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The importance of p53 location: nuclear or cytoplasmic zip code?

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Abstract

The regulation of p53 functions is tightly controlled through several mechanisms including p53 transcription and translation, protein stability, post-translational modifications, and subcellular localization. Despite intensive study of p53, the regulation of p53 subcellular localization although important for its function is still poorly understood. The regulation of p53 localization depends on factors that influence its nuclear import and export, subnuclear localization and cytoplasmic tethering and sequestration. In this review, we will focus on various proteins and modifications that regulate the location and therefore the activity of p53. For example, MDM2 is the most important regulator of p53 nuclear export and degradation. Cytoplasmic p53 associates with the microtubule cytoskeleton and the dynein family of motor proteins; while Parc and mot2 are involved in its cytoplasmic sequestration. Finally, a portion of p53 is localized to the mitochondria as part of the non-transcriptional apoptotic response. In this review we strive to present the most recent data on how the activity of p53 is regulated by its location.

Keywords: p53; Cytoplasm; MDM2; Microtubules; Nucleocytoplasmic

1. Introduction

Almost three decades ago, p53 was identified as one of the first tumor suppressor genes (Lane and Crawford, 1979; Linzer and Levine, 1979) and has since remained in the spotlight of cancer research. p53, often referred to as the "guardian of the genome," is a sequence-specific transcription factor activated by diverse stress signals (Lane, 1992). p53 activation regulates genes involved in many cellular functions, the most important being cell cycle arrest and apoptosis. Loss of p53 function confers radiation and chemotherapy resistance and is often associated with more aggressive tumor phenotypes. It is often remarked that 50% of cancers have mutated or inactivated p53; however, the real number is probably much higher when the involvement of the entire p53 pathway in tumorigenesis is examined. A quick search on PUBMED reveals over 25,000 publications on p53 for just the last decade. While of loss of p53 function by inactivating mutations has been the most widely studied (Fojo, 2002), a relatively new area in the p53 field has been p53 regulation through intracellular localization (Vousden and Van der Woude, 2000; Lain et al., 1999; Fabbro and Henderson, 2003). In this review we will present the most recent findings in this emerging area of p53 regulation.

2. Regulation of p53 protein localization

The regulation of p53 function is tightly controlled through several mechanisms including p53 transcription and translation, protein stability and post-translational modifications (Gu and Roeder, 1997; Rodriguez et al., 1999). As mentioned above, the importance of p53 cellular localization for its function has gained momentum in recent years. For example, relocation of p53 to the nucleus after cellular stress is desirable to inhibit the growth of malignant cells. Moreover, in a number of tumor types that retain wild-type p53, loss of p53 activity is associated with a cytoplasmic "zip code." In such cases, p53 is excluded from the nucleus as a result of cytoplasmic sequestration, or hyperactive nuclear export. Tumor types with cytoplasmic confinement of p53 are less responsive to genotoxic stress induced by radiotherapy or chemotherapy; while cytoplasmic accumulation of p53 is an independent unfavorable prognostic factor in cancer (Bosari et al., 1995; Moll et al., 1995; Schlamp et al., 1997; Sembritzki et al., 2002; Ueda et al., 1995). The regulation of p53 cellular localization depends on factors that influence its nuclear import and export, subnuclear

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Table 1
Proteins regulating p53 subcellular localization

