MINIREVIEW

Regulation of p53 localization

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Despite intensive study of p53, the regulation of p53 cellular localization is still poorly understood. This is an overview of the elements and molecules involved in p53 nucleocytoplasmic transportation. These include the nuclear import and export signals of p53, inhibition of p53 nuclear import and export by oligomerization, MDM2-mediated p53 nuclear export, and possible roles of p53 phosphorylation in regulating p53 cellular localization. Finally, questions regarding p53 cellular trafficking will also be discussed.

Keywords: p53 tumor suppressor; nucleocytoplasmic transportation; oligomerization; cytoplasmic sequestration.

The p53 tumor suppressor is one of the most important molecules in regulating cell growth and protecting cells from malignant transformation. The importance of p53 is reflected by the fact that over 50% of all human cancers have p53 gene mutations [1,2]. Besides structural mutation, the functional inactivation of wild-type p53, such as the disruption of pathways involved in regulating p53 function, can also contribute to malignancy. A thorough understanding of the mechanism controlling p53 function will not only increase our knowledge of cancer biology but also may lead to the development of new cancer therapies targeting aberrant p53 pathways.

In a normal cell, the p53 protein is kept at a low concentration by rapid degradation. In addition, p53 probably exists in a latent, inactive form [3,4]. Several stressful situations, including genotoxic DNA damage, hypoxia, deprivation of growth factors, and loss of cell anchorage or cell–cell contact, can induce the formation of functional p53 [5]. Activation of p53 can be modulated at three levels: (a) an increase of the p53 protein concentration either by enhanced translation or elongated half-life; (b) the transformation of the p53 protein from a latent to an active conformation; and (c) the translocation of the p53 protein from the cytoplasm to the nucleus. While a number of studies have been performed to elucidate the modulation of p53 activity at the first two levels, few have addressed the regulation of p53 cellular localization. In this paper, we review what is known about p53 cellular localization and discuss the remaining issues regarding regulation of p53 nucleocytoplasmic transportation.

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THE p53 NUCLEAR IMPORT IS TIGHTLY REGULATED AND ESSENTIAL FOR ITS NORMAL FUNCTION

It was demonstrated in the early 1980s that p53 is cytoplasmic in most normal cells [6,7]. In transformed cells or rapidly growing normal cells, however, p53 is primarily located in the nucleus [6,7]. It was later reported that the nucleocytoplasmic translocation of p53 is tightly regulated during the cell cycle in normal fibroblasts [8]. In these cells, p53 accumulates in the cytoplasm during the G_1 phase and enters the nucleus during the G_1/S phase transition. A short period after the beginning of S phase, p53 cycles back to the cytoplasm. These data imply that p53 activity can be spatially regulated during the cell cycle. Indeed, it has been demonstrated that p53 is subject to both nuclear import and export via a fast, energy-dependent pathway [9].

Working principally as a transcription factor, the p53 nuclear import or retention is essential for its normal function in growth inhibition [10,11] or induction of apoptosis [12]. Thus, cellular mechanisms must exist to regulate the dynamic nucleocytoplasmic transportation of p53 either through enhanced nuclear import or decreased nuclear export. Studies have shown that wild-type p53 is abnormally sequestered in the cytoplasm in a subset of human tumor cells such as breast cancers, colon cancers, and neuroblastoma [13–15]. Although it is not clear whether this phenotype is directly linked to the malignancy, it suggests that tumorigenesis could result from a defect in the regulation of p53 nuclear import.

NUCLEAR IMPORT AND EXPORT SIGNALS OF p53

The nuclear localization signal (NLS) and nuclear export signal (NES) are the essential elements for a protein subjected to nucleocytoplasmic transportation. The typical NLS is either monopartite or bipartite (Table 1). The monopartite NLS consists of a single, short stretch of

Table 1. Signals for protein nuclear import and export. Bold type indicates residues shown to be required for signal function.

