MINI REVIEW

Interplay between p53-family, their regulators, and PARPs in DNA repair

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Summary Abnormalities of the p53 tumor suppressor gene are among the most frequent molecular events in human neoplasia. p53 is consequently one of the most studied proteins, and is the subject of over 55,500 scientific papers. In this review, attention is focused on the functions of p53 in DNA repair. We highlight the recent progress in the analysis of protein signals to p53, including PARPs, and ubiquitination cascade proteins MDM2, CRM1, USP10 and 14-3-3/α. © 2010 Elsevier Masson SAS. All rights reserved.

Introduction

Chromosomal and microsatellite instability ([CIN], [MIN]) are a characteristic of almost all human cancers. CIN refers to chromosome structure and number of changes over the time, and MIN refers to changing numbers of oligonucleotide repeats in microsatellite sequences. In hereditary cancers, the presence of both CIN and MIN is linked to mutations in DNA repair genes. Each day, endogenous and environmental assaults generate more than 10⁴ DNA lesions in any given cell, and these are corrected by DNA repair pathways responsible for maintenance of DNA integrity. The tumor suppressor gene TP53, which encodes the p53 protein, is considered as a DNA repair gene because of its function in the DNA damage response. When cells encounter substantial DNA damage, critical cell-cycle events are halted while DNA repair mechanisms are activated to restore genome integrity [1–2].

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The DNA damage response is a network of pathways that rapidly modulates many aspects of cellular metabolism, particularly following the induction of cytotoxic lesions such as DNA double-strand breaks (DSBs). A central part of the DNA damage response is the activation of p53 resulting in cell-cycle arrest, apoptosis, or senescence. These responses are important barriers to tumor progression and malignancy. Indeed, p53 tumor suppressor activity is attenuated in almost all types of human tumor cells, either by inactivating mutations in the TP53 gene or by altered expression of p53 modulators and effectors. Stability and appropriate intracellular localization of p53 is essential for its tumor suppressor function.

p53 is actively transported between the nucleus and cytoplasm. The ubiquitin ligase, MDM2, induces p53 nuclear export and degradation [3–6] whereas the nuclear protein kinase ataxia telangiectasia mutated (ATM) induces p53 stabilization in response to DSBs [7]. ATM phosphorylates several proteins, including p53 and its negative regulators: MDMX, MDM2, and COP1. Phosphorylation of MDM2 and MDMX enhances their degradation by reducing their interaction in the nucleus with the deubiquitinating protease USP7. COP1, another E3 ligase, has also been described as a direct ubiqu-
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Uituit ligase for p53 [8]. COP1 is also a p53-inducible gene. Further, COP1 depletion by siRNA enhances p53-mediated G1 arrest and can sensitize cells to ionizing radiation [3].

Oncogene activation could lead to genomic instability and selection for TP53 mutations. Unlike other tumor suppressor genes, most p53 oncogenic mutations are missense mutations within the core domain, leading to the expression of a full-length mutant p53 protein [9–11]. Accumulating evidence indicates that p53 cancer mutants are not only restricted to a loss of tumor suppressor activity but can also include gain-of-function phenotypes that promote tumorigenesis. The exact mechanism underlying gain-of-function phenotypes remains however unclear. Transfection of mutant p53 into TP53-null cells was shown to enhance their ability to form tumors in mice. Mutations in TP53 are associated with drug resistance in several malignancies and cell lines, a phenomenon that may be partially attributed to transcriptional activation of the multidrug resistance 1 (MDR1) gene by mutant p53 and to interference with apoptosis. This influence is clinically manifested as association of TP53 mutations with poor prognosis and drug resistance in a growing number of malignancies (for review, see [9–13]). Several gain-of-function properties of p53 mutants are mediated not through DNA binding, but rather through modulation of nontranscriptional processes. A recent study revealed that the hotspot DNA contact mutants p53R248W and p53R273H can bind MRE11, an upstream component of the ATM-dependent DNA damage response pathway and, consequently, inhibit the cellular response to DNA double-stranded breaks. The apparent phenotypes in mutant TP53 knock-in mice are augmented genetic instability, increased levels of interchromosomal rearrangements in premalignant thyomocytes and development of lymphomas, which are not observed in TP53-null mice. The clinical implications of this finding are the enhanced resistance of some tumors harboring mutant p53 to cancer therapies that induce double-stranded breaks and the observed association between mutant p53 and chromosomal instability in human cancers [9,14].