Subcellular localization	Protein interaction	Effect on p53	References
Nuclear import and export	p53–importin-α	Nuclear import is mediated by importin-α and enabled by the three nuclear localization signals (NLS) of p53	Liang and Clarke (1999), Roth et al. (1998)
	p53–CRM1	Nuclear export is mediated by CRM1 and enabled by the two nuclear export signals (NES) of p53	Stommel et al. (1999), Zhang and Xiong (2001)
	p53–MDM2	MDM2 binds and ubiquinates p53 in the nucleus, resulting in p53 nuclear export and degradation	Boyd et al. (2000), Geyer et al. (2000), Stommel et al. (1999)
	p14ARF–MDM2	p14ARF sequesters MDM2 into the nucleolus, resulting in nuclear retention of p53	Tao and Levine (1999b), Weber et al. (1999), Xirodimas et al. (2001a)
	PI3K/Akt–MDM2	PI3K/Akt pathway phosphorylates MDM2 allowing for its nuclear import, resulting in p53 nuclear export.	Mayo et al. (2002), Mayo and Donner (2001), Ogawara et al. (2002), Sabbatini and McCormick (1999), Stambolic et al. (2001), Zhou et al. (2001)
	c-Abl–MDM2	c-Abl binds to MDM2 and prevents its interaction with p53 resulting in nuclear retention of p53	Goldberg et al. (2002)
Cytoplasmic anchorage and trafficking	p53–actin	p53 binds to F-actin in a cell free system	Metcalfe et al. (1999)
	p53–vimentin	Cytoplasmic accumulation of p53 correlates with the presence of full length vimentin in rat glioma cells; nuclear p53 was found in vimentin-negative rat glioma cells	Klotzsche et al. (1998)
	p53–microtubules	SV40 large T-antigen and p53 form a protein complex with tubulin	Maxwell et al. (1991)
		p53 binds tubulin and requires functional microtubules to traffick to the nucleus via dynein	Giannakakou et al. (2000)
		Suppression of microtubule dynamics enhances p53 nuclear accumulation	Giannakakou et al. (2002)
	p53–Parc	Cytoplasmic anchor for p53	Nikolaev et al. (2003)
	p53–mot2	Cytoplasmic anchor for p53	Wadhwa et al. (1999), Wadhwa et al. (2002)
Mitochondrial localization of p53	p53–mitochondria	A portion of p53 can localize to mitochondria to induce apoptosis in a transcription independent manner	Marchenko et al. (2000), Mihara et al. (2003), Sansome et al. (2001)

localization, and cytoplasmic tethering and sequestration (Table 1).

3. Regulation of p53 nuclear import/export

p53 function depends on its nuclear localization and both nuclear import and nuclear export of p53 are tightly regulated (Ryan et al., 2001; Vousden and Van der Woude, 2000). Nuclear import of p53 is enabled by its three nuclear localization signals (NLS) (Roth et al., 1998; Liang and Clarke, 1999) while nuclear export is enabled by its two nuclear export signals (NES) (Stommel et al., 1999; Zhang and Xiong, 2001). When DNA damage occurs, p53 gets imported into the nucleus via its NLS and undergoes tetramerization (el-Deiry et al., 1992), binds and activates DNA damage-response genes, along with MDM2 (Jimenez et al., 1999). The tetramer state of p53 obscures its NES thereby blocking nuclear export (Stommel et al., 1999). There are several proteins that that influence p53 nuclear import and export, one of the most important being MDM2.

3.1. The p53–MDM2 feedback loop

MDM2 is a negative regulator of p53, which promotes p53 degradation by its E3 ubiquitin ligase function and ultimately inhibits p53 transcriptional activity. As MDM2 is a transcriptional target of p53, an autoregulatory feedback loop is generated in which p53 activity leads to increased expression of its own negative regulator (Kubbutat et al., 1997). When p53 is induced it transactivates MDM2 that in turn binds, ubiquinates and targets p53 for degradation. The activation and stabilization of p53 go hand in hand with the inhibition of the ubiquitin ligase function of MDM2 (for review see Alarcon-Vargas and Ronai, 2002; Haupt et al., 2003).

The importance of this regulatory loop is demonstrated in mice, where loss of MDM2 leads to early embryonic lethality due to aberrant p53-driven apoptosis (de Rozieres

et al., 2000). On the other hand, tumors with wild-type (wt) p53 and hyperactive MDM2, display a "p53-null" phenotype due to the rapid degradation of p53 (Cuny et al., 2000).

Both MDM2 and p53 move between the nucleus and the cytoplasm of the cell. Upon activation they localize in the nucleus where they activate target genes (Chen et al., 1995; Liang and Clarke, 1999), but they must also re-enter the cytoplasm for various reasons: degradation of p53 and MDM2 occurs mainly in the cytoplasm, while p53 must exit the nucleus to stop its transactivation function and allow the cell to continue its chosen course (cell cycle arrest or apoptosis). Although the MDM2-dependent p53 nuclear export is critical for p53 function, it is not yet clear how it occurs.

Two main models have been proposed to explain MDM2-dependent p53 nuclear export. The first model suggests that MDM2 binds p53 in the nucleus and both proteins shuttle into the cytoplasm where they get degraded (Roth et al., 1998; Freedman and Levine, 1998; Tao and Levine, 1999a). In this early model, the role of p53 ubiquination in the degradation process was not well defined.

