nuclear export sequences (NESs) respectively, present on cargo proteins. Tumorigenesis relates to the unbalanced nucleocytoplasmic shuttling of cargo proteins. The nuclear localization of p53 executes a DNA damage effect while cytoplasmic p53 executes an apoptotic effect. However, cytoplasmic p53 is entrapped by antiapoptotic protein Bcl-xl. The entrapped-p53 cannot initiate the mitochondrial membrane to execute anticancer effects until PUMA liberates the sequestered-p53 protein. Many transporter inhibitors are under investigation for their anticancer effects such as withacnistin inhibits nuclear localization of signal transducer and activator of transcription 3 (STAT3) and suppresses antiapoptotic proteins (X. Zhang, Blaskovich, Forinash, & Sebti, 2014). Hence, the PEST motif contributes to the diverse features of nuclear proteins, leading to multifarious molecular and physiological roles of PEST-NPs.

2 | MULTIFACETED BEHAVIOR OF PEST-NPs

In this review, we have summarized the impact of PTMs of PEST-NPs on their activation/inhibition, stability/degradation, and intracellular localization to execute the tumor-promoting or tumor-suppressive response. This review mainly addresses the multifaceted behavior of PEST-NPs in cancer biology by highlighting protein–protein interactions at the molecular level. Among various NPs, some important PEST-NPs such as p53, PTEN, Bmi-1, Myc, MeCP2, GLTSCR2, and PCNP have been discussed individually as model PEST-NPs. The structure of a protein defines its origin, mechanism of function, and fate. Moreover, the "PEST score," calculated by the computer-aided program "PEST-Find," defines the genuine PEST domain with a proteolytic signal. Here, the structural comparison of these proteins is given in Table 1. However, some of their important functions in cancer metabolism and transcription are summarized in Tables 2 and 3, respectively.

2.1 | p53

The human p53 is one of the most important tumor suppressor proteins. It is famous as the "guardian" of the genomes playing a vital role in controlling the fundamental processes of the hallmarks of cancer. The human p53 protein consists of 393 amino acids, starting from the amino acid 1 of the amino-terminal to amino acid 393 of the carboxyl-terminal. The N terminal is composed of five repeats of Proline-XX-Proline sequences (X is the amino acid, which varies from species to species of p53) and several phosphorylation sites (from 62 to 94 amino acids). The proline-rich area called the transactivation domain (TAD) is important for transcriptional activation of the protein. The TAD is divided into two parts, TAD1 (residues 1–39) and TAD2 (residues, 40–61). Although TAD1 is generally sufficient to execute p53-dependent cell cycle arrest and apoptosis in response to acute DNA damage, each TAD can induce senescence and suppression of tumor initiation in response to oncogenic signaling. The N terminal in general and TAD2, in particular, interacts with the DNA-binding domain at or near the DNA-binding surface of the host cell, and thus blocks the DNA binding (Miller Jenkins et al., 2015; Walker & Levine, 1996). The DNA-binding domains regulate the nuclear and cytoplasmic functions of p53 (Follis et al., 2014). The affinity and specificity of DNA-binding sites mediate competitive inhibition between binding domains (F. He et al., 2019). The phosphorylation sites at 22 and 23 amino acids are responsible for the

TABLE 1 Structural differences of PEST sequence enriched nuclear proteins (PEST-NPs)

Proteins	PEST score	Number of amino acids	Chromosome number	Number of transcript	Cytoband	Chromosome location (bp)
p53	-3.7–1.7	393	17	25	p13.1	7661779–7687550
Bmi-1	n.d.[a]	326	10	4	p12.2	22321211–22331484
PTEN	19.41–20.49	403	10	2	q23.31	87863113–87971930
Myc	2.3–11.8	439	8	9	q24.21	127735434–127741434
MeCP2	n.d.[a]	MECP2_e1(498) and MeCP2-e2(486)	Xq28	10	q28	154021573–154137103
PCNP	n.d.[a]	178	3	3	q12.3	101574095–101594437
GLTSCR2	7.23	478	19	5	q13.33	47745522–47757058

Abbreviations: GLTSCR2, glioma tumor suppressor candidate region gene 2; PCNP, PEST-containing nuclear protein; PEST, proline (P), glutamic acid (E), serine (S) and threonine (T); PTEN, phosphatase and tensin homolog.
[a]n.d., no data available.

degradation of p53. The p53 ubiquitin ligase MDM2 interacts with TAD1 (amino acids 22 and 23) and suppresses tumor activity (Lin, Chen, Elenbaas, & Levine, 1994; Walker & Levine, 1996). It has been reviewed that approximately 80% of the mutations found in the p53 gene occur in the proline-rich region of p53 (Bouaoun et al., 2016).

The DNA damage, metabolic variations, telomere corrosion, hypoxia, mitotic spindle malfunction, deficits in ribosomal biogenesis, and the mutational activation of other oncogenes such as Myc, Ets, and Ras trigger transcription of p53. The type of acetylation, methylation, and phosphorylation (PTM) specifies p53 associated cellular activities such as apoptosis, cellular senescence, differentiation, cell cycle regulation, ferroptosis, cellular repair mechanisms, autophagy, extrinsic signaling and metabolic mutation. Physiological conditions and types of cells are additional factors that further define the cellular activities of p53.

The p53 protein maintains a required level in normal cells, as it is a potent inducer of apoptosis. In normal circumstances, the level of p53 protein in the cell is very low due to a short half-life of about 620 min. In response to a variety of stress conditions, the p53 level is stabilized through different PTMs, which often regulate p53 binding with its natural destructor human double minute 2 (HDM2) and create multiple feedback loops. The transcription and oncogenic properties of p53 interplay between protein expression, while MDM2 and ADP-ribosylation factor p14 (p14ARF) are the chief players in p53 stability, where MDM2 degrades the p53 by proteasomal degradation (Lahav et al., 2004) while p14ARF inhibits MDM2 and increases p53 levels (Brown, 2009). Similarly, the stress-responsive kinase p38 MAPK, which phosphorylates p53 at serine 33 and serine 46, also contributes to p53 stabilization and activation. In contrast, the activated p53 induces Wip-1 phosphatase that facilitates a negative regulatory feedback on p38 MAPK/p53 signaling (Stramucci, Pranteda, & Bossi, 2018).

