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PHYSIOLOGICAL AGEING: ROLE OF p53 AND PARP-1 TUMOR SUPPRESSORS IN THE REGULATION OF TERMINAL SENESCENCE

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Ageing of organisms is among the most complex processes currently known. Understanding the molecular mechanism of physiological ageing is one of the most essential issues in biology and medicine because it is not possible to predict when and how a certain individual will start ageing. In the past centuries human life expectancies increased. Extension of life span is associated with increased susceptibility to a number of chronic diseases. Insight into the cellular and molecular targets of the ageing process would offer the opportunity to prevent at least some of the destructive processes. In the present paper the involvement of two tumor suppressor proteins: wild-type p53 and poly(ADP-ribose)polymerase-1 (PARP-1) in the regulation of cellular senescence and physiological ageing was reviewed. Moreover, the interaction and cross-talk between p53 and PARP1-1 was discussed.

Key words: *ts p53 mutant; high p53 upregulation; apoptosis; FACS analysis; organismal longevity; life-span; ageing; cellular senescence; PARP-1; cell cycle*

Abbreviations: PARP-1, Poly(ADP-ribose)polymerase-1; ROS, reactive oxygen species

IS AGEING PROGRAMMED?

What causes ageing? Current hypotheses on the problem of physiological ageing of organisms generally fall into one of two categories (1, 2). The first category invokes alterations in gene expression that are either programmed or that are modulated by non-mutational changes in DNA structure; the second invokes extrinsic and intrinsic factors that damage intracellular molecules. There is evidence that cells accumulate damage over a lifetime what induces a gradual

reduction of growth rate and impairment of functions of the cells (3). To what extent these hypotheses overlap or intersect, is a matter of extensive discussion.

A CHOICE BETWEEN LIVE FAST - LIVE LONG? LINK BETWEEN METABOLIC RATE AND LONGEVITY

However, regardless of the hypothesis, the putative link between energy metabolism via mitochondrial oxidative stress and longevity has been an important tool for testing the ideas concerning the causes of ageing of organisms.

Cellular components such as proteins, lipids, and DNA are in constant danger of being damaged by oxidants that are generated either as by-products of normal aerobic metabolism or as second messengers in various signal transduction pathways. Reactive oxygen species (ROS) produced endogenously as a consequence of normal cellular metabolism or derived from external sources result in severe damage of cellular components that lead to degenerative processes and subsequently to ageing. Cells contain a number of antioxidant defenses to reduce fluctuations of intracellular ROS. However, the levels of produced ROS often exceed the cell's intrinsic antioxidant capacity and lead to a condition known as oxidative stress. Mitochondria are primary targets for oxidative damage because they are responsible for metabolizing most of the oxygen taken up by cells. Interestingly, the mitochondrial oxidant production can be lowered by reducing food intake (2). Caloric restriction - reduction of the food intake of animals by 50 to 70%, reliably extended the mean and maximum life-span of several species, including mammalian species. It is thought that this is accomplished primarily by lowering oxidative stress and tissue damage by ROS. Contradictory predictions come from a more recent study (4). The authors demonstrated that within a cohort of mice there is a positive correlation between metabolic intensity and life span. They provided a trustworthy explanation why such a positive correlation might exist: activation of uncoupling proteins may increase the metabolic rate, but decrease the overall production of ROS (4).

ROLE OF p53 IN THE REGULATION OF CELL CYCLE PROGRESSION AND APOPTOSIS

Wt p53 phosphoprotein, the product of a tumor suppressor gene, responds to a variety of cellular and environmental stresses inducing either transient cell cycle arrest or apoptosis (for reviews, see 5-7). Thus, it prevents cancer development. However, enhanced wt p53 activity during a prolonged time is also able to induce a terminal cell cycle block in cultured cells, defined as senescence, as well as organismal ageing (8-12). In normal unstressed cells wt p53 protein is maintained at low levels primarily due to the action of mdm-2 protein, its downstream transcriptional target (13-15). Under stress conditions distinct signaling pathways can be activated that directly target p53 for post-translational modifications and

