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Review Article

p53 at the crossroads between cancer and neurodegeneration

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ABSTRACT

Aging, dementia, and cancer share a critical set of altered cellular functions in response to DNA damage, genotoxic stress, and other insults. Recent data suggest that the molecular machinery involved in maintaining neural function in neurodegenerative disease may be shared with oncogenic pathways. Cancer and neurodegenerative diseases may be influenced by common signaling pathways regulating the balance of cell survival versus death, a decision often governed by checkpoint proteins. This paper focuses on one such protein, p53, which represents one of the most extensively studied proteins because of its role in cancer prevention and which, furthermore, has been recently shown to be involved in aging and Alzheimer disease (AD). The contribution of a conformational change in p53 to aging and neurodegenerative processes has yet to be elucidated. In this review we discuss the multiple functions of p53 and how these correlate between cancer and neurodegeneration, focusing on various factors that may have a role in regulating p53 activity. The observation that aging and AD interfere with proteins controlling duplication and cell cycle may lead to the speculation that, in senescent neurons, aberrations in proteins generally dealing with cell cycle control and apoptosis could affect neuronal plasticity and functioning rather than cell duplication.

Contents

Introduction

Cancer and neurodegenerative disorders are common age-related conditions. Cancer arises from a sequence of genetic and/or epigenetic events that regulate cellular differentiation and proliferation [1–3]. DNA methylation, histone acetylation, and other epigenetic modifications play roles in the activation and suppression of cancer genes and recent evidence suggests that defects in these events are linked to the progression of neurodegenerative disorders [2]. As far as changes in DNA methylation, the most studied aspect of epigenetic events, are concerned, a global hypomethylation of DNA has been found in various human cancers when samples were compared to healthy tissue counterparts [4]. Also, in Alzheimer disease (AD), the promoter region of the amyloid precursor protein (APP) gene, the precursor of β-amyloid (Aβ) peptide, has been shown to be hypomethylated with age [5,6]. The gene for neprilysin, the major Aβ-degrading enzyme in the brain [7], is hypermethylated in cerebral endothelial cells after treatment with high concentrations of Aβ [8]. Furthermore, two recently identified genes, S100A2 and SORBS3, that have been implicated in memory storage in the central nervous system showed significantly different levels of DNA methylation in AD and control cases [9].

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Recent data from the literature further support the concept that one or more common molecular mechanisms may be involved in the development of both neurodegenerative diseases and many cancers [10–12]. In particular, we focused on the relationship existing between cancer and AD. Because cancer is a disorder characterized by uncontrolled cell growth, whereas AD, as well as other neurodegenerative diseases, is denoted by atrophy and neuronal death, common signaling pathways regulating cell death and survival have been suggested to influence the development of these conditions. Epidemiological data from the literature suggest an inverse correlation between cancer and AD. In particular, older adults with prevalent clinical AD have been reported to develop incident cancer with slower growth rates compared to older adults without dementia and that individuals without dementia with a cancer history may be less prone to develop clinical AD [13,14]. On the other hand, in the vast majority of studies, cancer seems to be a prevalent comorbidity for patients with AD [15]. Furthermore, in a postmortem histopathological study, unreported signs of AD were found in 42% of patients diagnosed with brain cancer and in 48% of cases without glioblastoma, suggesting that coexistence of both diseases is often uninvestigated and consequently underreported in the clinical literature [16]. Alternatively, it is possible that brain cancers give rise to a brain milieu favoring amyloid deposition and neurofibrillary tangle formation.

Taking into account that cancer and AD share common signaling pathways directing cell fate toward either death or survival, the identification of the putative common mechanisms may be useful to better understand these two disorders as well as to develop more appropriate therapeutic strategies. It is noteworthy that the balance of cell survival versus death is at least in part regulated by a fine timing of checkpoint proteins, the preservation of DNA integrity, and correct repair [17,18]. Among cell cycle proteins, p53 is particularly significant because of its role in stopping cells in G0/G1 and G2/M phases, thus either allowing DNA repair or activating a programmed cell death. p53 dysfunctional activity is involved in cancer progression, but also in aging and AD. In mammals, loss of p53 increases carcinogenesis, whereas specific gain-of-function alleles reduce the incidence of cancer but accelerate aging, suggesting a trade-off between cancer surveillance and stem cell maintenance [19]. Yang and co-workers demonstrated the existence of aberrant neurons in AD brain by showing that neurodegeneration is correlated with neurons reentering a lethal cell cycle [20], which suggests that dysfunctional p53 in nondividing cells may play a role in aberrant cell cycle progression.