Signal	Sequence	Reference
Monoparite NLSs		
SV40 T antigen	P**KKKRK**V	[56]
Yeast histone H2B	G**KKR**SKV	[57]
Human c-myc	**PAAKR**V**KLD**	[58]
Bipartite NLSs		
Nucleoplasmin	**KR**PAATKKAGQA**KKKK**L	[59]
Human IL-5	**KK**YIDGQKKKCGEE**RRR**VNQ	[60]
Human RB	**KR**SAEGSNPPKPL**KKL**R	[61]
Human p53	**KR**ALPNNTSSSPQP**KKK**P	[24]
Leucine-rich NESs		
HIV-1 Rev	**L**PP**L**ER**L**T**L**D	[62]
Rat PK1	**L**AL**K**LAG**L**D**I**	[63]
Human p53	**M**FRE**L**NEA**L**E**L**K	[27]

several basic amino acids. The bipartite NLS contains two separated clusters of basic residues. Nuclear import is initialized by binding of the NLS to the importin α and β complex that mediates the docking of proteins at the nuclear pore complex [16–18]. On the other hand, the best understood NES is characterized by a leucine-rich sequence (Table 1) to which the exportin 1 or CRM1 can bind and direct the protein nuclear export [19–21].

Three monopartite NLSs were originally identified in the C terminus of p53 based on sequence and mutagenesis analyses [22]; NLSI [PQPKKKP(316–322)] is the most active of the three in directing p53 nuclear import. The other two NLSs, II and III, are much weaker. NLSI was shown to be able to direct the nuclear import of a cytoplasmic protein, pyruvate kinase [23]. However, it was recently found that NLSI alone is not functional for p53 nuclear import [24]. A basic motif containing Lys305–Arg306 and a spacer between this motif and the NLSI are necessary for the entrance of p53 into the cell nucleus [25,26]. In addition, a complete sequence from Lys305 to NLSI has stronger binding to importin α than that of NLSI alone [24]. Thus, the functional NLS of p53 is bipartite rather than monopartite.

A highly conserved leucine-rich NES has been identified in the tetramerization domain of p53 (Fig. 1). This intrinsic NES is both necessary and sufficient to direct p53 nuclear export [27]. Similar to other proteins containing a leucine-rich NES, p53 nuclear export is mediated by exportin 1 and could be inhibited by leptomycin B, a drug known to bind to exportin 1 [28].

REGULATION OF p53 NUCLEOCYTOPLASMIC TRANSLOCATION BY OLIGOMERIZATION

The p53 NLS is adjacent to, and the NES is contained within, the oligomerization domain (Fig. 1). It is thus possible that oligomerization of p53 may regulate p53 nucleocytoplasmic transport by affecting the accessibility of the NLS and/or the NES to their respective receptors. It has been demonstrated that the cytoplasmic localization of p53 in neuroblastoma cells is the result of enhanced nuclear export [27]. Ectopic expression of a tetramerization domain, however, was able to restore the nuclear retention of p53, suggesting that tetramerization can inhibit p53 nuclear export, presumably by masking the NES.

We have identified a cytoplasmic sequestration domain (CSD) that can reduce the efficiency of p53 nuclear import by blocking the importin α binding [24,25]. This CSD contains the p53 oligomerization domain and hence implies that p53 tetramerization may be also involved in the regulation of p53 nuclear import. Although p53 was able to enter the nucleus in an oligomerized form [24], it was observed that mutation of residues that can disrupt p53 tetramerization but not nuclear export could increase the efficiency of p53 nuclear import (S.-H. Liang and M. F. Clarke, unpublished data), indicating that the p53 monomer can enter the nucleus more efficiently than the oligomer.

It seems that oligomerization of p53 can block both NLS and NES binding to their receptors. It is thus possible that p53 oligomerization itself is regulated, switching p53 from a tetramer to a monomer at the time that p53 needs to transport in or out of the nucleus.

MDM2 AND p53 CELLULAR LOCALIZATION

The MDM2 protein is an important regulator of p53 function. MDM2 binds to the transcriptional activation domain in the N-terminus of p53 [29]. It can also promote the degradation of p53 by the ubiquitin–proteasome system [30,31]. MDM2 has been linked to the regulation of p53 nucleocytoplasmic translocation in two ways. First, it was demonstrated that MDM2 interacts with p53 in the nucleus and shuttles p53 from the nucleus to the cytoplasm where it mediates the degradation of p53 [32,33]. Alternatively, MDM2 could mediate ubiquitination of p53 in the nucleus, which turns on p53 nuclear export by unmasking the NES of p53 (C. Maki, Harvard School of Public Health, Boston, MA, USA, personal communication).
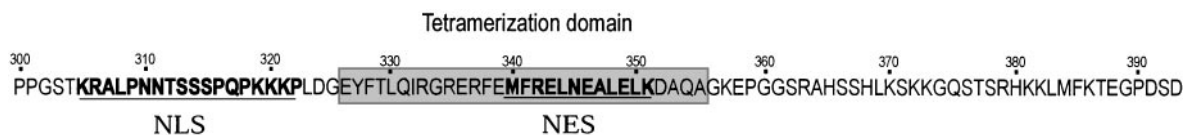

