Chapter 11

The Role of P53 Exonuclease in Accuracy of DNA Synthesis and Sensitivity to Nucleoside Analogs in Various Compartments of Cells

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Additional information is available at the end of the chapter

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1. Introduction

Genomic DNA is susceptible to a variety of mutagenic processes. The maintenance of the stability of genetic material, which is an important and essential feature of every living organism, depends on an accurate DNA replication [1]. Organisms across all kingdoms have developed diverse and highly efficient repair mechanisms to safeguard the genome from deleterious consequences of various kinds of stresses that might tend to destabilize the integrity of the genome. DNA is constantly being damaged. A low fidelity of DNA synthesis in various compartments of the cells by main replicative DNA polymerases leads to genomic instability (mutator phenotype) [2]. The errors produced during DNA synthesis could result from three fidelity determining processes: a)nucleotide misinsertion into the nascent DNA, b)lack of exonucleolytic proofreading activity, i.e the mechanism to identify and excise incorrect nucleotide incorporated during DNA synthesis, and c)extension of mismatched 3′-termini of DNA [3]. Failure to repair DNA can lead to mutations, genomic instability, chromosomal abnormalities, progression of cancer and premature aging.

Mutator phenotypes (with the potential for cancer progression) have been reported for cells that lack a proofreading 3′→5′ exonuclease activity associated with the DNA polymerase [4]. Certain organisms with a deficiency of exonucleolytic proofreading, have an increased susceptibility to cancer, especially under conditions of stress. Since cancer cells typically have many mutations compared to a non-cancer cell, it was proposed that one of the earliest changes in the development of a cancer cell is a mutation that increases the spontaneous mutation rate [5]. Inactivation of 3′→ 5′ exonuclease activity in the mouse DNA pol δ in nucleus appears to produce replication errors that can drive evolution of a cancer. Mitochon-

drial DNA (mtDNA) alterations have been associated with various human diseases with impaired mitochondrial function [6]. Mitochondrial DNA polymerase γ (pol γ) is responsible for replication of mtDNA and is implicated in all repair processes [7]. Mitochondrial DNA is prone to mutations, since it is localized near the inner mitochondrial membrane in which reactive oxygene species are generated. Additionally, mtDNA lacks histone protection and the highly efficient DNA repair mechanisms [8]. The mutation rate of mtDNA is estimated to be about 20-100-fold higher than that of nuclear DNA [9]. The mutagenic mechanisms were shown to be replication errors caused by misinsertion (as a result of a dNTP excess), or decreased proofreading efficiency [10,11]. The biological importance of the 3′→5′ exonuclease activity of pol γ to mtDNA integrity is illustrated by the fact that mice encoding an exonuclease-deficient form of pol γ have strongly elevated rates of base substitutions in mtDNA and undergo accelerated aging [12].

Virulence, pathogenesis and the ability to develop effective antiretroviral drugs and vaccines are largely dependent on genetic diversity in viruses [13]. Retroviruses are RNA viruses that replicate through a DNA intermediate in a process catalyzed by the viral reverse transcriptase (RT) in cytoplasm [14]. Human immunodeficiency virus type 1 (HIV-1), the etiological agent of AIDS, exhibits exceptionally high mutation frequencies [15]. The accepted explanations for the inaccuracy of HIV-1 RT are the relatively low fidelity of the enzyme during DNA synthesis and the deficiency of intrinsic 3′→5′ exonuclease activity [16-18]. A strong mutator phenotype is also observed for herpes viral DNA polymerase mutants with reduced intrinsic 3′→5′ exonuclease activity [19].

Thus, in various compartments of the cell increased DNA replication accuracy provided by DNA polymerase proofreading activity is an essential activity for the maintenance of genomic integrity for many organisms.