In this review we discuss the multiple functions of p53 and how these correlate between cancer and neurodegeneration, focusing on various factors that may have a role in the regulation of p53 activity.

p53: a transcription factor critical to decisions about cell fate

p53 is a very short-lived protein that exists in a wild-type latent conformation and is activated in response to a great variety of stresses that can damage the integrity of the cell genome [21,22]. Among these, DNA damage, hypoxia and activation of oncogenes are potent activators of p53 protein. Stabilization and induction of p53 transcriptional activity depend mainly on posttranslational modifications together with protein/protein interactions [23]. The regulation of p53 activity is crucial in determining the cellular outcome. For example, depending on the tissue, the same acetylation of Lys at position 320 of p53 can promote neurite outgrowth in neuronal cells [24] or cell cycle arrest in other tissues [25]. The transcriptional network of p53-responsive genes produces proteins that are able to interact with a large number of other signal transduction pathways in the cell [26]. Activation of cell cycle checkpoints by p53 leads to transient cell growth arrest [27]; once it is bound to sites of DNA damage, p53 promotes DNA repair [28] and simultaneously stimulates the transcription of direct effectors of cell growth arrest (e.g., the cyclin-dependent kinase inhibitor p21) as well as effectors required for efficient DNA repair of complex lesions that require longer processing (e.g., GADD45) [29]. At this point, there are several potential cellular outcomes, most of which are heavily influenced by the cell type as well as by the severity of the DNA lesions: transient cell cycle arrest (when DNA damage is not severe), defective repair (resulting in mutation, such as chromosomal aberrations), permanent cell cycle arrest (cellular senescence), or cell death (apoptosis) [30,31]. Thus, p53 protects the genome by promoting the repair of potentially carcinogenic lesions in the DNA, thereby preventing mutations. In addition, p53 eliminates or arrests the proliferation of damaged or mutant cells by the processes of apoptosis and cellular senescence [32,33]. Taken together these results show that the regulation of p53 activity in cells is extremely important in determining the fate of cells or tissues. Activated p53 integrates the various incoming signals that register different forms of cellular stress. Therefore, it is conceivable that small deregulations toward one or the other side may favor cell survival or cell death/senescence.

Loss of function in p53 is usually associated with many common human cancers [34]. The p53 gene is mutated in almost half of all human cancers; in the rest of human cancers, p53 is mostly inactivated by the disruption of pathways regulating its activation. Mutant p53 is almost always defective for sequence-specific DNA binding and thus for transactivation of genes upregulated by the wild-type protein [34]. However, accumulating evidence indicates that p53 cancer mutants not only lose the cancer suppression activity of wild-type p53, but also gain novel oncogenic activities to actively promote cell growth and survival [35–37].

p53 in cancer: mutations as more than a loss of function

The predominant mode of p53 inactivation in about 50% of human primary cancers is by point mutation rather than by deletion or truncation [38]. Mutational analysis of the p53 gene, conducted using the International Agency for Research Cancer database, revealed that almost all hot-spot mutations are located within the DNA-binding domain of the protein. These mutations may have structural consequences: DNA contact is lost, conformation of the DNA-binding domain is locally perturbed, or the entire core domain is unfolded [39]. Crystallography studies allowed one to classify p53 mutant proteins in two large categories: (a) DNA contact-defective mutations, which include mutations harbored on the residues composing the DNA/protein interaction surface (i.e., residues His273 and Trp248) and (b) structure-defective mutations, whose point mutation determines an important conformational alteration (residues His 175 and His 179). Verifying whether the structural classification of p53 mutants reveals similar differences in terms of biological activity has proven to be quite difficult. *In vitro* studies have shown that overexpression of conformational mutants renders cell cultures more resistant to etoposide, whereas DNA contact-defective mutants increase their resistance to cisplatinum [40–42]. Furthermore, the presence of conformational mutants has been associated with breast cancer patients, whose relapse rates after conventional chemotherapy treatment are higher with respect to those of patients carrying DNA contact mutations [43–45]. Altogether, these findings strongly indicate that p53 mutants not only lose their oncosuppressor activity but also contribute to development, maintenance, spreading, and resistance to anti-cancer treatments. Indeed, the concept that some p53 mutations not only result in loss of wild-type activity, but also acquire a gain of function started to find support at the beginning of the 1990s. Many studies have focused on specific gene networks transactivated by p53 mutants. Through genome-wide expression profile techniques mutant p53 proteins have been shown to modulate a set of genes that could serve as transcriptional mediators of its oncogenic activities [46–48]. Chromatin immunoprecipitation experiments have shown that p53 mutants can be bound to the regulatory regions (promoter, intron region, and 5′/3′ UTR) of potential target genes, but the identification of the specific DNA-binding consensus of mutant p53 is still missing. It is also still unclear whether p53 mutants bind directly to the promoters of these genes or are recruited to these promoters through interactions