Tetramerization domain

300   310   320   330   340   350   360   370   380   390

PPGST**KRALPNNTSSSPQPKKKP**LDGEYFTLQIRGRERFE**MFRELNEALELK**DAQAGKEPGGSRAHSSHLKSKKGQSTSRHKKLMFKTEGPDSD

NLS   NES

Fig. 1. Amino-acid sequences of the C-terminus of p53 protein. The nuclear localization signal (NLS) is from residues 305–322. The nuclear export signal (NES) is from residues 340–351. The tetramerization domain, as marked in a box, is from residues 326–355.

The involvement of MDM2 in regulating p53 cellular localization is further demonstrated by the observation that the MDM2 function is directly linked to the nuclear exclusion of p53 in a subset of tumors such as neuroblastomas and breast carcinomas [34]. Inhibition of MDM2 expression by an antisense oligonucleotide, or inhibition of MDM2 function by P19 (ARF) tumor suppressor protein result in a relocation of wild-type p53 from the cytoplasm to the nucleus. On the other hand, it has been reported that p53 nuclear export is independent of MDM2 binding, based on the observation that mutant p53 that cannot interact with MDM2 still undergoes nucleocytoplasmic translocation [27]. It appears that MDM2 could, in some circumstances, control p53 nucleocytoplasmic translocation by distinct mechanisms that may depend on the cell context.

INVOLVEMENT OF PHOSPHORYLATION IN THE REGULATION OF p53 CELLULAR LOCALIZATION

Protein phosphorylation has been shown to affect nuclear import or export of several proteins such as the SV40 large T antigen [35,36], cyclin B1 [37], nucleolin [38], and MAPKAP kinase 2 [39]. p53 is known to be phosphorylated at multiple sites located in both the N- and C-terminal regions [5,40]. It has been reported that insulin-like growth factor I could induce an elevated level of phosphorylation of p53 and a translocation of p53 from the nucleus to the cytoplasm in MCF-7 human breast cancer cells [41]. Although it is not clear whether this elevated p53 phosphorylation is directly involved in the p53 nucleocytoplasmic translocation, it is possible that phosphorylation may affect p53 localization.

There are several specific phosphorylation events which may be related to the regulation of p53 cellular localization. As MDM2 is associated with p53 cellular trafficking, phosphorylation(s) that could inhibit MDM2 binding to p53 may be involved in regulating p53 localization probably by

enhancing the nuclear accumulation of p53. These include phosphorylation at Ser15 by ataxia telangiectasia mutated kinase and dsDNA-activated protein kinase [42–44], Thr18 by casein 1-like kinase [45], Ser20 [46,47], and Ser37 by A–T related kinase and dsDNA-activated protein kinase [44,48].

Phosphorylation of Ser392 was able to stabilize p53 tetramer formation, and Ser315 phosphorylation largely reversed the effect of Ser392 phosphorylation [49]. Because oligomerization may affect p53 nucleocytoplasmic transportation, phosphorylation of Ser315 and Ser392 could thus work together to regulate p53 cellular localization. In some cellular compartments, phosphorylation of p53 at Ser392 may reduce the efficiency of p53 nuclear import or export by enhancing oligomerization of p53.

Phosphorylation that can directly or indirectly influence the accessibility of NLS or NES to their receptors may also involved in the regulation of p53 cellular localization. One potential target is Ser315, a residue located within the bipartite NLS (residues 305–322) of p53, by cdc2/cyclinB and cdk2/cyclinA [50,51]; phosphorylation of Ser315 may therefore may affect p53 nuclear import. Studies are underway to test the relationship between these phosphorylations and p53 cellular localization.