increase its stability resulting in nuclear accumulation of wt p53 protein (16, 17). Up-regulation and transcriptional activation of p53 protein leads to elevation of cell cycle inhibitors e.g. $p21^{waf1}$ blocking the progression of the cell cycle (18). The induction of a cell cycle block at G_1 and G_2 by p53 provides necessary time for the cell to repair genomic damage before entering the critical stages of DNA synthesis and mitosis. However, in tissues where the stressors generate a severe and irrevocable damage, p53 can initiate apoptosis, thereby, eliminating damaged cells (19, 20). A number of pro-apoptotic factors such as bax, caspase-9, APAF-1 or p53AIP have been shown to be under transcriptional control of wt p53 protein (20). Therefore, depending on the cell type and kind of stressors, wt p53 may initiate apoptosis or promote and accelerate the execution of apoptosis at different stages.

Alternatively, wt p53 may mediate a terminal cell cycle arrest called senescence (9, 11, 12). Senescence observed in cultured cells is irreversible and is accompanied by enhanced p53 activity.

SIGNIFICANCE OF p53 IN ORGANISMAL AGEING

Additionally, to its central role in DNA damage response and cell cycle regulation, p53 was suggested to play an important part in organismal ageing (21). *In vivo* studies aimed to elucidate the role of p53 in organismal ageing and based on manipulation of the *p53* gene have been difficult due to the fact that p53 knock-out mice exhibit genomic instability and are extremely prone to cancer development. Consequently, they usually die early from neoplasia and are therefore not suitable for ageing experiments (22). On the other hand, transgenic mice overexpressing high levels of p53 die at an early embryonic stage due to an enhanced rate of apoptosis resulting in severe developmental defects.

Important insights about the impact of p53 on organismal ageing came from 2 variants of p53 mutant mice. One mouse strain had a truncated *p53* gene, termed *p53 m* that was missing the first 6 exons and only expressed the remaining 5 exons (23-24). The generated p53 protein was truncated and showed a markedly elevated half-life. p53 m was able to increase the transcriptional activation and growth suppression activity of wt p53 protein. This led to a decreased cancer incidence. However, this beneficial effect came at a high price: the induction of accelerated ageing. The mutant $p53^{+/m}$ mice displayed early ageing-associated phenotypes and their life span was reduced by 20 %. Interestingly, onset of premature ageing became obvious only in mice that were older than 12 months. Recently, a short p53 variant has been detected in certain tissues which shows strong similarity to p53 m and also leads to an elevated p53 stability and activity (25).

The other mutant mouse line expressed a temperature-sensitive (ts) p53 with an alanine to valine substitution at position 135 of the protein. Depending on the temperature, the $p53^{135Val}$ mutant protein adopts wild type conformation at 32°C and mutant conformation at 37°C. Interestingly, the mice showed only signs of

premature ageing in tissues which were localized to compartments of the animal (e.g. skin) exposed to the lower ambient temperatures (23). This can be explained by the nature of the p53 protein which adopts wt conformation in the cooler parts of the animal close to the surface whereas it adopts the non functional mutant conformation in the central regions of the animal with elevated temperature.

What is the mechanism by which elevated p53 activity leads to a reduced life span of mammalian organisms? The recent findings clearly suggest that an enhanced p53 activity leads to a higher rate of apoptosis and cell cycle arrest. This in turn seems to exhaust the organism's capability to renew certain tissues, predominantly tissues with a high turn over rate which rely on the capacity of stem cells to replace lost cells. It seems that the inability of an organism to keep up this cellular homeostasis leads to ageing.

However, unexpectedly, the findings of a Spanish group that excess levels of wild-type p53 protect mice against cancer and ageing offer a ray of hope that increased levels of p53 may protect organisms against cancer without the undesirable side effects of premature ageing (26). Garcia-Cao and collaborators generated novel transgenic mice, called "super p53" (26). The "super p53" mice expressed wild-type endogenous p53, and additionally carried one or two copies of a normal *p53* gene, inserted as transgenes in the form of large genomic fragments. The additional p53 gene copies were expressed from their own promoter. This implicates that transgenically expressed p53 was regulated in the same fashion as the endogenous *p53* gene. The "super p53" mice were more sensitive to DNA damage because higher levels of p53 led to enhanced apoptosis and were more efficiently protected from chemically induced cancers. However, in contrast to the predictions from data on mutant p53 $^{+/m}$ animals, these "super p53" mice did not show any signs of accelerated ageing. One of the explanations for the absence of undesirable side effects of premature ageing in "super p53" mice may be that the transgenically espressed p53 was regulated from its endogenous promoter and that therefore this p53 was appropriately regulated. These data provide hope that protection of cells against cancer might be possible by introducing large fragments of DNA containing the p53 tumor suppressor in stem cells.