with other proteins. As an example, p53 mutants can interact with other p53 family members such as p73 and p63 to inhibit their activities in both human cancer cell lines and knock in mouse cells [49,50].

Mutation-independent conformationally altered p53 in cancer development: the role of oxidative stress

Stabilization and overexpression of p53 have been often considered markers of mutant p53. Mutant p53 stabilization depends on impaired ubiquitination due to the loss of wild-type (wt) p53 structure. The molecular basis of a prolonged half-life of mutant p53 might partially depend on the inefficient degradation exerted by the E3 ubiquitin ligase MDM-2, whose gene is a direct transcriptional target of wt p53 [see for review 51]. However, the corresponding mutant p53 accumulation of protein is not always true, because many cancers with p53 mutations do not show accumulation of mutant p53, and the increased content of p53 in some types of cancer is not due to mutation on p53. Webley et al. [52] analyzed p53 mutations with respect to their conformational state in 38 different colorectal cancers and found that 22 of them expressed p53 mutants, whereas 16 retained a wild-type p53 gene. Among 16 colorectal cancers with wild-type p53 gene, 7 presented the mutant conformation and exhibited resistance to apoptosis. These findings lead to two main observations: (a) conformational alterations in p53 protein occur also in the absence of p53 mutations and (b) conformational alterations in wild-type p53 may be involved in cancer development.

How can p53 change its tertiary structure in the absence of gene mutations? This issue has been well investigated by Méplan and co-workers. They demonstrated that the exposure of wild-type p53 synthesized *in vitro* to metal chelators, such as EDTA or orthophenanthroline, resulted in a rapid switch to an unfolded "mutant-like" isoform [22]. The conformational phenotypes were analyzed using two specific conformational anti-p53 antibodies, which discriminate folded vs unfolded tertiary structure. Transition from folded to an unfolded state was accompanied by loss of DNA-binding activity at the canonical DNA sequences. The DNA-binding domain consists of two sheets supporting two loops and a loop–sheet–helix motif that forms the DNA-binding surface [22]. The two loops are connected by a zinc atom coordinated with residues Cys176, Cys179, Cys238, and Cys242. Metal chelation affects Zn^{2+} binding that is crucial for the stabilization of p53 in the wild-type conformation and induces the oxidation of thiol into disulfides and the cross-linking of p53 in high-molecular-weight aggregates. The observation that p53 is intrinsically sensitive to redox regulation and to transition metals is also supported by evidence that p53–DNA bound *in vitro* requires thiol reductants (such as dithiothreitol) and by the role of two disulfide-reducing proteins, Ref-1 and thioredoxin reductase, which are able to keep p53 in a reduced state inside cells [53]. Furthermore, the switch from wild-type to unfolded p53 conformation can also be experimentally regulated by cadmium, synthetic nitric oxide donors, such as *S*-nitroso-*N*-acetylpenicillamine and *S*-nitroglutathione, and low concentrations of H_2O_2 [54–56].