Upstream events of p53 protein to integrate cellular responses to stress

Under normal conditions within the cell, the p53 protein is present at low concentrations due to its relatively short half-life of about 20 minutes [15]. The proteases that are responsible for p53 degradation are unknown although there is some evidence that ubiquitin-mediated proteolysis is involved. As well as being present at low concentrations, in some cells, p53 may also exist in a form that is inactive for transcription. In these conditions, the p53 protein requires a signal or alteration to be able to function. The upstream events or signals that are targeted to p53 are mediated by various stressful situations, including double-strand breaks in DNA produced by γ-irradiation and DNA repair intermediates following ultraviolet irradiation or chemical damage [16]. This damage is associated with a rapid rise in cellular p53 due to increase in the half-life of the protein and possibly the rate of translation of p53 transcript. The increase in the level of p53 protein is related not only to the extent of DNA damage, but also to the type of damage. This is possibly because the cell utilizes different proteins to recognize specific types of damage to DNA, which are then repaired through the recruitment of specific enzymatic pathways. The cellular proteins involved in the recognition of DNA damage may also be directly involved with p53 activation. For example, in in vitro studies, it was shown that cells, which are defective in the ATM gene, have a delayed and attenuated p53 response to ionizing radiation. This implies that the ATM protein, which is a protein kinase involved in coordinating DNA repair, may regulate p53 through protein phosphorylation [7]. The C-terminus of the p53 protein binds to double- and single-stranded DNA ends, excision-repair damage sites, and internal deletion loops [17–18]. It is therefore possible that phosphorylation or other activating signals for p53 take place at DNA damage and repair sites where p53 and damage-detector proteins are colocalized.

DNA repair requires functional ubiquitin specific peptidase 10 (USP10)

A variety of molecular pathways and their associated proteins are involved in the stabilization and activation of p53. These include phosphorylation (Abl, ATM, CDK2, Chk1, Chk2, GSK3-β, JNK1, p38, PLK1, HIPK2), dephosphorylation (PP2A, WIP1), poly(ADP-ribosyl)ation, sumoylation (Ubc9, PIAS1, TORORS), acetylation (p300, PCNA, TIP60), deacetylation (HDAC1/2, Sirt1), methylation (Smyd2, SET7/8, PRMT5), demethylation (LSD1), ubiquitination (MDM2, MDMX, COP1, RIPH2, synoviolin, ARF-BP1, CHIP, WWP1, E4F1, TRIM24), deubiquitination (USP7/HAUSP, USP10), and poly(ADP-ribos) polymerisation (PARP-1, PARP-2) [19–20]. Dysfunction of any of these p53 regulatory pathways may result in the development of cancer. Under normal conditions within the cell, p53 is continually degraded via the ubiquitin-mediated proteasome pathway. However, when DNA damage is detected, degradation of p53 is decreased. Ubiquitin carboxyl-terminal hydrolase 7 (USP7) is a deubiquitylating enzyme that regulates p53 in two ways: directly through deubiquitination of the p53 protein, and indirectly through deubiquitination and stabilization of the MDM2 and MDMX proteins which downregulate the activity and amount of p53 within the cell (Fig. 1 (i)) [21]. Recent studies have indicated that USP10 also plays an essential role in regulating p53 stability [22]. In unstressed cells, USP10 is mostly localized in the cytoplasm where it deubiquitinates p53 thereby allowing its re-entry into the nucleus (Fig. 1 (iii)). However, following DNA damage and ATM-activation, USP10 is phosphorylated and accumulates in the nucleus, where it acts alongside USP7 to deubiquitinate p53 (Fig. 1 (iii)). USP10 stabilizes both wild type and mutant p53 (Fig. 1 (i)) and so can act as either a tumor suppressor or as an oncprotein. Indeed, overexpression of USP10 has been associated with poor prognosis in patients with glioblastoma multiforme as a result of increased levels of mutant p53 [22].