2. Exonucleases in protecting genome stability

The effect of misinsertion of a wrong nucleotide on the polymerase reaction can be either inhibitory, leading to nascent chain termination and primer dissociation or non-inhibitory, leading to mispair extension (resulting in the fixation of either transition or transversion mutations) (Fig 1). Exonucleolytic proofreading of polymerization errors is one of the major determinants of genome stability [20]. The physiological role for the exonucleolytic proofreading has been proposed to be to increase the fidelity of DNA synthesis by excising incorrectly polymerized nucleotides. Following the incorporation of a non-complementary nucleotide at the 3′ end of the primer, exonucleolytic correction can occur by intrinsic exonuclease through intramolecular shuttling of the DNA substrate from the polymerase to the 3′→5′ exonucleolysis active site of the enzyme (*e.g.* pol γ, pol δ and pol ε) [1,4]. However, there are DNA polymerases that do not possess an intrinsic proofreading function, *e.g.* cellular DNA polymerases α and β, retroviral RTs [17,21,22]. Hence, during *in vitro* DNA synthesis by an inaccurate DNA polymerases, following the polymerase dissociation at a mispair, misincorporated nucleotides could be removed by two kinds of an "external" proofreading

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carried out by the 3′→ 5′ exonuclease activity of other DNA polymerase [23] and/or by separate protein serving as a proofreading exonuclease [24,25]. The lack of intrinsic proofreading, combined with delayed chain elongation of mispaired 3'-ends could provide the opportunity for a separate exonuclease to bind to the nascent DNA ends and excise the mispaired nucleotides. Enzymes that contain 3′–5′ exonuclease activities are involved in maintaining genome stability. Proofreading in trans is a very efficient process, which has a potential to allow exonuclease-proficient enzyme/protein to proofread for 3′→ 5′ exonuclease-deficient DNA polymerases. Proteins with intrinsic proofreading activity may be important for both, 3′→5′ exonuclease-deficient and exonuclease-proficient DNA polymerases. The p53 protein is a member of external proteins that by intrinsic 3′→5′ exonuclease activity may serve as proofreader and could be actively involved in DNA repair thereby significantly expanding the role of p53 as a guardian of the genome [26].

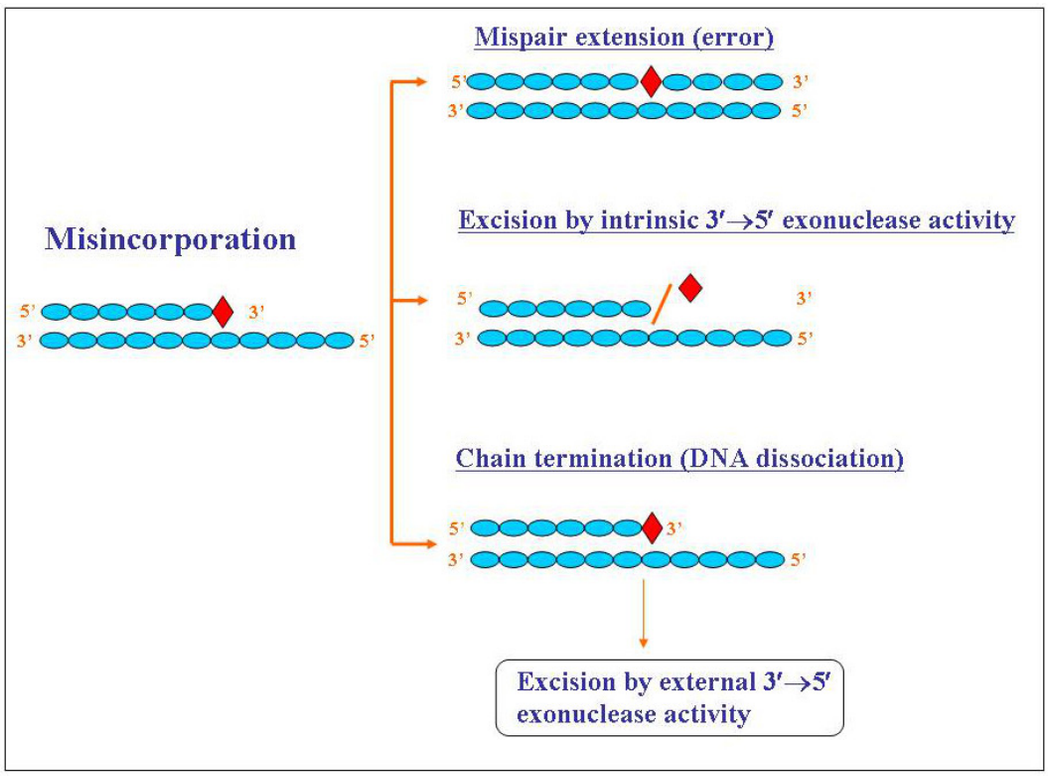

Figure 1. The outcomes of the misincorporation. DNA polymerases following a misincorporation of the wrong nucleotide, can either continue chain elongation beyond the mismatch or remove the mispaired terminus (if a proofreading exonuclease is associated with DNA replication machinery) or block the DNA synthesis by dissociating from the template-primer.