SOME OTHER MOLECULES ASSOCIATED WITH p53 CELLULAR LOCALIZATION

Several studies have shown that other proteins may also be involved in the regulation of p53 cellular localization. Using a protein synthesis inhibitor, it was demonstrated that protein synthesis is required to anchor a temperature-sensitive p53 mutant for nuclear transport [52]. It was suggested that there are short-lived proteins that can tether p53 in the cytoplasm. Supporting this theory, it has been shown that a hsp70 family member, Mot2, could interact with p53 and inhibit its nuclear import [53]. Transfection of NIH3T3 cells with *mot-2* resulted in an abrogation of

Fig. 2. Postulated pathways of p53 nucleocytoplasmic transportation. (A) By binding to MDM2 in the nucleus, p53 and MDM2 are together translocated to the cytoplasm, mediated by binding of CRM1 to MDM2. (B) MDM2 mediates p53 ubiquitination in the nucleus which unmasks the NES of p53 for CRM1 binding and nuclear export. (C) p53 tetramerization, which may be enhanced by phosphorylation at Ser392, reduces nuclear export by masking the NES. p53 can resume the monomer form (by mechanisms that are unclear), which is able to interact with CRM1 and nuclear export. (D) A cytoplasmic 'tether' that binds to p53 can inhibit p53 nuclear import probably by blocking the NLS binding to importin α. (E) Phosphorylation of p53 at Ser315, for example, may also blocking the NLS accessibility to importin α. (F) Tetramerization may mask the p53 NLS and reduce the efficiency of p53 nuclear import.
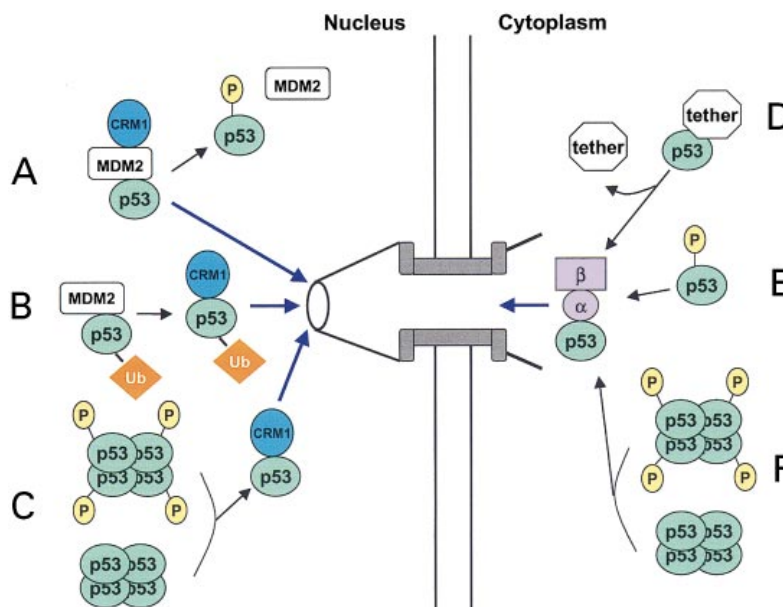
nuclear translocation of wild-type p53 and the repression of p53-mediated transactivation.

The cell death inhibitor, Bcl2, has been implicated in p53 nucleocytoplasmic translocation. In murine erythroleukemia cells, coexpression of *bcl-2* and c-*myc* can totally inhibit p53 function by cytoplasmic sequestration [12]. Overexpression of *bcl-2* in LNCaP human prostate carcinoma cells suppressed cell death following DNA damage and inhibited p53 nuclear import [54]. It appears that, depending on the cell type, the Bcl2 alone or working together with other factors can modulate p53 cellular localization.

In addition to Bcl2, it was reported that the calcium-dependent protein kinase C could cooperate with a calcium binding protein, S100B, in regulating p53 nuclear accumulation [55]. It was suggested that cPKC activation by calcium signalling, together with the expression of S100B, could result in p53 nuclear translocation in early G_1 phase to initialize a p53-dependent G_1 checkpoint control.

CONCLUSIONS

It appears that p53 cellular localization can be modulated by a variety of ways (Fig. 2). It's our belief that more and more molecules or elements will be identified as being involved in this process. Remaining questions include the following. Do these molecules work alone or interact with each other to function? What signals trigger distinct mechanisms in controlling p53 cellular localization? How do cells integrate these varied mechanisms and find a efficient way to regulate p53 localization? Understanding all these issues will provide a new insight into the sophisticated pathways of the regulation of p53 function.

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