The second model proposes that MDM2 binds and ubiquinates p53 in the nucleus which unmasks the p53 NES resulting in p53 nuclear export and degradation (Stommel et al., 1999). In this model MDM2 ubiquinates p53 but does not have to physically "carry" p53 out of the nucleus as was previously thought (Boyd et al., 2000; Geyer et al., 2000; Inoue et al., 2001).

Accumulating body of evidence favors the second model as it has been shown that a RING domain mutant of MDM2 unable to ubiquinate p53 did not cause re-localization of p53, indicating that MDM2 ubiquitin ligase activity is required for p53 nuclear export (Kawai et al., 2003; Lohrum et al., 2001). Furthermore, the MDM2-dependent p53 nuclear export requires an intact NES for p53 but not for MDM2 (Boyd et al., 2000; Geyer et al., 2000), indicating that MDM2 and p53 do not shuttle together and that MDM2 does not have to re-localize to the cytoplasm for p53 degradation.

The cellular "zip code" of MDM2 is very important for the regulation of p53, thus factors that influence MDM2 localization also affect p53 function. Two such factors are the p14ARF tumor suppressor gene and the PI3K/Akt pro-survival signaling pathway.

3.1.1. p14ARF–MDM2: a positive regulator of p53

p14ARF is a tumor suppressor gene (Kamijo et al., 1997) which has been identified as a positive regulator of p53. p14ARF, binds and inactivates MDM2 thereby stabilizing p53 (Kamijo et al., 1998; Zhang et al., 1998; Honda and Yasuda, 1999). p14ARF sequesters MDM2 into the nucleoli preventing MDM2 from physical interaction and ubiquination of p53 (Tao and Levine, 1999b; Weber et al., 1999). It has been also shown that p14ARF can sequester the entire p53–MDM2 complex into the nucleolus, thereby inhibiting the nuclear export of the complex and degradation of p53 (Xirodimas et al., 2001a). Accumulating data on the p14ARF–MDM2–p53 feedback loop suggest that p14ARF affects both MDM2 and p53 by re-localizing them within a nucleus in a way that leads to p53 stabilization.

3.1.2. PI3K/Akt–MDM2: a negative regulator of p53

As mentioned above, for MDM2 to regulate p53 it must enter the nucleus. MDM2 nuclear import is regulated by the PI3K/Akt pathway. Mitogen-induced activation of PI3K and its downstream target, Akt, results in phosphorylation of MDM2 on serines 166 and 168 (Mayo and Donner, 2001; Ogawara et al., 2002; Zhou et al., 2001). Phosphorylation on these sites is necessary for translocation of MDM2 from the cytoplasm into the nucleus. Phosphorylation of MDM2 enhances is nuclear localization and its interaction with CBP/p300 while it inhibits its interaction with p14ARF, thus increasing p53 degradation. In addition, expression of constitutively active Akt promotes nuclear entry of MDM2, diminishes cellular levels of p53 and decreases p53 transcriptional activity (Mayo and Donner, 2001). In breast cancer tissue with HER-2/Neu and Akt positive staining, MDM2 was mainly present in the nucleus (Zhou et al., 2001), suggesting that MDM2 was actively degrading p53. This result may explain why Her-2/Neu overexpressing tumors are often resistant to chemotherapy-induced p53-mediated apoptosis. Furthermore, many human tumors show enhanced activity of the PI3K/Akt signaling cascade due to loss of PTEN, a tumor suppressor gene and a negative regulator of this pathway (Singh et al., 2002). Similarly to the Her-2/Neu overexpressing tumors, cancer chemotherapy that relies on p53 function is less effective in PTEN null tumors.

On the other hand, inhibition of the PI3K/Akt pathway by either a dominant negative PI3K/Akt, PTEN expression or pharmacologically with LY294002, prevents nuclear translocation of MDM2 leading to increased p53 levels and activity (Sabbatini and McCormick, 1999; Mayo et al., 2002; Stambolic et al., 2001).

3.1.3. c-Abl/MDM2

c-Abl, a nonreceptor tyrosine kinase, is another important regulator of p53. It interacts in vivo and this interaction is further enhanced by DNA damage (Yuan et al., 1996). However, a novel indirect role for c-Abl in the stabilization of p53 was recently established. c-Abl neutralizes the ability of MDM2 to promote p53 for degradation and to inhibit the transcriptional and apoptotic activities of p53. This is a key role for c-Abl in the activation of p53 by stress, by regulating the nuclear export of p53 and the extent of its ubiquitination (Sionov et al., 2001). c-Abl binds directly to MDM2 and phosphorylates it at tyrosine 394 imparing the ability of MDM2 to interact with p53, leading to increased transcriptional activity (Goldberg et al., 2002; Ciechanover and Schwartz, 1998; Fuchs et al., 1998; Kashuba et al., 2003; Lai et al., 2001; Lohrum et al., 2001).