3. p53 and DNA repair

The tumor suppressor protein p53 represents a central factor for the maintenance of genome stability and for the suppression of cancer [27,28]. Under normal conditions within the cell

p53 is present at low levels, but after exposure to various stress signals, the protein is stabilized and functionally activated by a series of post-translational modifications, resulting in p53 accumulation at nuclear and extranuclear sites [29,30]. The cellular level of p53 and the nature of DNA damage can dictate the response of the cell. As p53 is a pleiotropic regulator, it affects many processes. The biological outcomes of p53 functions as a sequence-specific transcription factor include cell cycle arrest, apoptosis or DNA repair [31]. Apparently, cell cycle arrest mediated by p53 in response to DNA damage allows time for the cells to repair DNA. If the cells are unable to repair DNA damage, apoptosis is triggered by a p53-dependent pathway to eliminate the cells that contained damaged DNA. These processes together ensure the integrity of the genome. p53 can affect DNA repair processes through its ability to transactivate genes involved in these processes [28]. Mutations in p53 are the most frequent molecular alterations detected in human cancers. The loss of the functional p53 may be responsible for genetic instability and the development of cancer [32].

Appropriate subcellular localization is critical for regulating function of p53. p53 is actively transported between the nucleus and cytoplasm. Furthermore, p53 translocates to mitochondria. The sub-cellular localization of p53 and the interaction with other cellular or viral proteins plays a central role in the regulation of its various biological activities [26]. p53 may modulate DNA repair through processes, which are independent of its transactivation function. p53 can directly interact with DNA repair related cellular factors including DNA polymerase β, AP endonuclease, Rad 51, and mammalian homologs of the RecQ helicase family and Wrn proteins [33-36]. In addition, full range of various intrinsic biochemical features of the p53 protein support its possible roles in DNA repair. After DNA damage: (a) p53 is able to recognize and bind sites of DNA damage, such as single-stranded (ss) DNA and double-stranded (ds) DNA ends [37,38], (b) p53 catalyzes DNA and RNA strand transfer and promotes the annealing of complementary DNA and RNA single-strands [39,40], (c) p53 binds insertion/deletion mismatches and bulges [41] and (d) it can bind DNA in a non-sequence-specific manner [42]. Evidence suggesting a direct role in DNA repair is supported by observations that (1) p53 increases transcription-coupled nucleotide excision repair [43]; (2) p53, like classical mismatch repair factors, checks the fidelity of homologous recombination processes by specific mismatch recognition [44]; (3) p53 can markedly stimulate base excision repair [33,45]; (4) p53 exhibits 3'→ 5' exonuclease activity and wild-type p53, but not mutant p53, enhanced the replication fidelity of various DNA polymerases in an *in vitro* replication assay, strongly supporting the idea that p53 can act as an exogenous proofreader for the replicases [46,47].

4. Characterization of p53 exonuclease activity

Highly purified p53 protein from different sources displays 3′→ 5′ exonuclease activity. p53 has no associated polymerase activity and catalyzes the excision of nucleotides from DNA exclusively in the 3 ′to 5′direction [46]. This activity is dependent on the presence of Mg^{2+} and is intrinsic to the wtp53, since no exonuclease activity was detected with mutant p53 protein, *e.g.* 273His and 175His mutant p53s. Importantly, the exonuclease activity could be

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reconstituted from SDS gel-purified and urea-renatured p53 protein. While p53 exhibits optimal transactivation as tetramer, it displays exonuclease activity as monomer [48]. Notably, the oligomarization status of p53 may be important in determining whether protein may act as transcription activator (tetramer) or as exonuclease (monomer).