The protein p53 mediates cytoplasmic and nucleoplasmic responses upon active accumulation in the respective regions. Cytoplasmic accumulation induces mitochondrial permeability and apoptosis while nuclear localization initiates DNA damage response or transcription of target proteins. The nucleo-cytoplasmic shuttling is a strictly regulated process and slight mutations can abruptly disturb the normal processes. The nuclear export of p53 is mediated by PTMs such as phosphorylation at threonine 155 mediates nuclear export (E. W. Lee, Oh, Song, & Kim, 2017), phosphorylation at serine 392 mediates mitochondrial localization (Castrogiovanni, Waterschoot, De Backer, & Dumont, 2018) while a kinase inhibitor inhibits nuclear export. Acetylation at lysine is another modification of p53 that induces activation, nuclear localization and DNA binding of p53 protein (Ai et al., 2016).

2.2 | PTEN

PTEN is a tumor suppressor nuclear protein, which is first found in glioblastoma cell lines and xenografts, prostate cancer cell lines, and breast cancer cell lines and xenografts in the mutant form (J. Li et al., 1997). The PEST-NP PTEN, also called MMACI/TEP1 consists of 9 exons, 1,212 nucleotides and 403 amino acids with 47 kDa molecular mass (Haddadi et al., 2018; D. M. Li & Sun, 1997; Steck et al., 1997). The two domains, N-terminal phosphatase and C-terminal with a small N-terminal tail constitute the major portion of the protein. The C-terminal region consists of the lipid-binding domain called the PDZ-binding C2 domain that confers the affinity for the phospholipid membrane and is essential for the right placement of PTEN at the plasma membrane. It also consists of two PEST sequences, which are responsible for its tumor-suppressive activity (Georgescu, Kirsch, Akagi, Shishido, & Hanafusa, 1999; Myers et al., 1998). The tail of the C-terminal comprises about 50 amino acids that are responsible for active phosphorylation. Lipid-phosphatase activity is mainly responsible for the antitumor function of PTEN. The suppression of the enzymatic activity of PTEN illustrates the loss of function. The phosphatase activity correlates with gene expression and invasion. The PEST region of PTEN is not only responsible for proteasomal degradation of protein by ubiquitination but also correlates with improved stability of the protein and its

TABLE 2 Nuclear proteins act via oncogenic pathways

Proteins	Oncogenic pathways	Tumor type	Mechanism	Effect
p53	PI3k dependent AKT kinase	Breast cancer MCF-7. MDA-MB468, SKBR3	↓ AKT kinase induced BCL-2-modifying factor and ↑ mitochondria-dependent apoptosis	Tumor suppressor
		Epithelial BRKs	↑ AKT kinase delayed DNA degradation and cell death kinetics without effecting Bcl-2, Bcl-xl, and Bax.	Delaying the onset of p53-mediated apoptosis
	mTOR pathway	Adenocarcinomic alveolar epithelial A549	Activated p53 ↑ transcription of IFN regulatory factor 9	Tumor suppressor
		Mantle cell lymphoma (MCLs)	p53 activation ↓ AKT/mTOR pathway via AMP kinase	Tumor suppressor
		Murine erythroleukemia (MELs)	↑ Dephosphorylation of the eIF4E-binding protein 4E-BP1, ↓ ribosomal protein S6 kinase	↓ Initiation of translation
	p38 MAPK pathway	Neural crest cells (NCCs)	↑ Levels and phosphorylation of p53	Tumor suppressor
PTEN	PI3k dependent AKT kinase	Renal cell carcinoma786-oBreast cancer MCF-7	↓ AKT kinase	G1 arrest
		Embryonal carcinoma NT2/D1	↑ Cytoplamic re-localization of cyclin-dependent kinase inhibitor $p27^{kip1}$	G1 arrest
		Fibroblast cell NIH3T3	↓ H-Ras R12 transformation	↓ Morphological transformation and anchorage-independent growth
		Endometrial epithelial cells	↑ Smad3-dependent activation of PTEN transcription	TGF-β induced apoptosis
	mTOR pathway	Colon cancer HCT116Prostate cancer DU145	Dephosphorylation of 4E-BP1/4E-BP2 and increases their association with eIF4E to suppress translation	Hypoxia induced cell death
	mTORC1 pathway	Prostate adenocarcinoma PCa	Activation of PI3K, MAPK and WNT	Androgen derived therapy resistance
	MAPK	Colon cancer HCT116	Oridonin induced PTEN drives MAPK/p38 phosphorylation	Cell arrest and apoptosis
Bmi-1	PI3k dependent AKT kinase	Esophageal squamous cell carcinoma ESCC	Bmi-1 knockdown increased cell apoptosis, downregulated MCL-1 and p-AKT and upregulated Bax.	↓ Cell viability and ↑ radio-sensitivity
		Human liver carcinoma HepG2, MHCC97-H	Bmi-1 knockdown increased PTEN expression and down regulate vascular endothelial growth factor (MM2, MM9)	↓ Invasiveness
	AKT/GSK3b	Human mammary epithelial cells HMECs	↑ Mesenchymal markers, ↓ expression of epithelial markers	↑ Tumorigenesis and lung metastases
Myc	PI3k dependent AKT kinase	Prostate cancer DU145, PC3, C4-2B	SIRT3 mediated ↓ ubiquitination and degradation c-Myc	↑ Cell survival
		Chronic lymphocytic leukemia	EZH2 directly binds to the *IGF1R* promoter to increase IGF1R via Myc	Poor prognosis
GLTSCR2	AKT/mTOR/p70S6K pathway	Glioma U251, Breast cancer MCF-7	Inhibition of rRNA transcription	Pro-death autophagy
	PTEN-PI3k dependent AKT kinase	Human cervical cancer Hela	Binds and stabilizes PTEN	Antitumor

Abbreviations: GLTSCR2, glioma tumor suppressor candidate region gene 2; IFN, interferon; MAPK, mitogen-activated protein kinase; mTOR, mammalian target of rapamycin; PI3k, phosphoitide 3-kinase; rRNA, ribosomal RNA; TGF-β, transforming growth factor-β.