Taken together these results clearly show that p53 plays a central role in organismal ageing, additionally, to its role in DNA damage response and cell cycle regulation. Nevertheless, very thorough investigations will be necessary to eventually elucidate all the proteins and pathways involved in ageing.

ADDITIONAL FACTORS COOPERATING WITH WT p53

If wt p53 protein plays an essential role in regulating senescence, how might it perform these functions? It seems clear that several additional proteins may regulate the stability and activity of wt p53 protein known to accelerate the process of ageing.

One of the putative candidates is hSIR2 (SIR1), the human homologue of the *S. cerevisiae* Sir2 protein known to be involved in cell ageing (27-29). hSIR2, an NAD-dependent deacetylase binds and deacetylates the p53 protein with specificity for its COOH-terminal Lys382 residue, a modification which has been implicated in transcriptional activation. Sir2 has been shown to enhance longevity when overexpressed (28-29). In contrast, inactivation of Sir2 causes a reduction of the mean life span. A reduction of Sir2 mediated gene silencing with age leads to an increase in the generation of extrachromosomal rDNA circles, which appear to shorten the life span.

Recent reports also show that the histone deacetylases hSIR2 and hDAC1 are negative regulators of ageing in mammals (27). They achieve their anti-aging activity by transcriptional inactivation of p53 and p21 promoters. hSIR2 deacetylates p53 on Lys382, thereby, changing its transcriptional activity. When overexpressed, SIR2 enhances longevity and leads to a decrease in age-related conditions.

POLY(ADP-RIBOSE)POLYMERASE-1 (PARP-1): GUARDIAN OF THE GENE-POOL OR LONGEVITY FACTOR?

PARP-1 is the first and by far best investigated member of the PARP family of protein modifying enzymes. The other members comprise PARP-2 (30-31), PARP-3 (31), Tankyrase 1 (32) and 2 (33-34), vPARP (35) and TiPARP (36). PARP-1 accounts for 80-90 % of the poly(ADP-ribosyl)ation activity within a given cell (37) and is able to form homodimers or heterodimers with PARP-2. Modification of proteins catalyzed by PARP-1 results in the covalent attachment of oligo- or poly(ADP-ribose) (PAR) chains to target proteins (38). Covalently bound PARs change the stability, activity and DNA-binding capabilities of the targets (39-40). Among the proteins covalently modified by PARP-1 are p53, waf-1, XRCC1, DNA ligase III, histones, DNA-polymerase-ε, DNA-PKC, Ku70, NF-κB, XPA, iNOS and telomerase (41). The list of targets already points to an involvement of PARP-1 in DNA-repair and maintenance of genomic integrity. Most importantly, together with XRCC1, DNA-polymerase-β and DNA ligase III, PARP-1 constitutes the protein complex responsible for base excision repair (BER) (42), one of the main pathways involved in the repair of damaged DNA. Interestingly, it was also found that PARP-2 is able to interact with all four members of the BER and that PARP-2 in fact is necessary for this DNA-repair pathway (43).

PARP-1 is a very abundant nuclear protein. Its activity is stimulated up to 500-fold by single- or double-strand DNA breaks. Poly(ADP-ribose) chains do have a short half-life due to the action of poly(ADP-ribose)glycohydrolase (PARG) the enzyme that counteracts the action of PARP-1 and degrades the polymers. Human PARP-1 is an enzyme of 113 kDa and is composed of 3 domains. An N-terminal DNA binding domain with two zinc-fingers and a nuclear localisation sequence, a central automodification domain containing a BRCA1 C-terminal motiv and,

finally, a C-terminal catalytic domain including the PARP signature sequence, the key region defining all members of the PARP family.