Although the role of oxidative stress in cancer development has been documented, there is no linear correlation between reactive oxygen species (ROS) and cancer. High levels of oxidative stress affect cell viability, inhibiting cell proliferation and leading to apoptosis/necrosis cell death, whereas low intermediate levels of oxidative stress are more effective in stimulating cancer development and spreading [57]. Indeed sublethal levels of ROS increase DNA damage, which in turn triggers mutagenesis via DNA base modification and mismatch repair, finally affecting structure and function of proteins [58]. An impairment of p53 activity, mediated by the effects that ROS and reactive nitrogen species (RNS) have on its tertiary structure, may be involved in cancer development. It is becoming clear that ROS in low concentrations may act as second messengers, regulating gene transcription involved in proliferation and triggering redox-responsive signaling cascades [59]. p53 belongs to a growing list of transcriptional factors that are subject to redox modulation [60–62]. ROS play at least two distinct roles in the p53 pathway. First, they are important activators of p53 through their capacity to induce DNA strand breaks [63–65]. Second, they regulate the DNA-binding activity of p53 by modulating the redox state of a critical set of cysteines in the DNA-binding domain, which in turn induces conformational changes [22,66,67]. The duration and the degree of ROS signaling can influence one event or the other. An intriguing hypothesis is that the p53 conformational state affected by ROS/RNS may not be only a mere consequence of oxidative stress, but a fine gene transcription mechanism allowing specific adaptive responses.

p53: a delicate balance between cancer-suppressive and age-promoting functions

Recent observations suggest that p53 may play a central role in aging and in neurodegenerative disorders [68–73]. In this review we mainly focused on the p53 role in AD [74], yet we must not forget that this protein has been related to other neurodegenerative diseases [75,76]. Even if it is still a matter of controversy whether organism aging is due to a programmed process or is the consequence of failed mechanisms involved in regeneration or repair tissues, p53 can promote select aspects of the aging process because of its role in establishing senescence and in determining organism aging when its activity is increased [77–79]. Studies with mouse models suggest a delicate balance between cancer-suppressive and age-promoting functions of p53 [30]. In several mouse models, altered p53 activity has been associated with premature/accelerated aging under some circumstances (such as stress) or otherwise with cancer suppression [for a review see 30]. However, how exactly this balance is achieved is a topic in need of further elucidation. Insights into the role of p53 in aging also derive from human population studies. A polymorphism Pro/Arg in codon 72 of the p53 gene results in a slight reduction in p53 activity and is associated with enhanced cancer risk but also with increased longevity [80]. Humans carrying the Arg72 allele have a survival advantage despite a higher risk of cancer in longer living individuals [81], even if the association seems to be dependent on the type of cancer and on the genetic background of the population under study. In this regard, it has recently been reported that the Pro72 genotype is more frequent in patients with colorectal cancer with respect to age-matched controls and centenarians [82]. Evidence from mice and other mammals suggests that p53 acts as a longevity-assurance gene, by basically shutting down carcinogenesis [83,84]. Taking this hypothesis into account, one can postulate that the increased age-related incidence of cancer could be due not only to an accumulation of mutations, but also to a possible age-related decrease in p53-mediated responses, as previously cited [84]. Seluanov et al. [85] showed an impairment of p53-induced cellular responses against cytotoxic agents in aged normal diploid human fibroblasts, but not in young cells. A decline in p53 responses has also been observed in aged mice after γ-irradiation, which has been ascribed to the decreased stabilization of p53 protein due to decreased ATM function [84]. Therefore, this age-related decline in p53-mediated responses suggests a putative explanation for the correlation between carcinogenesis and the aging process.

p53 and Alzheimer disease: not only a killer

Evidence of the pivotal function of p53 in neuronal death is provided by data from both *in vitro* and *in vivo* models. A strong correlation between p53 expression and excitotoxic neuronal death induced by glutamate, kainic acid, and *N*-methyl-D-aspartate has been established [86,87]. Also in 1998 our group demonstrated that glutamate- and kainate-induced neuronal death was p53-dependent [88]. Furthermore, increased p53 immunoreactivity associated with neuronal death was observed in models of cerebral ischemia stroke, traumatic brain injury, and epilepsy [see for review 89]. According to these data, many studies demonstrated that inhibition of p53 prevents cell death in a variety of neurodegenerative models. For example pifithrin-α, a

compound that inhibits p53 activity, attenuated neuronal death in several different rodent models of stroke [90–92] and in kainate-induced seizure in cultured neurons exposed to DNA-damaging agents, glutamate, and Aβ [90,92,93]. All these studies, aimed at demonstrating the killer role of p53, share a common feature: use of an acute toxic insult.