TABLE 3 Nuclear proteins act via transcription pathways

Protein	Transcription pathways	Tumor type	Mechanism	Effect
p53	p38 MAPK pathway	Non-small-cell lung cancers NSCLCs	↑ Degradation of MDM2 and stabilization of p53, ↑ transcription of EGFR	↑EGFR expression and tumor growth
Bmi-1	NF-κB	Gliomaa1-72, LN229	NF-κB mediated ↑ MMP-9 expression and activity	↑Invasiveness, ↑ migration
		Nasopharyngeal carcinomaTW01		
		Osteosarcoma U-2OS		
Myc	CDK/Rb/E2F	Nasopharyngeal carcinoma 5–8 F	↑ Activity of cyclin D1	↑Tumor growth, ↑cell cycle progression
		Human diploid fibroblastsHs 27	Ceramide induced ↓ transcription of c-Myc, ↑ p21 WAF1/CIP1/Sdi1 kinase inhibitor↓ cyclin-D1 associated kinase activity	Tumor growth arrest
	MAPK	Chondrocytes	Micro-RNA-24 induced ↓ C-Myc, IL-1β, TNF-α, p38, p-p38, ERK, p-ERK, JNK, and p-JNK	↓Apoptosis ↑ proliferation
		Pancreatic cancer AsPC-1, BxPC-3, PANC-1	Calreticulin mediated ↑ EGF-induced epithelial mesenchymal transition	↑Tumor growth
	WNT/β-catenin	Prostate cancer	AR-independent castration-resistant prostate cancer through β-catenin activation, with PTEN loss	Resistant prostate cancer
MeCP2	Wnt5a/β-catenin	Gastric cancer	↓ Transcription of FOXF1 and MYOD1	↑Tumor growth
	5-hydroxymethylcytosine dependent transcription	HMECs, BPECs	↑ MAPK ↑ PI3K	Anchorage-independent growth

Abbreviations: NSCLC, non-small-cell lung cancer; BPEC, breast progenitor epithelial cell; CDK, cyclin-dependent kinase; E2F, early-region-2 transcription-factor; EGFR, epidermal growth factor receptor; ERK, extracellular signal–regulated kinase; FOXF1, forkhead box protein F1; HMEC, human mammary epithelial cell; IL-1β, interleukin-1β; JNK, Jun N-terminal kinase; MAPK, mitogen-activated protein kinase; MDM2, murine double minute 2; MMP-9, matrix metalloproteinase-9; MYOD1, myogenic differentiation 1; NF-κB, nuclear factor-κB; PI3k, phosphoitide 3-kinase; TNF-α, tumor necrosis factor-α.

deletion decreases the expression of the PTEN protein considerably. The ubiquitination regulates the degradation, nuclear import, and tumor-suppressive activity of PTEN (Trotman et al., 2007). Mono-ubiquitination at lysine 13 and/or lysine 289 is responsible for nuclear import of PTEN. However, upon nuclear localization, PTEN-Ub deubiquitinates and antagonizes the mammalian target of rapamycin (mTOR) pathway. PTEN also antagonizes the mTOR pathway in the cytoplasm only if it is not ubiquitinated. Otherwise, polyubiquitination readily degrades cytoplasmic PTEN by proteasomal degradation.

Although PTEN is the second most effective tumor suppressor protein, its tumor-promoting effects are also observed (Ma et al., 2019; Mukherjee et al., 2018). PTEN nuclear localization through NLS is responsible for increased cell viability in endometrial adenocarcinoma Ishikawa cells (Mukherjee et al., 2018). Nuclear PTEN encourages DNA repair and chromosomal stability (J. O. Lee et al., 1999; Tibarewal et al., 2012; J. Zhang et al., 2019). Another study (J. Zhang et al., 2019) demonstrates that dimethylation of PTEN recruits PTEN to DNA-damage sites where it mediates efficient repair of DNA double-stranded bands via dephosphorylation.

In addition to phosphorylation and ubiquitination, SUMOylation also regulates the activity and localization of PTEN. PTEN-SUMOylation regulates its nuclear retention, which is sensitive to genotoxic stress (Bassi et al., 2013). SUMOylation of PTEN not only increases nuclear retention but also positively correlates with the DNA damage response. The loss of SUMO-PTEN is responsible for the loss of the DNA damage response. PTEN-mediated DNA damage response is a major cause of poor prognosis under radiotherapy.

2.3 | Bmi-1

A polycomb group (PcG) protein, Bmi-1 plays a pivotal role in epigenetic regulation of various cellular processes such as proliferation, differentiation, self-renewal of stem cells, and chemoresistance to different anticancer drugs. The PEST-NP Bmi-1 encodes 326 amino acids with a molecular weight of 44–46 kDa. The polycomb group protein PRC1 commonly known as Bmi-1 consists of two domains, one of which is Bmi-1 that covers the Ring1b while the tail of Ring1b wraps around the Bmi-1. The oncogene Bmi-1 attaches to the substrate protein and ubiquitinates the lysine of the substrate protein by E3 ligase Ring1b (Alkema, Wiegant, Raap, Berns, & van Lohuizen, 1993; Z. Li et al., 2006). For example, Bmi-1 ubiquitinates p53 via RNF2/Ring1b and decreases the stability of p53 (Calao et al., 2013; W. J. Su et al., 2013). The protein Bmi-1 also contains two NLSs, NLS1 and NLS2 that are responsible for nuclear localization of Bmi-1. Among these signals, NLS2 plays an essential role in the nuclear localization of Bmi-1 (Alkema et al., 1993). According to functions, Bmi-1 is divided into three portions or regions: a conserved ring domain at the N terminal, a central helix-turn-helix (HTH) domain and a carboxyl-terminal containing a PEST-like domain (Aikema, Wiegant, Raap, Berns, & Lohuizen, 1993; Alkema et al., 1993; Z. Li et al., 2006). In response to DNA damage, the ring domain of Bmi-1 concentrates on DNA strand breaks and together with HTH avoids senescence in cells (Ginjala et al., 2011; Itahana et al., 2003). Thus, it increases the replicative lifetime and accumulation of cells at the G2/M phase, hence increasing the proliferation of cells (Yadav et al., 2010). The Bmi-1 also increases the survival time of the tumor cell through Myc-N-activation.