3.1.4. CBP/p300–MDM2

It has to be emphasized that despite the central role that p53 nuclear export plays in p53 inactivation by degradation,

nuclear export by itself is not always synonymous with *p53 degradation*. For example, studies have shown that CRM1-induced nuclear export of p53 without prior ubiquination by MDM2 did not lead to degradation (Lohrum et al., 2001). Furthermore, p53 ubiquination without nuclear export lead to partial degradation (Xirodimas et al., 2001b; O'Keefe et al., 2003). It is clear now that p53 needs to be ubiquinated to subsequently be degraded. Although MDM2 is required for p53 ubiquination, there are additional factors contributing to p53 degradation. One such factor is the CBP/p300 (CREB Binding Protein transcriptional coactivator/p300), which was recently identified as the E4 ubiquitin ligase for p53. CBP/p300 is responsible for adding additional ubiquitin molecules to the monoubiquinated p53 by MDM2 so that it becomes polyubiquinated and subsequently degraded (Grossman et al., 2003). It has been shown that proteins must be polyubiquinated to be degraded by the proteasome (Ciechanover and Schwartz, 1998; Thrower et al., 2000). MDM2-induced monoubiquination of p53 is required for CBP/p300 to exert its polyubiquinating function (Grossman et al., 2003). In addition, it was shown that in cells harboring mutant MDM2 unable to bind CBP/p300, p53 can be ubiquinated but not degraded (Zhu et al., 2001). It remains to be clarified whether in vivo, in the absence of CBP/p300 monoubiquinated p53 is able to be degraded via other E4 ubiquitin ligases.

3.2. Post-translational modifications and p53 nuclear export

A variety of genotoxic stresses initiate signaling pathways that transiently stabilize the p53 protein, cause it to accumulate in the nucleus, and activate it as a transcription factor. There are various *post-translational modifications* that occur to p53 in response to cellular stress. The amino-terminal of p53 houses multiple phosphorylation sites, while the carboxy terminal houses several phosphorylation, acetylation, glycosylation and sumoylation sites. Among all post-translational modifications, N-terminal phosphorylation and C-terminal acetylation are the most important for p53 stabilization and modulation of the nuclear import/export process (reviewed in Appella and Anderson, 2001).

3.2.1. Phosphorylation of p53

N-terminal phosphorylation of p53 at serines 15 (Shieh et al., 1997) and 20 (Unger et al., 1999; Chehab et al., 1999) prevents p53 nuclear export and leads to enhanced p53 nuclear accumulation and transcriptional activity. Inhibition of nuclear export occurs due to both, inhibition of p53–MDM2 binding, which is known to occur at the N-terminus of p53, and masking of the N-terminal p53 nuclear export signal. Distinct kinases mediate p53 phosphorylation, including checkpoint kinase 1 (Chk1), JNK or Chk2 (reviewed in Appella and Anderson, 2001). Two studies recently found that nitric oxide (NO) also causes phosphorylation of p53 serine 15, accounting for the induction and nuclear accumulation of p53 after NO treatment (Schneiderhan et al., 2003; Wang et al., 2003). In addition, the substitution of serine 20 with alanine resulted in enhanced binding to MDM2, thus making p53 more prone to MDM2-mediated degradation (Shieh et al., 1997; Unger et al., 1999). These reports suggest that the nuclear-cytoplasmic shuttling of p53 protein is regulated in part by phosphorylation at serines 15 and 20 by diverse stimuli.