DNA repair requires functional 14-3-3σ, c-Abl, and p53: protein-protein interactions

How do cells co-ordinate specific biological outcomes of p53 activation that are appropriate to specific types of cell
The c-Abl protein contains an Src homology activity. Overexpression of c-Abl in normal cells has been shown to block cell-cycle progression in a p53-dependent manner [25–26]. The c-Abl protein contains an Src homology domain 3 (SH3) binding domain. The five amino acid motifs, proline-X-X-proline, located at residues 61–94 of the human p53 protein binds to the SH3 domain of c-Abl [27]. Recently, a family with Li-Fraumeni syndrome with late-onset breast cancer has been reported to have a mutation in the proline at codon 82, disrupting one of the proline-X-X-proline repeats [20,28].

DNA repair requires functional poly(ADP-ribose) polymerase-1 and p53

Poly(ADP-ribose) polymerase 1 (PARP-1) is an important DNA strand break sensor that regulates p53 function [29]. PARP-1 is involved in various types of DNA damage repair including base-excision-repair (BER), homologous recombination (HR), and nonhomologous end-joining (NHEJ) [30]. Under basal conditions, p53 is transported out of the nucleus to the cytoplasm via the chromosomal region maintenance 1 (CRM1) pathway (Fig. 1 (vi)). This transport is facilitated by a nuclear export signal located at the N-terminus of p53. During DNA repair or response to DNA damage, PARP-1 activity is upregulated in the nucleus and this results in rapid poly(ADP-ribosyl)ation of both PARP-1 itself (Fig. 1 (vii)) and multiple sites of p53 at amino acid residues Glu255, Asp256, and Glu268 of the C-terminal domain of this tumor suppressor protein (Fig. 1 (viii)). This inhibits the interaction of p53 with CRM1 thereby increasing its nuclear abundance, which results in increased expression of the cell-cycle inhibitor p21, an inhibitor of the cell-cycle and a key downstream effector molecule of p53-dependent signalling, and ultimately leads to the DNA damage-dependent checkpoint response [31]. In addition, p53 is also transported to the nucleus through nuclear pore complexes (NPCs) by transportin1, which recognizes nuclear localization signals. The nuclear import and export of p53 is facilitated by RanGTPase activating protein (RanGAP1), and Ran guanine nucleotide exchange factor (RanGEF)/regulator of chromosome condensation (RCC1). Therefore, the regulation of cell-cycle progression in response to DNA damage requires both functional PARP-1 and poly(ADP-ribosyl)ation—susceptible p53, which allows proper nuclear accumulation of p53 and appropriate stimulation of p21 gene expression important to avoid the formation of tumors and ensure proper genomic maintenance. PARP-1 regulates the stability of the wild type p53 protein and its enzymatic activity is necessary for this stabilization. The molecular determinants that switch between DNA repair, cell-cycle arrest or apoptosis in PARP regulation for mutant p53 are not fully understood [32–33].

In cancer cells, increased PARP activity confers tumor resistance to DNA damaging agents, such as platinumos, topoisomerase inhibitors, and radiation treatments. PARP inhibitors have been shown to counteract this resistance and thereby potentiate the antitumor effects of drugs in vitro and in vivo [34–35]. PARP inhibitors are also used as single-agent therapies in tumors with specific DNA repair defect such as BRCA mutations. Because triple-negative estrogen-, progesterone- and erbB2-receptors negative breast cancer exhibit some of the properties of BRCA1- or BRCA2-deficient cells, PARP inhibitors are being tested against this type of tumors [36–37]. Gene expression profiling in human breast
cancer cells treated with PARP-1 short-hairpin RNA reveals that PARP-1 downregulation alters the expression of many genes involved in cell-cycle control and stress response, including p53.