As an immediate reaction to DNA-damage, PARP-1 binds to the strand break, is activated and starts to covalently modify a plethora of nuclear proteins. PARP-1 also poly(ADP-ribosyl)ates itself and then leaves the strand breake to allow other repair proteins to access the site of damage. In cases where the damage is too strong, PARP-1 can deplete the cell of NAD+, the substrate for the enzymatic reaction. This also leads to ATP depletion and together with other changes in a severely damaged cell this can initiate apoptosis, the regulated suicide program of cells. In this case PARP-1 can be cleaved by activated caspase-3. Then the DNA-binding domain of PARP remains at the DNA break and does not allow repair enzymes to access the site. A fragment of 89 kDa is cleaved off. The cleaved product of the protein is often used as an indicator for apoptosis. When the damage to the cell is too severe and the pathway of regulated suicide cannot be pursued, cells can also become necrotic.

Knock-out mice deficient in PARP-1 are sensitive to γ-irradiation and to DNA-damage inflicted by genotoxic substances (44-45). Depending on the mouse model, PARP-1 deficient mice do have more or less severe deficiencies in DNA-repair and they also show developmental defects and are prone to some forms of cancer. Actually, it was rather surprising that the knock-out mice did not show more severe deficiencies but it seems clear that there is some redundancy in the functions of PARP-1. Nevertheless, *in vitro* and *in vivo* experiments showed that PARP-1 is a key component for the maintenance of genomic integrity. Interestingly, PARP-1 has been found to have a detrimental effect in very specific situations. This happens after pathological conditions like ischemia-reperfusion, myocardial infarction and inflammation when severe local damage occurs (46). In this setting the apoptotic effect induced by PARP-1 can destroy whole tissues and, subsequently, even lead to the death of the whole organism. This effect is not as pronounced in knock-out mice (47) and it can also be counteracted by administration of PARP-1 inhibitors (48).

A role of PARP-1 in ageing has been proposed due to several highly interesting studies. It was found that a positive correlation between the poly(ADP-ribosyl)ation activity of mononuclear blood cells and the longevity of 13 mammalian species exists (49). Obviously, there was an evolutionary pressure to provide a better protection for the DNA in somatic cells of long-lived species. In another study the assumption that PARP-1 activity might be higher in centenarians than in the average population, was tested (50). For this purpose the poly(ADP-ribosyl)ation capacity of lymphoblastoid cell lines established from peripheral blood was measured. The enzymatic activity in cells derived from centenarians was significantly higher than in control cells. At the molecular level it was so far not possible to assign the differences in activity to any specific polymorphism in the coding region of the PARP-1 gene. Taken together, these observations clearly establish a role for PARP-1 in longevity (51-52).

From an evolutionary point of view it seems clear that PARP-1 has evolved to be one of the guardians of the genome. The fact that some functions of the protein are redundant is a hint that those activities are too important for the cell to rely on one particular protein and not that PARP-1 is easily dispensable by the organism. As the experiments, especially from the knock-out mice show, the fundamental function of PARP-1 can clearly be seen when cells are challenged e.g. by γ-irradiation or genotoxic substances (44-45). In pathological situations where an exhaustive activation of PARP-1 can lead to the local destruction of tissue, a spatially and chronologically clearly defined treatment with a PARP-1 inhibitor might be helpful to prevent damage (48). In terms of evolution, it might be more important in such extreme situations to protect the gene-pool and to risk the health or even life of one organism than to try to repair severely damaged DNA under all circumstances and risk the accumulation of mutations. Nevertheless, the fundamental importance of PARP in DNA-damage repair and protection of the genomic integrity clearly is a positive contribution to longevity.

CROSS TALK BETWEEN p53 AND PARP-1

The stability and nucleocytoplasmic translocation of p53, which are essential for its functional competence, are regulated by multiple factors. The interaction of p53 protein with poly(ADP-ribose) polymerase 1 (PARP-1) represents one of several alternative pathways controlling the stability of wt p53 in unstressed cells.

The role of PARP-1 in the regulation of basal expression of wt p53 protein was demonstrated in two different cell systems. First, the lowered expression (53) or inactivation of PARP-1 by gene disruption resulted in a reduction in the baseline level of wt p53 protein (40). This was attributable to the approximately tenfold shortening of the half-life of the p53 protein (40, 54-55). The reconstitution of PARP-1 deficient mouse cells with the human counterpart abrogated the reduced stability of p53 protein. A second line of evidence revealed that elevated expression of PARP-1 in combination with ts $p53^{135Val}$ mutant increased the stability of wt p53 in transformed primary rat cells (56). The feature of this ts $p53^{135Val}$ mutant is temperature-dependent switching between mutant (at 37°C and 39°C) and wild-type (at 32°C) phenotype (57). Increased wt p53 stability in cells possessing a high level of PARP-1 had functional consequences. It delayed the reentry of G1 arrested cells into the cell cycle (56).