4. Regulation of p53 subnuclear localization

Subnuclear localization of p53 itself or components of the p53-response pathway, is also important for p53 activity. p53 can accumulate in subnuclear structures termed nuclear bodies (NBs). For example, the nuclear body promyelocytic leukaemia gene (PML) has been shown to contain p53 (Ferbeyre et al., 2000; Fogal et al., 2000; Guo et al., 2000; Pearson et al., 2000). It has been postulated that PML nuclear bodies serve as a "meeting place" for p53 and p53-interacting proteins. Several p53 post-translational modifications, critical for its function, occur in the PML bodies. Phosphorylation of serine 43 of p53 occurs primarily in the PML bodies by homeodomain-interacting protein kinase-2 (HIPK2) and leads to enhanced p53-mediated apoptosis (D'Orazi et al., 2002; Hofmann et al., 2002). Acetylation at lysine 382 by CBP/p300 also occurs within the PML bodies via the formation of a trimeric p53–PML–CBP/p300 complex (Pearson et al., 2000; Ito et al., 2001). Other proteins frequently contained in the PML body are: Sp100, SUMO1, HAUSP, Daxx and Chk2 (Maul et al., 2000; Yang et al., 2002). The small ubiquitin-like protein SUMO1 is an important protein in the ubiquination and degradation step of both MDM2 and p53 (Rodriguez et al., 1999; Xirodimas et al., 2002). MDM2 becomes conjugated through the mediation of p14ARF to SUMO1 and prevents the self-ubiquitin ligase function of MDM2, thus increasing the levels of MDM2 and decreasing the levels of p53 (Buschmann et al., 2000; Xirodimas et al., 2002). On the other hand, localization of SUMO1 in the PML bodies results in p53 stabilization, as it is physically separated from MDM2 allowing for MDM2 self-ubiquination and degradation. The herpesvirus associated ubiquitin-specific protease (HAUSP) de-ubiquinates p53, leading to p53 nuclear accumulation (Li et al., 2002). A recent paper found that PML can protect p53 from MDM2 degradation by colocalization of Chk2 and p53 in the PML bodies (Louria-Hayon et al., 2003). This co-localization facilitated p53 phosphorylation and activation by Chk2.

5. Regulation of p53 cytoplasmic anchorage and trafficking

In normal cells under non-stressed conditions, p53 is a short-lived protein which shuttles between the nucleus and the cytoplasm in a cell-cycle specific manner (reviewed in

Table 2
Tumors with aberrant protein localization of p53 or MDM2

Protein	Mislocalization	Tumor type	References
p53	Cytoplasmic sequestration, unable to perform nuclear functions	Colorectal carcinoma	Bosari et al. (1995)
		Undifferentiated neuroblastoma	Moll et al. (1995)
		Retinoblastoma	Schlamp et al. (1997)
		Breast carcinoma	Kim et al. (2000), Lilling et al. (2002)
		Glioblastoma	Sembritzki et al. (2002)
		Hepatocellular carcinoma	Ueda et al. (1995)
MDM2	Tumors with hyperactive MDM2 display a p53-null phenotype due to rapid degradation of p53	Breast carcinoma	Cuny et al. (2000)
		Leukemia	Konikova and Kusenda (2003)
		Melanoma	Polsky et al. (2002)
		Sarcoma	Cordon-Cardo et al. (1994)
		Glioblastoma	Reifenberger et al. (1993)

Hayon and Haupt, 2002) and is maintained in a latent form. In response to stress, however, this shuttle is biased towards nuclear accumulation, which is essential for p53 to elicit its biological effects. Interference with this regulation is sufficient to inactivate p53. Indeed, wild-type p53 becomes functionally inactivated by aberrant cytoplasmic sequestration and lack of response to DNA damage in many tumor types, including inflammatory breast adenocarcinoma, undifferentiated neuroblastoma, retinoblastoma and colorectal carcinoma (Table 2). Constitutive cytoplasmic localization of p53 in these tumor types has been associated with poor response to chemotherapy, tumor metastasis and poor long-term patient survival (Bosari et al., 1995; Moll et al., 1995; Schlamp et al., 1997; Ueda et al., 1995). The specific mechanisms, however, governing p53 cytoplasmic tethering and abnormal cytoplasmic accumulation are not well understood.

5.1. p53 cytoplasmic anchorage and sequestration

p53 is synthesized in the cytoplasm and must transport into the nucleus to exert its transcriptional effect on downstream targets after cellular stress. However, the signals/proteins that direct p53 "navigation" through the cytoplasm towards the nucleus are not entirely clear. The aberrant cytoplasmic sequestration of p53 in many tumor types together with their poor response to chemotherapy and radiation therapy has prompted several groups to investigate which of the major cytoskeletal filament systems, namely actin filament, microtubules and intermediate filaments, could serve as a backbone for cytoplasmic anchorage and sequestration of p53 molecules.