Genomic integrity also depends on proper assembly and functioning of the bipolar mitotic spindle, which is required for equal chromosome segregation. During cell division, centrosome duplication occurs only once at the G1/S transition stage. The presence of multiple centrosomes in cancer cells suggests that this process is deregulated in carcinogenesis. Centrosome function can be regulated by post-translational modifications of centrosomal proteins. Indeed, poly(ADP-ribosyl)ation of p53 located in centrosomes by PARP-1 plays an important role in the regulation of centrosome function [38]. The p53 protein regulates the initiation of centrosome and DNA duplication as well as the suppression of centrosome re-duplication through p21/CIP/Waf1-dependent and independent pathways. Studies of cultured cells as well as tumor tissues have shown that chromosome instability associated with inactivation of p53 is primarily attributed to deregulation of the centrosome duplication cycle and subsequent centrosome hyperamplification. Nonetheless, direct induction of chromosome instability by loss or mutation of p53 has yet to be demonstrated. More recently, p53 has been shown to regulate the G2/M cell-cycle checkpoint. When mitotic spindle inhibitors such as nocodazole are added to cells with wild-type p53, the cells are blocked in G2 phase. However, in the absence of wild-type p53, DNA synthesis is reinitiated, which increases the ploidy of the cells. This suggests that through regulating the G2/M checkpoint, p53 prevents premature entry into another S phase and may account for the phenotype of genomic instability that is commonly associated with p53 mutation.

Gain of oncogenic function of p53 mutants and PARP regulates E-cadherin expression and epithelial to mesenchymal transition

Both PARP and mutant p53 tumor suppressor genes contribute to the enrichment of epithelial to mesenchymal transition (EMT) during malignant transformation. EMT is a process by which epithelial cells acquire mesenchymal properties, dissociate from the epithelium and migrate to secondary sites [39–41]. EMT is an essential process during early embryonic development, but also in several fibrotic diseases and possibly metastatic progression in many human cancers. Importantly, the loss of E-cadherin expression and the associated disruption of cell-cell junctions are the key steps in the process of EMT, a marker of poor patient prognosis in many solid cancers. Loss of E-cadherin may result from genetic mutations, promoter hypermethylation or transcription factor dysregulation. The Snail transcription factor is a known transcriptional repressor of E-cadherin and a well-characterized inducer of EMT, silent in normal epithelial cells. Snail expression is regulated at the level of transcription by several cell signalling effectors, including Akt, ET-1, Gli, and Integrin Linked Kinase (ILK). ILK stimulates Snail and zeb-1 expressions and regulates E-cadherin through PARP-1 in prostate PC3 cancer cells [42]. In this connection, we have recently found that PARP and p53 are overexpressed in MCF-7 cells undergone epithelial mesenchymal transition (data not shown [43]).

p53 acts as the guardian of the genome

p53 acts as a ‘‘molecular node’’ in the DNA damage response where signals converge in response to either double-strand DNA breaks or stalled replication forks. p53 will subsequently determine which effector role is assumed, and this likely depends on its subcellular localization, the cell-cycle status, as well as the type and extent of DNA damage. Within the cell, p53 protein has a dual role in the regulation of cell function. First, p53 acts as a transactivator and transrepressor of genes that are involved in cell-cycle arrest, apoptosis and senescence [44]. Second, p53 modulates DNA repair and recombination through processes, which are either dependent or independent of its transactivation function. The transactivation-independent processes regulated by p53 through protein interactions include homologous recombinational repair (HR), nonhomologous end-joining (NHEJ), mismatch repair (MMR), base-excision-repair (BER), and nucleotide-excision-repair (NER) [45]. It can be hypothesized that when the extent of DNA damage is low, the latent pool of p53 (unmodified or post-translationally modified) interacts directly with the DNA repair machinery, either alone or in combination with other repair-specific factors. This graded response prevents over reaction to the low level of any DSBs and DNA distortions arising during the cell-cycle, and to low level exposure to mediators of DNA damage. When the extent of DNA damage cannot be successfully handled by p53 alone, the tumor suppressor undergoes stabilization (dependent on post-translational modification), and functions as a sequence-specific transcription factor. It thereby activates a set of genes that arrest the cell-cycle so that the DNA repair-processes can successfully correct the lesion. During this stage, p53 can also interact and modulate the different repair-process-specific proteins. Apoptotic-specific genes are induced by p53 if the DNA damage persists or is irreparable, resulting in cell death. In these processes, upstream signalling pathways play a key role in recognizing the extent of DNA damage and in post-translational modification of p53. In this way, p53 is able to act as the ‘‘guardian of the genome’’ by functioning as a ‘‘cellular rheostat’’ that modulates its multivariant functions according to the specific in vivo situation [46].