p53 removes 3′-terminal nucleotides from various nucleic acids substrates: ssDNA, dsDNA RNA/DNA template-primer, ssRNA and dsRNA [46-52]. A unique property of p53 is its ability to excise nucleotides non-processively (on DNA <17 nucleotides) and processively (on DNA >17 nucleotides) [48]. The purified wtp53 exhibits all hallmarks of a genuine proofreading activity [49]. First, the protein shows a preference for degradation of ssDNA over dsDNA substrate. Second, on partial duplex structures, the p53 exonuclease activity displays a marked preference for excision of a mismatched versus a correctly paired 3′ terminus, which enables the protein to act as a proofreader. The intrinsic ability of p53 exonuclease to sequentially remove incorrect 3′ terminal nucleotides from DNA strands before primer extension is important for subsequent elongation of primers during error correction and renders the p53 protein essential in DNA replication, repair, and recombination. Third, p53 acts coordinately with the DNA polymerase to enhance the fidelity of DNA synthesis by excision of mismatched nucleotides from the nascent DNA strand.

The proofreading capacity of p53 was observed during ongoing DNA synthesis *in vitro;* p53 exonuclease has a marked impact on the extent of mispair formation and on the extension from specific mispaired termini by DNA polymerase [49]. Recombinant, as well as endogenous wtp53 can proofread for exonuclease-deficient cellular or viral DNA polymerases (*e.g.* DNA polymerase α, DNA polymerase α-primase, HIV-1 RT) and exonuclease-proficient DNA polymerase (*e.g.* pol γ), thus enhancing the accuracy of DNA synthesis by excising incorrectly polymerized nucleotides [53-57]. Apparently, the exonuclease activity of p53, by removal of a mismatched nucleotide incorporated by a DNA polymerase, might provide a biochemical basis for its direct involvement in the correction of replication errors. Notably, the exonuclease activity of p53 must not be restricted to its non-induced state, but might also be exerted by a subclass of p53 after DNA damage when the protein is able to display its full range of possible biochemical activities [26]. Remarkably, p53 exonuclease excises nucleotides from RNA/DNA template-primers, a property which distinguishes it from the large majority of the known exonucleases [50]. The fact that p53 is reactive with both DNA/DNA and RNA/DNA suggests that it may functionally interact with substrates participating in the reverse transcription process during the replication of retroviruses.

p53 is capable of excising 3'-terminal mispaired nucleotides in direct exonuclease assay independent of DNA polymerase; p53 is very active when first binding to a 3′-terminus [49,50].. Some template-primers with terminal mispairs remain unextended by the polymerase. Interestingly, unextended free template-primers (already dissociated from the enzyme following the misinsertion) may be further recognized by other DNA polymerase (*e.g.* HIV-1 RT) molecules and undergo a rebinding process with a subsequent 3'-mismatch extension [58]. The fact that p53 excises terminal nucleotides independent of DNA polymerase [49,55] suggests that the dissociated unextended 3'-mismatch containing template-primer may be recognized and utilized by p53 to remove terminal mispairs generating the correctly base-

paired 3'-termini necessary for continued DNA synthesis [49]. The recognition and binding to 3′ mismatched ends is a prerequisite for the excision of mismatched or damaged nucleotides [42]. Endogenous p53 displays intrinsic 3'-terminal mispaired DNA binding activity. Since p53 binds directly to various 3'-terminal purine:pyrimidine and purine:purine mispairs to an equal extent, it can be considered a general 3'-mismatched DNA binding protein. Intrinsic 3'-terminal mismatched DNA binding capacity of p53 extends the spectrum of DNA damage sites that p53 can recognize and bind. Through the binding p53 participates in damage recognition, which serves as a signal for DNA repair. Thus, the role of p53 in proofreading is two fold — to excise terminal mismatches, but also to prevent extension of mismatched primer ends by DNA polymerase.

p53 intrinsic exonuclease activity, like sequence-specific DNA binding, was mapped to the central conserved core domain of protein, which is the target for most of the missense mutations inactivating the tumor suppressor function of p53 [59]. It is noteworthy that bacterially expressed, i.e., nonphosphorylated, p53 is virtually devoid of sequence-specific DNA binding activity but exerts exonuclease activity [46], pointing to the possibility that the p53 exonuclease activity might be exerted by hypo- or even nonphosphorylated p53. Treatments activating sequence-specific DNA binding of full-length p53 strongly inhibited its exonuclease activity, indicating that p53 exonuclease and sequence-specific DNA binding are distinct features of the p53 core domain, regulated in opposite manners. Apparently, p53 exerts two complementary functions in maintaining the integrity of the genome. After damage different functional subclasses of p53 will exist within the same cell, then the increase of p53 protein levels not only will activate the potential of p53 to transcribe p53 target genes, leading to growth arrest, but will also increase the amount of p53 with a 3'→5' proofreading exonuclease activity. As its basal function in maintaining genetic stability, p53 participates actively in repair processes of endogenous DNA damage and the prevention of mutational events resulting from such damage, through activities not related to sequence-specific DNA binding, specifically through its exonuclease activity [26]. Such p53 then could enhance the accuracy of DNA repair synthesis performed by the error-prone DNA polymerases, e.g. pol α and β. At another level of control, cellular stress activates the functions of p53 generally associated with growth arrest and apoptosis.