What type of cell death neurons undergo in AD is still a matter of controversy, but it is clear that there is progressive atrophy of the brain due to cell and synaptic loss. The leading explanation for the pathologic changes associated with AD is the "amyloid hypothesis," which holds Aβ as the main pathogenetic factor of AD because the aberrant metabolism of APP and the subsequent aberrant production and deposition of the peptide in extracellular sites are responsible for a concatenate series of events resulting in neurotoxicity and subsequent neuronal death [94,95]. AD neurodegeneration takes place over many years, and neuronal death is not the result of a single acute insult, but is more probably the consequence of many triggers inducing compensatory responses over long periods of time until the last detrimental event occurs. Based on these speculations, we wonder whether high levels of p53 in certain neurons, as observed in *postmortem* autopsy samples from AD patients, are coincidentally related to cell death or whether they are the results of the adaptive responses mentioned above. Furthermore, high expression of p53 around Aβ senile plaques may find another interpretation in addition to that of a marker of occurred death. The pro-oxidant environment induced by Aβ, well established in AD pathology [96–99], may contribute to affecting cysteine residues in the DNA-binding domain of p53, impairing its conformational structure and finally its functional activity.

Nevertheless, accumulating evidence highlighted various roles for p53 in addition to the one-sided view of its proapoptotic activity. p53 functions in a stimulus-dependent and cell-type-dependent manner. This is made possible by the multiple posttranslational modifications that target p53 on its N- and C-termini, thus resulting in conformational changes that affect protein/protein interactions with transcriptional cofactors. The consequences of specific patterns of p53 posttranslational modifications are also context-dependent, meaning that specific p53 codes might lead to different biological outcomes depending on the transcriptional context of a given cell or tissue [100]. An intriguing nonapoptotic role for p53 has been proposed by recent research carried out by Di Giovanni and co-workers. Surprisingly, p53 has been demonstrated to be required for axonal outgrowth in primary neurons as well as for axonal regeneration after neuronal injury in mice, probably through a different posttranslational pathway [101,102]. *In vivo* analyses of axonal injury and regeneration suggested that some "atypical" p53-dependent cellular functions could depend on specific patterns of p53 posttranslational modifications, such as acetylation in its C-terminus. In particular, acetylation of lysine 320 (K320) of p53 is involved in the promotion of neurite outgrowth and in the regulation of the expression of the actin-binding protein coronin 1b and the GTPase Rab13, both of which associate with the cytoskeleton and regulate neurite outgrowth [101]. Furthermore, acetylated p53 at K372-3-82 drives axon outgrowth and growth-associated protein 43 (GAP-43) expression and binds specific elements on the neuronal GAP-43 promoter in a chromatin environment through CBP/p300 signaling. [103]. Hence, the loss of the p53 wild-type conformation may compromise the brain's ability to overcome a toxic insult by restoring new axonal connections.

Conformational mutant p53 in aging and Alzheimer disease

Focusing on the study of p53-induced signaling responses in peripheral cells of AD patients and age-matched controls, the lack of p53 functional activity has been observed in AD fibroblasts after cytotoxic insults. Such impairment was demonstrated to be due to conformational changes in p53 tertiary structure, selectively occurring in AD cells [63,104]. Furthermore, p53 has been studied in blood cells of aged controls and demented patients, with data demonstrating an age-related increase in conformational altered p53, which is more pronounced in AD patients, to the point that it has been proposed as a putative biomarker in the early stages of the disease [105]. Similar results were reported by Zhou and Jia, who demonstrated a p53-mediated G1/S checkpoint dysfunction in lymphocytes from AD due to the expression of the unfolded p53 conformation [106]. Furthermore, Serrano et al. [107] demonstrated a significant increase in unfolded p53 in older AD transgenic mice compared with younger APPswe/PS1A246E animals and wild-type counterparts of comparable age.