5.2. p53–actin interaction

Katsumoto et al. described a potential association of p53 with cytoplasmic actin filaments in a cell-cycle dependent manner (Katsumoto et al., 1995). However, no direct binding between the two proteins nor any biological significance of such association was demonstrated. Two additional reports examined the potential p53–actin association. The first showed calcium-dependent binding of wild-type p53 protein to F-actin in a cell-free system (Metcalfe et al., 1999). Although no cellular experiments were performed the authors proposed that actin could regulate cellular p53 biological function at some level. To complicate matters further a more recent paper showed that wild-type p53 binds to nuclear matrix following DNA damage via its association with nuclear F-actin (Okorokov et al., 2002). In this study, the authors showed the p53 nuclear F-actin association to be important for p53 nuclear retention rather than p53 cytoplasmic sequestration. At this point we feel it is fair to say that no conclusive functional relationship between p53 and actin has been successfully demonstrated.

5.3. p53–vimentin interaction

Klotzsche et al. reported that a temperature-dependent mutant p53 associated with the intermediate filament vimentin in rat glioma cells (Klotzsche et al., 1998). The authors of this paper used a temperature-sensitive mutant p53 (ts-p53) to study parameters influencing the subcellular localization of both wild-type and mutant p53. The ts-p53 was shown to localize in the cytoplasm at the non-permissive 37 °C temperature, while at the permissive 30 °C temperature, translocated into the nucleus assuming its wild-type conformation. Using this p53 system in vimentin-expressing or vimentin-negative rat glioma cells the authors claimed that in vimentin-negative cells only nuclear p53 was observed in contrast to the strong cytoplasmic p53 staining in vimentin-expressing cells. The authors also attempted to exclude potential involvement of the actin or microtubule cytoskeleton; however, these studies were not well controlled and were therefore non-conclusive. Most importantly, we do not believe that the use of the ts-p53 system can serve as a substitute to study stress-induced wild-type p53 nuclear translocation.

5.4. p53–microtubules interaction

A possible interaction between p53 and tubulin has been proposed by Maxwell et al. in a study where the cellular proteins that associate with SV40 large T-antigen (T-ag) were

examined (Maxwell et al., 1991). Partial protease mapping and microsequencing experiments revealed that both T-ag and p53 were part of a protein complex containing tubulin. Furthermore, both p53 and T-ag co-purified with microtubules through two cycles of temperature-dependent disassembly and assembly, while immunoelectron microscopy showed that they both were localized to microtubules in the cytoplasm of SV40 transformed mouse mKSA cells. Although no direct binding of p53 and tubulin was reported, this study prompted us to investigate whether wild-type p53 associated with the microtubule cytoskeleton in non-SV40 transformed human epithelial cancer cells in a biologically functional way. Our work demonstrated that wild-type p53 binds cellular microtubules (MTs) in a variety of human cancer cell lines of distinct origin, and that it requires an intact microtubule network for its intracellular trafficking and nuclear accumulation (Giannakakou et al., 2000). The first 25 residues of p53 protein mediate its binding to MTs, while p53 nuclear translocation requires the activity of the MT-based minus-end motor protein, dynein. We have also demonstrated that recombinant p53 protein binds purified MT protein in vitro and this binding is enhanced in the presence of MT-associated proteins (MAPs) including dynein. Most importantly, our study has significant repercussions in tumor therapy as we showed that disruption of the MT cytoskeleton by commonly used chemotherapy drugs like Taxol or vincristine resulted in impaired p53 nuclear localization and activity.

MTs are uniquely situated in the cell to serve as scaffolds or tracks for intracellular trafficking as they fill the entire cytoplasm spanning the whole area from the plasma membrane to the MT-organizing center (MTOC) located just outside the nucleus. Moreover, MTs have an inherent polarity in that they have a "minus" end associated with the MTOC and a "plus" end located at the cell periphery. This polarity allows for the directional flow of information within the cell as it is recognized by the minus- and plus-end directed motor proteins, namely dyneins and kinesins, which move cargos to and from the nucleus. Despite their structural role, MTs are highly dynamic and regulation of MT-dynamics is essential for many cellular functions, especially mitosis. In fact, it is well established that both MT-stabilizing (taxanes) (Yvon et al., 1999) and MT-destabilizing (vinca alkaloids) (Dhamodharan et al., 1995) chemotherapy drugs when used at very low concentrations they inhibit MT-dynamics without affecting the organization and integrity of the microtubule cell network (for review see Jordan, 2002). We have recently investigated the effects of suppression of MT-dynamics on p53 subcellular localization (Giannakakou et al., 2002). We found that suppression of MT-dynamics by low concentrations of Taxol or vincristine enhanced p53 nuclear accumulation and activity by facilitating minus-end directed MT-dependent trafficking and subsequent nuclear targeting.