The oncogenic function of Bmi-1 is evident as it inhibits the transcription of tumor suppressors such as p16, p19, p53 and PTEN (Jacobs et al., 1999; Song et al., 2009; Yadav et al., 2010). The protein Bmi-1 is upregulated in a variety of cancer types, including lymphomas, prostate cancer, non-small-cell lung cancer (NSCLC), colon cancer, breast cancer, and nasopharyngeal carcinoma. This upregulation of Bmi-1 in prostate cancer, NSCLC, and colon cancer occurs concurrently with downregulation of INK4A (also called p16) and ARF. During the development process, Bmi-1 plays a critical role in the self-renewal of neural stem cells and intestinal stem cells through inhibition of the p21CIP1 gene and INK4A/ARF locus respectively (Fasano et al., 2007). The upregulation of cyclin E contributes to the Bmi-1-mediated progression of neuroblastoma (Mao et al., 2013). Bmi-1 also enhances telomerase activity in mammary epithelial cells and prostate cancer cells (Dimri et al., 2002; Ismail et al., 2012). The PTMs of Bmi-1 at different serines regulate both INK4A/ARF-dependent and INK4A/ARF-independent functions of Bmi-1 (Voncken et al., 2005). The phosphorylation of Bmi-1 by AKT stimulates glioma and hepatic carcinogenesis through an INK4A/ARF-independent pathway (Bruggeman et al., 2007; Xu et al., 2009) and suppresses tumor growth through an INK4A/ARF-dependent pathway (Y. Liu et al., 2012). The modification of Bmi-1 by AKT kinase affects its oncogenic properties depending on the type of residual substrate (Y. Liu et al., 2012). Bmi-1 also phosphorylates by MAPK activated protein kinase 3 (MAPKAPK3). The activation or overexpression of MAPKAPK3 phosphorylates Bmi-1 and other polycomb proteins and subsequently recruits substrate proteins from chromatin, activates transcription of the INKA4/ARF locus; and hence promotes apoptosis (Cui et al., 2007; Sahasrabuddhe, Dimri, Bommi, & Dimri, 2011).

A large number of transcriptional and posttranscriptional regulators modulate Bmi-1 expression such as Myc-N, Myc-C, specific protein 1 (Sp1), Twist related protein 1 (Twist1), forkhead box protein M1 (FOXM1), E2F-1 and Sal-like protein 4 (SALL4) regulate the expression of Bmi-1 positively. While, Kruppel-like factor 4 (KLF4), Mel-18, and histone deacetylase inhibitors (HDACi) suppress Bmi-1 at the transcriptional level. The Notch and Wnt pathways also regulate the expression of Bmi-1 (Erika et al., 2015). The mRNA and protein levels of Bmi-1 are upregulated in PTEN-null prostate cancer, while the loss of Bmi-1 attenuates the oncogenic progression in PTEN-null prostate cancer. The ubiquitination breaks down Bmi-1. The protein Bmi-1 is ubiquitinated at tyrosine 18 of ring finger by ubiquitin ligase β-transducing repeat-containing protein (βTrCP) into proteasomes. In MCF10A cells, the overexpression of βTrCP increases the degradation of Bmi-1 and vice versa (Sahasrabuddhe et al., 2011; J. Zhang & Sarge, 2009).

2.4 | Myc

The PEST-NP Myc is probably the most prominent resident nuclear transcription factor that dimerizes with MAX to mediate various biological processes that influence normal cell growth and proliferation. However, when deregulated, Myc becomes an oncogene and transforms cells into malignant cells in concert with other genetic alterations including loss of tumor suppressor checkpoints. According to functions, Myc proteins consist of a large unstructured amino-terminal region with conserved boxes known as Myc boxes (MBI and MBII), which are involved in transcriptional activation. The middle segment of Myc proteins is composed of PEST sequences that are responsible for ubiquitination, two conserved Myc boxes (MBIII and MBIV), and one NLS for nuclear accumulation. The carboxyl-terminal region constitutes the basic helix-loop-helix leucine zipper (bHLHZ) domain of one hundred amino acids, which is essential for DNA–protein interaction initiating transcription (Adhikary & Eilers, 2005; Conacci-Sorrell, McFerrin, & Eisenman, 2014).

The protein Myc is generally overexpressed in human cancer cells. The vital downstream outcomes of Myc are protein translation, cell cycle progression, and differentiation, tumor metabolism, and ribosome biogenesis. It coordinates with a wide range of biological functions, such as cell differentiation, cell survival, immune surveillance, apoptosis, and cell proliferation. The Myc-associated cell cycle defects arise after enzymatic activation of Myc by SYMOylation (Kessler et al., 2012). The SUMOylation of Myc by E3 ligase, the protein inhibitor of activated STAT (PIAS-1) not only increases half-life by preventing its proteasomal degradation but also positively regulates the transcriptional activity of Myc. It is observed that Myc activates transcription of the WW domain-containing E3 ubiquitin-protein ligase 1 (WWP1), which poly-ubiquitinates PTEN at serine 27, thus inhibiting PTEN dimerization and membrane recruitment leading to degradation of PTEN and loss of PTEN-mediated tumor-suppressive activity (Y. R. Lee et al., 2019). The pharmacological inhibition of PI3K/AKT/mTOR pathway (Suter & Marcum, 2007) distinctly decreases the Myc level and reveals significant therapeutic value in Myc–driven cancers like small-cell lung carcinoma, breast cancer, hematopoietic cancer, and neuroblastoma.