Mutant H115N p53, showed markedly reduced exonuclease activity [60]. Surprisingly, purified H115N p53 protein was found to be significantly more potent than wild-type p53 in binding to DNA. Interestingly as well, non-specific DNA binding by the core domain of H115N p53 is superior to that of wild-type p53. Unexpectedly, in contrast to wtp53, H115N p53 was markedly impaired in causing apoptosis when cells were subjected to DNA damage facilitating apoptosis, further supporting the idea that the exonuclease activity and transcriptional activation functions of p53 can be separated. The impact of deficiency of exonuclease activity in p53 is not known. This might be partly due to the observation that tumor derived hot-spot mutants not only fail to function as transcriptional activators but also were reported to be deficient in exonuclease activity. p53 hot spot mutants were categorized into two classes; structural and functional mutants [61]. Since representative members of both classes were defective in exonuclease activity, it is likely that both, structural integri-

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ty of the protein and DNA binding activity are essential for each of these two biochemical functions.

5. p53 exonuclease provides proofreading during DNA synthesis in various compartments of cells

p53 activities are extended to normal and cancer cells and they efficiently contribute to genome stability even in the absence of stresses. p53 is expressed constitutively in the cell and is distributed in the nucleus, cytoplasm and mitochondria of unstressed and stressed cells.

5.1. p53 exonuclease activity in nucleus

The observation that p53 protein is co-located with the DNA replication machinery and may preferentially remove mismatched nucleotides from DNA, suggests a link between p53 and DNA replication fidelity [62]. The localization of p53 in nucleus is essential for its normal function in growth inhibition or induction of apoptosis. The low accuracy of DNA polymerases and imbalance of intracellular dNTP pools are major factors in causing replication errors [3]. The proofreading for such replication errors by the 3′→ 5′ exonuclease activity associated with the DNA replication machinery is extremely important in reduction the occurrence of mutations. DNA polymerase α is lack of proofreading activity and is prone to making replication errors [63]. p53 specifically interacts with DNA polymerase α and has been shown to preferentially excise mismatched nucleotides from DNA and enhance the DNA replication fidelity of DNA polymerase α *in vitro* [47]. The fact that p53 is able to enhance the replication fidelity of pol α *in vitro* suggests that p53 may serve a proofreading function during DNA replication in intact cells.

It is conceivable that cells lacking p53 exonuclease activity can demonstrate high mutation frequency under stress conditions and the mutations should be reduced by introduction of wild type p53 into the cells. Hydroxyurea (HU), an inhibitor of ribonucleotide reductase involved in the *de novo* synthesis of deoxynucleotides, was used to induce dNTP pool imbalance and to cause mutations in the cells due to misincorporation of unpaired deoxynucleotides into DNA [54]. Cells with different states of p53 expression, either endogenously or ectopically, were exposed to HU. The analysis of the rates of HU-induced mutations in H1299 (p53-null) and H460 (wtp53) cells revealed substantially increased mutation rates in H1299 cells. Furthermore, the HU-induced mutation frequency was significantly reduced by introduction of wild type p53 expression vector into the p53-null H1299 cells. Thus, wild type p53 expression was associated with a reduction of mutations caused by replication errors under the stress of dNTP pool imbalance [54]. p53, presumably, may play an important role in reduction mutations caused by misincorporation of unpaired nucleotides. This biological function of p53 in whole cells is consistent with its biochemical activity in preferential removal of mismatched nucleotides from DNA by 3′→ 5′ exonuclease activity and enhancing replication fidelity of DNA polymerase α in vitro. The reported association

of replication error phenotype with p53 mutations in mucosa-associated lymphoid tissue lymphomas is consistent with the proofreading function of p53 [64].