Collectively, we have shown that functional cellular microtubules are essential to "guide" p53 from the cytoplasm to the nucleus via the minus-end directed motor protein dynein. The role of the microtubule cytoskeleton on p53 cytoplasmic "navigation" upon nuclear export has not been addressed. It would be interesting to examine the potential involvement of kinesins, the plus-end directed motor proteins, in p53 targeting to distinct areas of the cytoplasm such as the proteasome or mitochondria.

5.5. p53–Parc interaction

A recent study described the identification of a novel cytoplasmic protein involved in p53 subcellular localization and function. The authors of this study biochemically purified p53-containing complexes of unstressed cells and have identified Parc (p53-associated, Parkin-like cytoplasmic protein), as the key component of these complexes (Nikolaev et al., 2003). Parc, a Parkin-like ubiquitin ligase, serves as a cytoplasmic anchor of p53 in unstressed cells. RNAi-mediated ablation of endogenous Parc-induced p53 nuclear accumulation, while ectopically expressed Parc promoted cytoplasmic retention of p53. Examination of Parc levels in neuroblastoma cell lines revealed high Parc expression, consistent with the aberrant cytoplasmic sequestration of p53 in this tumor type. The authors of this study propose Parc as a key cytoplasmic protein critically involved in the regulation of p53 subcellular localization and subsequent function.

Interestingly enough, Parkin a protein-ubiquitin E3 ligase linked to Parkinson's disease, was recently shown to tightly bind microtubules leading to increased ubiquitination and degradation of α- and β-tubulins (Ren et al., 2003). These data reveal Parkin as a novel tubulin-binding whose role in cancer has not been examined. Our data together with the high homology of Parc to Parkin led us hypothesize that Parc may also be associated with microtubules in a complex with p53. Further elucidation of this potential association is warranted.

5.6. p53–mot2 interaction

Another cytoplasmic protein implicated in p53 cytoplasmic sequestration is a member of the heat-shock protein Hsp70, namely mot2 or mthsp70 or GRP75 (for review see Zylicz et al., 2001). This protein, we will call it here mot2 for simplicity purposes, binds directly p53 and has been shown to either sequester p53 in the cytoplasm (Wadhwa et al., 1999, 2002) or mediate p53 association with mitochondria (Dumont et al., 2003; Marchenko et al., 2000). Wadhwa et al. showed that mot2 interacts with p53 and abrogates its nuclear translocation (Wadhwa et al., 1999) by binding to the carboxy terminus of p53 encompassing residues 312–352 (Wadhwa et al., 2002). The interaction with p53 is mediated by the N-terminal (residues 253–282) portion of mot2 (Kaul et al., 2001). Noticeably, this part of p53 contains a nuclear localization signal, NLS (residues 313–322), a nuclear export signal, NES (residues 337–348) and the cytoplasmic sequestration domain, CSD (residues 323–352). mot2 was

also found in p53 aggregates in the cytoplasm of certain neuroblastoma, glioblastoma and breast carcinoma cell lines that show high levels of cytoplasmic p53. These data suggest that p53–mot2 interaction may be important for p53 cytoplasmic sequestration while abrogation of such interaction may provide a therapeutic approach to restore the function of wild-type p53. An earlier study in support of this notion, showed that MKT-077, a rhodamine dye that binds mot2, can reinstate the nuclear localization and transcriptional activation of wild-type p53 in breast carcinoma cells (Wadhwa et al., 2000).

mot2 protein has been also shown to mediate p53 mitochondria localization. The mot2 protein is the major translocation motor that "pulls" proteins into mitochondria and refolds them before they get sorted into their final compartments within the membrane or the matrix (Pfanner et al., 1997). Studies have shown that p53 associates with mitochondria early on in p53-mediated apoptosis and settles in the membranous barrier compartment after an import step involving mot2 (Dumont et al., 2003; Marchenko et al., 2000). The mot2–p53 association in mitochondria may mediate in part the mitochondria-dependent p53-induced apoptosis.