Considering the involvement of PARP-1 in the regulation of the stability of wt p53 protein, the elucidation and characterization of the interaction between both proteins was of fundamental importance.

PARP-1 binds directly to p53 protein and forms tight complexes (56, 58). The complex formation does not depend on the p53 status: both wt and mutant p53 protein bind to PARP-1. Recently, the domains involved in the protein-protein interaction were identified (59). It has been found that the central- and the carboxy-

terminal domains of human p53 were essential for human PARP-1 binding, whereas the amino-terminal part harboring the transactivation domain failed to bind PARP-1 (59). The highest capacity to bind PARP-1 was exhibited by the distal carboxy-terminal fragment of p53 phosphoprotein. On the other hand, amino-terminal and central fragments of PARP-1 were necessary for complex formation with p53. It seems that complex formation between both proteins is regulated by phosphorylation of p53 (60). Dephosphorylated p53 failed to bind to PARP-1 (60). The marked phosphorylation of p53 at Ser392 observed in unstressed mouse cells implicates that the phosphorylated carboxy-terminal part of p53 undergoes complex formation with PARP-1 resulting in masking of the nuclear export signal (NES) and thereby preventing its export (60). The assumption that PARP-1 binding to the carboxy-terminal domain of p53 might regulate its export is consistent with the previous observation that the elimination of PARP-1 in mouse cells by gene disruption reduced only the stability of the regularly spliced form of p53 (40), whereas the alternatively spliced form of p53 was not affected (40). Remarkably, both splice variants of murine p53 differ solely in the distal carboxy-terminal sequence (61). Moreover, exposure of PARP-1 deficient mouse cells to leptomycin B (LMB) induced phosphorylation of p53 at Ser392 which coincided with the onset of accumulation of the p53 protein (54, 60). LMB which covalently modifies CRM1 protein at cysteine 529 (62) impairs the interaction between the NES and CRM1, thereby, efficiently blocking the nuclear export of proteins. This concept is in concordance with the recent results of Zhang and Xiong (63). The authors identified the new NES within the amino-terminus of p53 that seems to cooperate with the carboxy-terminal NES and observed that DNA damage-induced phosphorylation of p53 at Ser15 inhibited the nuclear export of p53. The interaction between p53 and PARP-1 was also of functional significance in the regulation of DNA binding and induction of apoptosis. In transformed primary rat cells overexpressing ts p53 mutant^{135Val}, only mutant p53 protein residing in the cytoplasm was poly(ADP-ribosyl)ated (58). Modification of cytoplasmic p53 protein resulted in an increase of its molecular weight to 64 kD indicating that a poly(ADP-ribose) chain of approximately 20 residues was covalently attached to the p53 protein (58). Moreover, mutant p53 protein sequestered PARP-1 in the cytoplasm. Surprisingly, wt p53^{135Val} protein remained unmodified despite its co-localization with PARP-1 in the nucleus (39). Additional experiments showed that only unbound wt p53 could be modified by PARP-1 (39). After its binding to DNA, wt p53 is no longer a target for covalent attachment of poly(ADP-ribose) chains (39). Interestingly, a link between modification of p53 by ADP-ribose chains and induction of apoptosis was observed in osteosarcoma cells (64). Transient poly(ADP)-ribosylation of p53 protein preceded the onset of spontaneous apoptosis (64).

On the other hand, PARP-1 which contributes to the regulation of the intracellular NAD+ level may directly control the activity of SIR2 and the

transcriptional competence of p53 protein and may additionally regulate the basal level of wt p53 in ageing cells.

Thus, the evidence that hSIR1 and PARP-1 directly modulate p53 activity and stability raise the intriguing concept that they may affect longevity in mammals, at least partially, through a p53-dependent pathway. Mouse models of organismal senescence support the idea that wt p53 may in part regulate the longevity of organisms.

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