The posttranslational phosphorylation and isomerization together are responsible for the nuclear localization of the Myc protein. The serine 62 phosphorylated-Myc accumulates at the nuclear pore followed by proline 63 isomerization of serine 62 phosphorylated-Myc in the presence of phosphorylation-dependent prolyl isomerase. This isomerization promotes internalization of Myc from the nuclear pore to the nuclear complex via mitogen-induced MYC–MAX–GCN5 pathway. The specific nuclear localization regulates resident genes according to external stimuli (Y. Su et al., 2018). Another study demonstrates that Myc accumulates at the nucleolus after threonine 58 phosphorylation where it ubiquitinates and degrades by FBW7, a component of an SCF-class ubiquitin ligase (E3) complex (Welcker, Orian, Grim, Eisenman, & Clurman, 2004). The SUMOylation is another way to regulate the stability of Myc by proteasome degradation, upon deSUMOylation by deSUMOylating enzyme; it promotes mono-ubiquitination and phosphorylation at serine 62 and threonine 58, thus regulating activity and expression of Myc (Sun et al., 2018). The reverse transcriptase catalytic subunit (TERT) regulates and stabilizes Myc expression at chromatin by ubiquitination, hence contributing to activation or suppression of the target gene (Koh et al., 2015). In addition to nuclear degradation, Myc degrades in the cytoplasm by calcium-dependent proteases (calpain) into C and N terminals (Conacci-Sorrell & Eisenman, 2011).

2.5 | MeCP2

The protein MeCP2 is another nuclear protein where PEST-Find detects the presence of the PEST motif. The methyl-CpG binding protein 2 (MeCP2) is a 43 kDa protein with an ability to bind with DNA (Lewis et al., 1992; Meehan, Lewis, & Bird, 1992). It consists of two functional halves; the N-terminal portion mainly constitutes DNA-binding domains called the methyl-CpG-binding domain (MBD). The C-terminal of protein harbors at least two independent DNA-binding domains and constitutes a chromatin specific binding domain that is mainly responsible for regulating nucleosomal array compaction and oligomerization (Ghosh et al., 2010; Ho et al., 2008). It contains the transcriptional repression domain. This region also consists of protein–protein interacting regions such as a dimerizing domain (Becker et al., 2013) and a WW domain-binding region (WDR; Buschdorf & Stratling, 2004). The protein MeCP2 also possesses one NLS (Nan, Tate, Li, & Bird, 1996) and two PEST sequences in the C-terminal with a strong positive PEST score as mentioned in Table 1 (Thambirajah, Eubanks, & Ausio, 2009). The phosphorylation of the PEST sequence at serine sequentially undergoes ubiquitination of the neighboring lysine leading to rapid proteasomal degradation of MeCP2. The PEST sequences and their phosphorylation are involved in protein turnover, hence responsible for maintaining an adequate level of MeCP2 within the nucleus.

The PEST-NP MeCP2 regulates cell development by mediating transcriptional activity as well as epigenetic activity. MeCP2 together with its MBD domains binds with the hydrophilic surface of methylated DNA to repress the transcriptional activity of DNA. The differential phosphorylation of MeCP2 is a key mechanism by which the MBD modulates its affinity for its partners. In response to stimuli, MeCP2 recruits other corepressors, such as HDAC and Sin3A, to the promoter site to mediate the hindrance to the expression of brain-derived neurotrophic factor (BDNF) and CDK15 (Bellini et al., 2014; Ho et al., 2008). On the other hand, it recruits transcriptional activator CREB1 at the promoter site of an activated target to activate BDNF promotion; hence transcriptional activation (Chahrour et al., 2008). The protein MeCP2 inhibits FOXF1 and MYOD1 transcription by binding with their promoters (L. Zhao et al., 2017). FOXFI is not only a potent tumor suppressor for breast cancer, gastric cancer, lung cancer, and esophageal carcinoma but also a member of the transcription family that regulates cell proliferation, differentiation, tissue repair, and embryo early development. It also promotes the growth of gastric cancer cells by suppressing miR-338-mediated antiproliferative effects (Tong et al., 2016).

The normal functioning of neuronal cells is another important function of MeCP2 (Amir et al., 1999). The disruption of MeCP2 functions in mouse and human neurons decreases ribosomal RNA (rRNA) and cell size that demonstrate poor cell health. It is an efficient gene that regulates the expression of other genes. It binds with methylated- and hydroxyl methylated-cytosine adenosine nucleotides of DNA with strong affinity and regulates expression of the largely distributed long gene in brain cells (Gabel et al., 2015). The neuronal activation stimulates calcium-dependent phosphorylation of MeCP2 with concomitant release of the methyl-binding protein from BDNF promoter III, thereby facilitating transcription (Bellini et al., 2014).

The PTMs of MeCP2 are likely to affect the binding of MeCP2 with DNA and protein partners and therefore contribute to the versatility of MeCP2. In spite of having a constitutive NLS, the strong affinity of MBD for DNA is sufficient for nuclear retention of MeCP2. The SUMOylation of MeCP2 at lysine 412 by the E3 ligase PIAS-1 degrades it into N and C terminals. However, the N terminal together with its MBD retains its activity of DNA binding while the C terminal loses its activity. The phosphorylation at serine 421 and threonine 308 also facilitates MeCP2 SUMOylation (Tai et al., 2016). MeCP2S80 is the most abundant phosphorylated-residue during resting conditions and neuronal activity induces its dephosphorylation. Serine 80 phosphorylation does not affect the overall subcellular localization of MeCP2 but seems to increase its affinity for chromatin (Bellini et al., 2014).

2.6 | GLTSCR2

The glioma tumor suppressor candidate region gene 2 protein, GLTSCR2, also known as protein interacting with carboxy terminus 1 (PICT-1), is a 60 kDa protein located at chromosome 19q13 with 478 amino acids. The PEST-NP GLTSCR2 shares its homology with the yeast 60S ribosomal protein. There is a putative PEST sequence with a score of +7.23 in GLTSCR2, which is functionally associated with ribosomal RNA processing (Kalt, Borodianskiy-Shteinberg, Schachor, & Sarid, 2010). The protein GLTSCR2 contains six NLS motifs along with extraordinary long sequences enriched with several arginine and lysine clusters sharing functional similarity with nucleolar localization signals (NoLSs).