Many recent investigations have characterized the transactivation-independent functions of p53 during DNA repair and recombination. NER factors (except CSB) together with p53 the transcription factor play a role in transcription process to activate promoters and facilitate chromatim modification, which can be distinguished from their role in DNA repair [47]. Estrogen levels act as a rheostat on p53 levels and modulate p53-dependent responses in breast cancer cell lines [10,48—49]. A considerable proportion of breast tumors (20—30%) carry mutations in the p53 gene. These are associated with poor survival and poor response to several types of chemotherapeutic treatments. There is increasing evidence of functional interactions between p53 and estrogen receptor α (ERα) pathways in breast and other tissues. Recent studies have demonstrated that estrogens, acting on ERα, influence the expression and activity of both wild type
and endogenously expressed mutant p53 protein. In colon cancer cells, mutations in p53 are associated with tumor progression and metastasis, downregulation E-cadherin, and upregulation of slug and zeb-1 [13].

p53 is part of a multigene family that also includes p63 and p73 [20,50]. The overall protein architecture of this family is highly conserved in humans and comprises a central sequence-specific DNA binding domain, an N-terminal transactivation domain, and a C-terminal oligomerization domain [50]. p63 and p73 can undergo the same modifications and interactions as p53 and are at least partially involved in the same regulatory networks. This implies that all p53-family proteins should be considered when analyzing the genetics of cancer cells. Changes in the levels of selected p53-family members or their isoforms may affect the relative availability of shared protein partners, as multiple p53-family proteins compete for interaction [50–53].

The role of p53 in tumor formation

Mutations in p53 are found in 50–55% of all human cancers [10] (http://www-p53.iarc.fr/). These mutations strongly select for p53 proteins that fail to bind to DNA in a sequence-specific fashion. The cell-cycle arrest functions of p53 require p53 transcriptional activity, while some of the apoptotic activities of p53 do not require p53-dependent gene products. This could mean that transcription of selected p53 target genes is critical to its tumor suppressor function or that the p53 direct signalling pathway, in the absence of transcription, requires p53 to bind to DNA in a sequence-specific fashion. Clearly, the enhancer elements recognized by p53 in the DNA are not all equally regulated by it. Some p53 mutant proteins can activate a p53 responsive sequence in the p21 gene but not the BAX gene [44]. This could indicate that BAX transcriptional activation (apoptosis) is selected against more frequently than are those of p21 and BAX together (G1 arrest). In addition to p53 mutations, some tumors inactivate p53 by the amplification of the MDM2 gene (about one third of all sarcomas) [54] or by the localization of p53 to the cell cytoplasm (a small number of breast tumors and neuroblastomas). In other cancers, p53 mutations are never selected (teratocarcinomas). In this case, the wild-type p53 protein in cancerous stem cells is not a functional transcription factor, and that is presumably the reason for the absence of selection of p53 mutations [44].

Conclusions and perspectives

DNA damage and replication stress lead to genomic instability and selective pressure for TP53 inactivation. Loss of p53 function allows evasion from cell death and proliferation of cancer cells. p53 was discovered 30 years ago with 55,500 publications in the PubMed database. The p53 pathway is still complex and much research need to be done to identify further regulatory interactions that may potentially target for new therapeutic molecules. However, some very promising therapeutic tools, including small molecules, PRIMA-1 (MET), Nutlin and RITA that target the MDM2–p53 interaction have been already discovered [6,55–57]. Micro-RNA-34a is one of the important components of PRIMA-1-induced apoptotic network in the cancer cells harboring mutant p53 [58].

What is the impact of p53 wild type and mutant, on normal and cancer stem cell survival, proliferation, differentiation, and resistance to chemotherapy? In 2008 Zhang, and colleagues identified a subpopulation of tumor-initiating cells in a syngeneic p53-null mouse mammary gland tumor model that mimics human breast cancer. Upon subsequent transplantation, this subpopulation generated heterogeneous tumors that displayed properties similar to the primary tumor [59]. However, inactivation of p53 potentially helped the survival of immortalized, premalignant cells [60].

Conflict of interest statement

Nothing declared.

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