Unfolded p53 found in AD fibroblasts has been demonstrated to be independent of gene mutations on the basis of sequence analysis of the p53 gene, thus suggesting that one of the peripheral events associated with the disease is responsible for p53 conformational changes [63]. The exposure to nanomolar concentrations of Aβ 1–40 and Aβ 1–42 peptides has been found to induce the expression of an unfolded p53 protein isoform in various cell lines [104,108]. These data suggest that the tertiary structure of p53 and the sensitivity to p53-dependent apoptosis are influenced by low concentrations of soluble Aβ. Furthermore, a correlation between Aβ peptides, oxidative stress, and p53 conformational changes has been demonstrated within stable transfected clones expressing APP751 wild-type protein [109]. On the basis of these findings, low amounts of soluble Aβ have been hypothesized to induce early pathological changes at the cellular level that may precede the neurodegenerative process. It is possible that this unfolded p53 conformation may participate in AD development and may thus be considered a specific marker of the early stage of the pathology. As a result of such a conformational change, p53 partially loses its activity and may no longer be able to properly activate an apoptotic program when cells are exposed to a noxious stimulus [63,110,111].

We cannot speculate as to whether conformationally altered p53 found in AD peripheral cells is also present in the brain of AD patients and what the relevance of such impairment is in terms of neuronal function. However, accumulating evidence indicates that p53 is perturbed in the central nervous system in a number of neurodegenerative disorders [75,76,112]. Furthermore, past *postmortem* studies suggest an involvement of p53 in degenerating neurons in AD. These include de la Monte et al. [113], showing increased p53 and Fas expression in specific populations of cortical neurons; Kitamura et al. [114], showing increased amounts of p53 in the temporal cortex, mainly localized in glial cells; and Seidl et al. [115], showing higher levels of p53 in the frontal and temporal lobes in Down syndrome patients. However, it is not known whether the increase in p53 observed in these papers occurs in degenerating neurons or reflects the expression of a conformational altered isoform of p53 as detected in blood cells and fibroblasts from AD patients [63,106,116].

What the contribution of a conformational change in a protein to the aging and neurodegenerative processes may be is still under investigation. We could also address the issue of whether a generalization of this phenomenon within the context of the "gain and loss of function" of protein conformers may be possible. Several studies demonstrated that conformational mutant p53 loses the ability to regulate the genes usually activated by wild-type p53 and acquires new transcriptional properties. As an example, conformational mutant p53 has been found to regulate some factors that are common targets of cancer mutant p53. For example, among genes regulated by mutant-cancer-associated p53, CD44 mRNA was found in those AD B lymphocytes expressing unfolded p53 [117]. CD44 is a surface antigen expressed by cells of the immune and central nervous system as well as of a variety of other tissues. Functioning as an adhesion molecule, CD44 is further involved in driving immune responses in infected tissues, including the central nervous system. Although more studies will need to demonstrate that CD44 is a target gene of unfolded p53, the correlation between CD44 and unfolded p53 in AD may assume an intriguing significance also in terms of the possible role of the immune system in AD pathology. That conformational altered p53 may acquire new transcriptional properties was suggested by Tassabehji et al. [118], who identified a number of noncanonical target