The phosphorylation by c-Jun NH2-terminal kinases (JNK) and poly-ubiquitination mediate the stability of GLTSCR2. Under stress conditions like DNA damage, GLTSCR2 localizes and aggregates to the nucleolus near ribosomal DNA via JNK phosphorylation (Kalt, Levy, Borodianskiy-Shteinberg, & Sarid, 2012; S. Lee, Cho, Kim, & Park, 2016) where it releases ribosomal proteins like fibrillarin. The ribosomal proteins are important to regulate the cell cycle, tumorigenesis, viral replication, senescence and stress response.

The antitumor effects of GLTSCR2 are dependent on the PTEN pathway. It exerts antitumor effects either directly interacting with the carboxyl-terminal of the tumor suppressor PTEN and promoting PTEN phosphorylation, therefore inhibiting AKT pathway or indirectly by increasing the stability of PTEN (Okahara, Ikawa, Kanaho, & Maehama, 2004). The phosphorylation of the carboxyl-terminal of GLTSCR2 inhibits phosphatase activity and subsequent recruitment of PTEN to the plasma membrane, both of which are essential for antitumor functions of PTEN. On the other hand, phosphorylation of the carboxyl-terminal of GLTSCR2 is also important to maintain the cellular level of PTEN (Okahara et al., 2004). It is important to know that GLTSCR2 downregulation promotes the tumorigenic transformation of cells and this impaired GLTSCR2 expression is associated with PTEN downregulation in human neuroblastoma. The putative tumor suppressor gene GLTSCR2 induces PTEN-modulated cell death (Yim et al., 2007).

GLTSCR2 is mainly located at the nucleolus due to presence of several NoLS while it also influences the localization of other proteins. However, little is known about this localization mechanism. Given that the NoLS motif must be present at the protein surface to interact with the relevant partner bringing it to the nucleolus, the efficacy of each NoLS in the context of the full-length protein may depend on posttranslational events. In other words, multiple NoLS motifs may function to secure nucleolar localization of GLTSCR2 in different conditions (Kalt et al., 2012; Okahara et al., 2006; Yim et al., 2007). This protein assumes distinct protein confirmations upon different PTM or protein–protein interactions. GLTSCR2 interacts with the Bcl-2 homolog (KS-Bcl-2) and selectively relocates KS-Bcl-2 from the mitochondria to the nucleolus.

2.7 | PEST-containing nuclear protein (PCNP)

PCNP is an under investigation member of the PEST-NPs. Recent research work explains that mRNA of PCNP is present in various kinds of cancer cells like WI-38 and TIG-7 normal fibroblast cells, HT-1080 fibrosarcoma cells, and HepG2 hepatoma cells, signifying that PCNP can be involved in various features of tumorigenesis (Mori, Li, Hata, Ono, & Kochi, 2002). PCNP is a novel nuclear protein consisting of 178 amino acids. It co-localizes with a ring finger protein NIRF in a homogenous manner in the peri-nucleus area, the avoiding nucleus (Mori et al., 2002). PCNP interacts with NIRF in vitro and in vivo. NIRF contains the ubiquitin domain in the N-terminus and the ring finger catalytic domain in the C-terminus, suggesting catalytic activity of NIRF. The association of PCNP with NIRF in vitro and in vivo highlights the ubiquitination of PCNP via NIRF, hence controlling the stability and regulation of PCNP. A similar relationship exists between p53 and ring finger protein MDM2. Moreover, the expression level of NIRF is higher in various cancers (Alhosin et al., 2011).

Our previous studies describe that PCNP mediates the proliferation, migration, and invasion of human neuroblastoma, ovarian cancer cells, and lung adenocarcinoma cells through MAPK and PI3K/AKT/mTOR signaling pathways (Dong et al., 2020; Wang et al., 2019; Wu et al., 2018). PCNP high levels reduce apoptosis via upregulating the expression levels of phosphosignal transducers and activators of transcription (STATs). It is also responsible for the immune response in rheumatoid arthritis in a positive relationship with TNF-α-inducible

protein 8-like 2 (Shi-Bai et al., 2017). PCNP actively executes apoptosis by mediating caspase activities. However, the underlying mechanism of inducing apoptosis is unknown. The induction of autophagy and apoptosis in different cell lines suggests the involvement of different nuclear transporters in the dual behavior of PCNP. However, the nuclear transportation of PCNP needs to be investigated (Afzal et al., 2019).

3 | CANCER GENE THERAPY

Worthwhile advances in technology have improved the diagnosis and treatment plans for cancer with improving survival rates (Siegel, Miller, & Jemal, 2018). However, the conventional methods are not free from the risk of metastasis and/or long-term adverse impacts on nearly every organ system. Gene therapy (delivery of genetic materials e.g. gene, DNA, siRNA, and mRNA) is an intriguing and effective approach to treat various diseases, of which 60% of the on-going clinical trials are related to cancer treatment (Wirth & Yla-Herttuala, 2014). Gene delivery is based on novel approaches such as siRNA delivery to block a critical pro-growth pathway or delivery of a gene coding for a proapoptotic inducer (Libutti, 2014). The tumor suppressor proteins are lost in many human tumors as in the case of p53, PTEN, and PCNP. Restoration of such types of tumor suppressor genes may be a suitable approach. The tumor suppressor protein p53 has been widely explored as a gene therapeutic and is now in Phase III clinical trials (Levine & Oren, 2009).

3.1 | Emerging role of gene therapy

It is important to understand the disease mechanism before starting its treatment. Insights into tumorigenesis demonstrate that it is a complex, multi-gene, multi-stage, and multi-factorial disease. Cancer development initiates upon imbalance between cell proliferation and apoptosis, cell differentiation and inhibition, immunity and avoidance of immunity, angiogenesis, and inhibition, as well as metastasis and suppression of metastasis. However, cancer gene therapy is a simple process involving the balance of oncogene and tumor suppressor nuclear proteins.