6. p53 mitochondrial localization and activation of apoptotic response

Mitochondria are central components of the intrinsic apoptotic pathway. Mitochondria contain various critical apoptotic activators and effectors of cell death, including cytochrome *c* (Goldstein et al., 2000), Smac/Diablo (a cytochrome *c*-dependent caspase co-activator) (Verhagen et al., 2000) and apoptosis inducing factor (AIF), a flavoprotein which activates nuclear endonucleases (Susin et al., 1999) and procaspases 2, 3 and 9. The release of these factors from the mitochondrial membrane constitutes the "point of no return" and triggers cell suicide through the apoptotic machinery (Zaffaroni and Daidone, 2002; Fulda and Debatin, 2003).

p53 induces apoptosis by target gene regulation and transcription-independent signaling. Although the latter mechanism is not well understood, recent studies have shown that a fraction of p53 protein in damaged cells translocates to mitochondria and that these cells duly undergo apoptosis (Marchenko et al., 2000; Sansome et al., 2001). It was further shown that targeting p53 to mitochondria was sufficient to induce apoptosis, just by the mere localization of p53 to mitochondria, in a transcription-independent manner (Marchenko et al., 2000; Moll and Zaika, 2000). A more recent study provided a better understanding of the mechanism responsible for transcription-independent apoptosis by p53 (Mihara et al., 2003). This study showed that endogenous p53 transclocates to mitochondria in vivo following irradiation, and induces permeabilization of the outer membrane of the mitochondria by forming an inhibitory complex with Bcl-X_L and Bcl-2 proteins, resulting in cytocrome *c* release. They further showed that four naturally occurring cancer-derived p53 mutants (R175H, L194F, R273H, R280K), failed to form a complex with Bcl-X_L and to subsequently release cytochrome *c*. Based on these data, the authors hypothesized that tumor development selects against both the nuclear and the mitchondrial apoptotic function of p53. Although these findings appear intriguing, it remains to be determined to what extent the lack of mitochondrial localization of mutant p53 is responsible for the lack of its apoptotic activity.

Is the fraction of p53 that translocates to mitochondria regulated by MDM2? This question becomes particularly important in light of another study that found a relationship between MDM2 ubiquination of p53 and its mitochondrial localization. Dumont et al. found that two different polymorphisms of p53 had different subcellular localization (Dumont et al., 2003). At position 72, wild-type p53 can encode either a proline or an arginine. They found that Arg72 was at least five times more effective at inducing apoptosis under stress than Pro72. They also found that this increase in apoptotic potential was due to enhanced mitochondrial localization of Arg72 following DNA damage or other types of cellular stress. The Arg72 variant was also more ubiquinated compared to Pro72, suggesting a stronger interaction with MDM2 and facilitation of nuclear export. Although one would expect enhanced proteasome degradation of the Arg72 variant, the authors observed enhanced mitochondrial localization of Arg72 and they postulated that this is due to its enhanced nuclear export. It is unclear why Arg72 does not get readily degraded in the cytoplasm. One potential explanation provided by the authors is that p53 can also become de-ubiquinated in the cytoplasm; albeit via an unclear pathway. An even more unclear question is how p53 gets localized to mitochondria in the first place. Since mitochondria are known to associate with microtubules via kinesins in order to "move around" the cytoplasm (Kondo et al., 1994; Liu et al., 2002) it is not unlikely that p53 may be "targeted" to mitochondria in a microtubule-dependent manner.

7. Conclusions

The great interest in p53 due to its pivotal relevance to human cancer has generated a flood of information addressing almost any possible aspect of p53 biochemistry and biology, in the past two decades. In recent years, it has become apparent that p53 function is also tightly regulated by its cellular localization. Often loss of wild-type p53 activity is associated with a cytoplasmic "zip code." Several factors that influence p53 cytoplasmic or nuclear localization as well as p53 nucleocytoplasmic shuttling have been presented herein. We believe that more and more molecules will be identified as being involved in these processes. Remaining outstanding questions are:

1. Do these molecules work alone or interact with each other in order to function?

2. How do cells integrate various and often conflicting signals and find an efficient way to regulate p53 localization?
3. How could we exploit this knowledge to develop therapies directed towards enhanced p53 nuclear localization following its release from cytoplasmic anchors?

Ultimately, the answer to these questions will promote our understanding of the basic biology of p53 dysregulation in cancer and will provide new opportunities for the successful development of p53-based therapies.

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