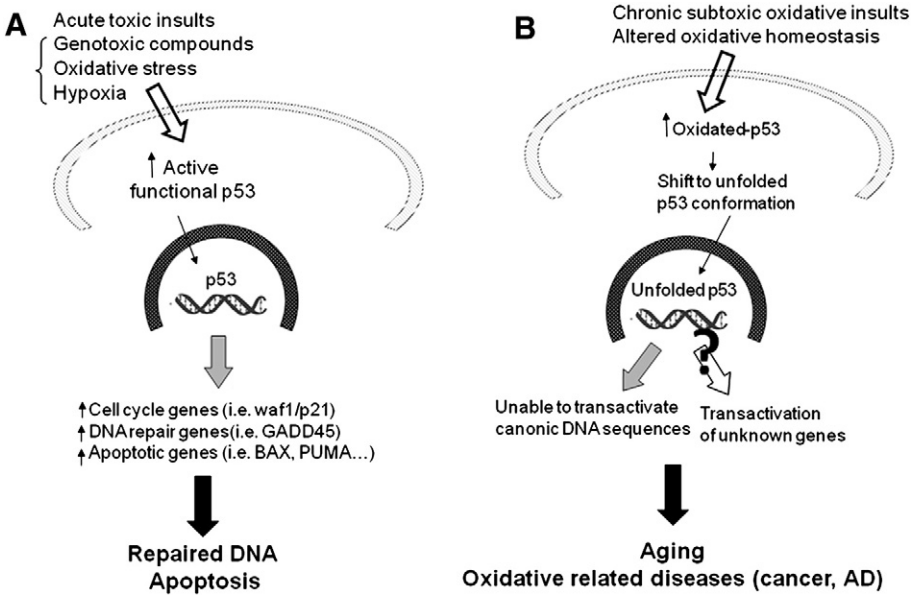

Fig. 1. The dual role of p53 after a ROS insult. (A) An acute toxic insult, such as oxidative stress but also genotoxic damage, activates the canonic p53 intracellular pathway. After acute damages, p53 induces cell cycle arrest, transactivating numerous genes, most of which are involved in cell cycle control, DNA repair, and apoptosis. Otherwise, if the damage is too extensive, p53 activates an apoptotic process via the transactivation of proapoptotic genes. (B) Lack of p53 activity can be due to posttranscriptional modifications altering p53 tertiary structure and preventing binding to specific wild-type p53 DNA sequences. An alteration in oxidative homeostasis, resulting in a subtoxic and chronic ROS exposure, impairs p53 tertiary structure and induces a shift in unfolded p53 conformation. Unfolded p53 is not able to transactivate the canonical genes of wild-type p53, but may acquire new transcriptional activity and possibly participate in the development of aging and age-related diseases.

genes of p53 in human hepatoma cells (Hep G2) after inducing p53 conformational changes toward a mutant phenotype with Cu^{2+} compound.

Conclusion

The type of cell death involved in AD is still controversial, but it is clear that there is progressive atrophy of the brain due to cell and synaptic loss. The average time course of AD is 10 years from first symptoms to death. If we consider that a typical apoptotic process takes roughly 12 h and that few neurons (fewer than 1/10,000 at any given time) exhibit signs of apoptosis [119], we could speculate that neuronal death is not the result of a single acute insult. Hence, like cancer, AD may be the result of serial insults that alone are insufficient to lead to disease. It is more probable that the neuronal death observed in AD brains may be considered the consequence of many triggers, inducing compensatory responses through time until the last detrimental event occurs. This observation is consistent with a "two-hit hypothesis" stating that the first hit makes neurons vulnerable and the second hit triggers the whole degenerative process [120]. The first hit may be cell cycle abnormalities or oxidative stress. Taking this into account, tolerable levels of oxidative stress have been proposed to provoke compensatory changes that lead to a shift in neuronal homeostasis, which is initially reversible if the oxidative stress is acute. With persistent oxidative stress, such as that seen in pre-AD and AD cases, which is significant compared to age-matched controls, it is likely that, after a certain threshold (in terms of severity and chronicity of oxidative stress), the majority of neurons recruit permanent adaptive changes but still function normally or slightly suboptimally in a pro-oxidant environment [119,121,122]. In such an oxidative environment, proteins highly sensitive to redox modulation, including p53, can be compromised [22,60–62]. In particular reactive oxygen/nitrogen intermediates play, at least, two distinct roles in the p53 pathway. First, they are important activators of p53 through their capacity to induce DNA strand breaks [64,123]. Second, they regulate the DNA-binding activity of p53 by modulating the redox state of a critical set of cysteines in the DNA-binding domain, which in turn induces conformational changes [55,66,67,124]. The duration and the degree of ROS signaling can influence one or the other event (Fig. 1). Based on these speculations, we wonder whether high levels of p53 in certain neurons, as observed in *postmortem* autopsy samples from AD patients, lead to cell death or are the results of those adaptive responses. However, we cannot speculate at this time whether conformational altered p53 found in AD peripheral cells is also present in the brain of AD patients and what the relevance of such impairment may be in terms of neuronal function. There are, however, a number of *postmortem* studies suggesting an involvement of p53 in degenerating neurons in AD [113–115]. It is not known, though, whether the increase in p53 observed in these papers occurs in degenerating neurons or reflects the expression of a conformationally altered isoform of p53 as we detected in blood cells and fibroblasts from AD patients [63,104,116].

The observation that aging and AD interfere with proteins controlling the duplication and cell cycle, such as p53, may lead to the speculation that, in senescent neurons, derangements in proteins commonly dealing with cell cycle control and apoptosis could affect neuronal plasticity and functioning rather than cell duplication.

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