3.1.1 | Gene therapy by restoring the expression of tumor suppressor proteins

Maintaining the expression of the tumor suppressor gene at the target site either by induction, increasing the stability, or intracellular localization is a significant strategy in cancer therapy. The induction of tumor suppressor PEST-NPs like p53 or PTEN through viral and nonviral vectors is an emerging field of cancer gene therapy. Induction of p53 through adenovirus is under clinical trials by the US-FDA while scientists are also interested in nonviral techniques like micelles, liposomes, nanoparticles, and exosomes. Some recent attempts in pursuit of efficient delivery of genetic material are listed in Table 4. Inhibiting the PTM-mediated proteasomal degradation of the tumor suppressor gene also restores the expression level of tumor suppressor proteins. For example, inhibition of p53 degradation by an MDM2 ligase antagonist (Lisachev et al., 2015) such as roscovitine (Lu, Chen, Peng, & Chen, 2001) and nutlin-3 (T. He et al., 2018) successfully restores the expression of p53. The antagonist of MDM2, RG7112 demonstrates clinical activities in treating acute myeloid leukemia and chronic lymphocytic leukemia (Andreeff et al., 2016). Enhancing mitochondrial permeability by maintaining cytoplasmic p53 expression level via PUMA-mediated modification of p53 is another strategy to restore P53 at the target site

TABLE 4 Gene delivery systems composed of PEST-NPs

Gene	Loaded genetic material	Carrier systems	Cell line
PTEN, TRAIL	Protein	Zein nanoparticles	HepG2, HCC
	mRNA PTEN	Polymer-lipid hybrid nanoparticles coated with a polyethylene glycol shell	PC3-luc
	Protein	Cationic lipidoids	PC3
	Plasmid	Antioxidant nanoliposomes	
	Protein	Silver nanoclusters was encapsulated within PEG coating	U-87 MG MCF-7
p53	pDNA	Chitosan-sodium deoxycholate nanoparticles	Human Caco-2 cells
		Magnetic nanoparticles	U-87
		Polyethyleneimine-modified calcium carbonate nanoparticles	Hep3B, QSG-7701, H1299, 293a and Hela cells
		Monodisperse double-walled microspheres	N/A
		Gold nanoparticles	WI-38, A549

Abbreviations: HCC, hepatocellular carcinoma; mRNA, messenger RNA; PEST-NP, PEST containing nuclear protein; PTEN, phosphatase and tensin homolog; TRAIL, TNF-related apoptosis-inducing ligand.

(Kim et al., 2019). Restoration of the anticancer gene at the site of action can be achieved also by regulating the localization of the gene by controlling NLS-/NES-mediated transporters or PTM-mediated accumulation of the gene. Similarly, specific inhibition of transcription factors like Bmi-1, which inhibit the transcription of tumor suppressors like p53 and PTEN, may also be fruitful.

3.1.2 | Gene therapy via inhibition of oncogene

On the other hand, to transform mutant PEST-NPs into a wild-type form with normal functions and to inhibit the transcription or to decrease the stability of oncogene are also important mechanisms in cancer gene therapy. The metallochaperone stabilizes the DNA-binding protein domain and restores the ability of p53 to bind with DNA. This drug is in preclinical trials (Yu et al., 2018). Inhibition of cyclin-dependent transcription of Myc via CDK7 or CDK9 inhibitors such as THZ1 or PC585, respectively, substantially reduces Myc expression and induces potent antitumor effects in Myc-overexpressing T-cell acute lymphoblastic leukemia (Kwiatkowski et al., 2014) and small cell lung cancers (Christensen et al., 2014). A potential strategy to target the stability of Myc is by inhibiting the kinases or deubiquitinases that stabilize Myc. Several deubiquitinating enzymes are involved in Myc stabilization. The ubiquitin-specific protease 7 interacts with N-Myc, induces deubiquitination and subsequent stabilization of N-Myc (Tavana et al., 2016). Phosphorylation in response to low levels of PI3K activity degrades N-Myc. (Otto et al., 2009).

3.1.3 | Gene therapy via immunity

To improve the balance between immunity and avoidance of immunity is a very important strategy in treatment of any disease especially cancer. Hydroxychloroquine and vorinostat improve antitumor immunity and inhibit autophagy in refractory colorectal cancer patients (Patel et al., 2016). Similarly, dendritic cells induce a strong adaptive immune response enough to generate a long-lasting immunological memory against the tumor, enabling the host immunity to prevent further relapses and metastasis (Lamberti et al., 2020). Upregulation of proapoptotic proteins or inhibition of anti-apoptotic proteins by mediating PTMs is also an acceptable strategy. Scientists might be interested in decreasing the immunity of cancer cells by interrupting oncogenes-mediated DNA damage response via PTMs.

3.2 | Limitations of gene therapy

To deliver genetic material into target cells/tissues and to express it with the intention to obtain therapeutic effects is an important advancement in the field of cancer treatment. A big investment is being made by the biotech companies on gene (DNA) based therapeutics in the fight against cancer. PEST-NPs like p53 and PTEN are promising candidates in gene therapy because of their distinct role in oncogenesis while other PEST-NPs are good targets to be explored as cancer therapeutics. However as discussed earlier, PEST-NPs participate in tumorigenesis as a tumor suppressor, oncogene, or transcription factors as well as immune modulators leading to protein–protein interactions.

3.2.1 | Protein–protein interactions

PEST-NPs are easy targets for ubiquitination, phosphorylation, glycosylation, methylation, and so on because of the PEST-motif. These PTMs are important for intracellular localization, activation/inhibition, and stability/degradation of PEST-NPs (Chipuk et al., 2005; Itkonen et al., 2013; H. B. Li, Tong, et al., 2017; Mori et al., 2002). PEST-NPs like MeCP2 and GLTSCR2 recruit other oncogenes to/from their active sites and behave as a promoter or inhibitor of that specific gene. The acetylation of MeCP2 at lysine 171 mediates its interaction with at least two other chromatin remodeling enzymes and may serve as a regulatory switch that can potentially modulate protein–protein interactions (Pandey, Simmons, Malyarchuk, Calhoun, & Pruitt, 2015). The PTMs of the PEST motif mediates the bimolecular responses of PEST-NPs. The PEST motif is a dual modulator of vascular endothelial growth factor receptor-2 (VEGFR-2); its phosphorylation at serine 1188/serine 1191 mediates ubiquitination and subsequent degradation via β-TRCP1, while its phosphorylation at tyrosine 1173 through PKA/p38 MAPK controls the stability of VEGFR-2 (Meyer et al., 2011). Some important protein–protein interactions have been summarized in Table 5. So, targeting the single gene as a cancer therapeutic is not as fruitful as is supposed like kinase driver oncogenes (Nastiuk & Krolewski, 2016). As a particular inhibitor cannot interrupt specific DNA binding with a particular transcription factor (Darnell, 2002), similarly the therapeutic effects of oncogene NPs generally and PEST-NPs specifically are uncertain in a large group of people owing to multiple functions of PEST-NPs. Not only the unprecedented effects of the target gene but the heterogeneity of cancer and patient condition also limit the efficacy of gene therapeutics.

3.2.2 | Gene delivery system

Pharma scientists are attempting to deliver the genetic material to the site of action as shown in Table 4. The entrapment efficiency of the carrier system and release of genetic material from the carrier system are other limitations of cancer gene therapy in addition to protein–protein interactions. It is obvious that until now US-FDA has not registered any tumor-suppressing protein as a cancer therapeutic and is awaiting critical Phase III clinical trials with Advexin. An adenovirus p53 gene therapy, gendicinan is the first-ever tumor suppressor gene therapy protocol approved by China FDA in 2004 for clinical use in humans. However, more experimental and clinical trials using well-designed and effective doses of vectors are required to

TABLE 5 Protein–protein interaction

Proteins	Interaction			
	PEST motifs nuclear proteins	Effect	Non-PEST motifs nuclear proteins	Effect
P53	↑ Myc	Myc dependent transcription	↓ Ets	↓ Ets-1-dependent transcription
			↓ Ras	↓ Ras dependent transcription
			NDN	↓ p53-dependent transactivation and Apoptosis
			SALL4	↑ Bmi expression via transcription hence cancer progression and metastasis
	Sp1	Binds with Bmi-1 promoter and increase Bmi 1 expression	E2F-1	Binds with Bmi and regulates its expression
			FOXM1	↑ Bmi expression via Myc transcription hence *avoids senescence*
PCNP	NIRF	Degradation of PCNP	HYPK	Acetylation of PCNP
Myc	p53 and MDM2	Induces p53 and MDM2 transcription evoking a DNA damage response	E2F-1	Binds with Myc-N and regulates *Myc-N* transcription
GLTSCR2	PTEN	↑ PTEN phosphorylation hence tumor suppression, regulates the stability of PTEN	RPL11, TP53	↑ nucleolar localization of RPL11 and ↑ TP53 accumulation, hence reducing cancer progression
	P53	Stabilizes p53 hence suppress tumor growth		
		↑ Nucleolar localization of RPL11 and ↑ TP53 accumulation, hence reducing cancer progression		
	Myc	↓ Transcription activity of Myc, hence, suppresses tumor growth		

Abbreviations: FOXM1, forkhead Box M1; GLTSCR2, glioma tumor suppressor candidate region gene 2; HYPK, huntingtin interacting protein K; MDM2, murine double minute 2; PCNP, PEST containing nuclear protein; PEST, proline (P), glutamic acid (E), serine (S), and threonine (T); PTEN, phosphatase and tensin homolog; RPL11, 60S ribosomal protein L11; SALL4, Sal-like protein 4.

ensure the therapeutic efficacy of gene therapy for its clinical use against a wide variety of cancers (Ajith, 2015).

4 | FUTURE PROSPECTS

Specially designed combination gene therapy based on a detailed understanding of the underlying molecular mechanism of tumorigenesis may present a satisfactory approach. Managing the internalization and accumulation of tumor suppressors at its target site in proliferating tumors is certainly conceivable. This intracellular compartmentalization can be achieved by (a) regulating nuclear transport of the target gene via importins and exportins as in the case of p53 (Chipuk et al., 2005). (b) To control localization of proteins by mediating PTMs like ubiquitination, phosphorylation, and dephosphorylation is also a feasible strategy. The PTMs control (bA) in and out of the protein, such as the proteasome inhibitors induce nuclear localization of proteasome target proteins directly or indirectly (Khan et al., 2018; Latonen, Moore, Bai, Jaamaa, & Laiho, 2011; Santiago-Josefat & Fernandez-Salguero, 2003), for example, MG-132 and Lactacystin in acute myeloid leukemia (Matondo et al., 2017). (bB) The regulation of PTMs is also important to control the stability of the protein; as decreased ubiquitination by NEDD8 inhibitor increases the stability of the protein, and in turn, increases mitochondrial localization (G. Liu & Xirodimas, 2010) while COX-2 inhibitors increase the stability of p53 and nuclear localization (Swamy, Herzog, & Rao, 2003). Also, (bC) regulation of DNA binding by PTMs is very important. For example, N-terminal phosphorylation and other PTMs regulate DNA-binding affinity and specificity of p53 (Follis et al., 2014; F. He et al., 2019). A careful selection of gene therapy in combination with transport inhibitors, kinase inhibitors and posttranslational modification inhibitors (Abd-Elhakim et al., 2019) may be a good choice. The nuclear protein p53 demonstrates synergistic activation by stimulating actinomycin-D kinase and preventing upregulated-MDM2 from binding to p53 by nutlin-3a in combination therapy (Zajkowicz, Gdowicz-Klosok, Krzesniak, Scieglinska, & Rusin, 2015). As PEST-NPs show multifaceted behavior in tumorigenesis, a pragmatic approach is required when employing these candidates as gene therapeutics.

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CONFLICT OF INTERESTS

The authors declare that there are no conflict of interests.

AUTHOR CONTRIBUTIONS

Conceptualization: M.S, A.A., D.D.W, S.P.J, S.F.D.; data curation: S.K, A.A., M.S., U.A.K.S, H.M.L; Q.Q.Z, A.M, K.H; funding acquisition: X.Y.Ji, D.D.W; writing-original draft: S.K, A.A. M.S; visualization and supervision: S.P.J, S.F.D. D.D.W, X.Y.J; writing-review and editing: all authors.

ORCID

Dong-Dong Wu http://orcid.org/0000-0001-6739-8437
Xin-Ying Ji http://orcid.org/0000-0003-0690-4206

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