It was shown that in the early steps of cellular transformation process high incidences of mutations occur, which may be due to misinsertion and proofreading deficiency of DNA polymerases [65]. The existence of complex pol-prim- p53 *in vivo*, identified by immonoprecipitation experiments, suggests that p53 might cooperate with DNA polymerase to maintain the genetic information in cells [53]. The functional interaction of DNA polymerase and exonuclease activity was observed with p53/pol-prim complex. p53-containing DNA pol-prim complex excised preferentially a 3′-mispaired primer end over a paired one and replaced it with a correctly paired nucleotide. In contrast, a pol-prim complex containing the hot spot mutant p53R248H did not display exonuclease activity and did not elongate a mispaired 3′-end, indicating that the p53 exonuclease from the p53/pol-prim complex was mandatory for the subsequent elongation of the primer by DNA polymerase. These findings support the view that p53 might fulfill a proofreading function for pol-prim and suggest that the defect in proofreading function of p53 may contribute to genetic instability associated with cancer development and progression.

Notably, the non-genotoxic stress may include a long-lasting, moderate accumulation of p53 in nucleus. In contrast, acute genotoxic stress may induce rapid and transient accumulation of very high levels of p53 with preferential activation of target genes involved in apoptosis. The *in vivo* experiments showed that while expression of low levels of p53 facilitate BER activity, higher levels reduced it and instead induced apoptosis, suggesting that p53 mediating various activities are correlated with the levels of the p53 protein in the cells [66]. In this regard, it is possible that the accumulation of p53 in nucleus allows the protein to function in several ways: as a regulator of transcription, as a facilitator of BER and as an exonucleolytic proofreader. Moreover, there is a possibility that both transcription-independent pathways act in synergy thereby amplifying the potency of involvement of p53 in DNA repair. The presence of p53 was demonstrated in different nuclear compartments and suggested that the p53 population not engaged in transcriptional regulation could exert functions other than induction of growth arrest or apoptosis and directly participate in processes of repair via its various biochemical activities [26].

5.2. p53 exonuclease activity in cytoplasm

p53 is retained in the cytoplasm during part of the normal cell cycle. Wild-type p53 occurs in cytoplasm in a subset of human tumor cells such as breast cancers, colon cancers and neuroblastoma [67-69]. Notably, cytoplasmic sequestration of p53 in tumor cells (that do not have mutated p53), besides structural mutation and the functional inactivation of wtp53, was suggested to be an important mechanism in abolishing p53 function and in tumorigenesis [67,70]. Shuttling between nucleus and cytoplasm not only regulates protein localization, but also often impacts on protein function. Analyses of various cell lines (MCF-7 human breast cancer cells – expressing high levels of wtp53 in nucleus, LCC2-subclone derived from MCF-7 cells-expressing high levels of wtp53 in cytoplasm, MDA cells-expressing high levels of mutant p53 or H1299-p53-null cells), demonstrated that the cytoplasmic extracts of

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non-stressed LCC2 cells, exert high level of 3′→ 5′ exonuclease activity [55,56]. Interestingly, the 3′→ 5′ exonuclease in the cytoplasmic fraction from LCC2 cells displays identical biochemical functions characteristic for recombinant wtp53 [56]: 1)it removes 3′-terminal nucleotides from various nucleic acid substrates: ssDNA, dsDNA, and RNA/DNA template-primers, 2)it hydrolyzes ssDNA in preference to dsDNA and RNA/DNA template-primers, 3)it shows a marked preference for excision of a mismatched vs correctly paired 3′ terminus with RNA/DNA and DNA/DNA substrates, 4)it exerts the preferential excision of purine-purine (transversion) mispairs over purine-pyrimidine (transition) mispairs, 5)it excises nucleotides from various nucleic acid substrates independently from DNA polymerase, 6) it fulfils the requirements for proofreading function; acts coordinately with the exonuclease-deficient viral (*e.g*. MLV RT, HIV-1 RT) and cellular DNA polymerases – (*e.g*. pol α and β) (unpublished results) to enhance the fidelity of DNA synthesis by excision of mismatched nucleotides from the nascent DNA strand [55,56]. It is noteworthy, that in non-stressed cells p53 is constitutively expressed and exists in transcriptional inert state. Thus, the protein exerts exonuclease activity independently of p53 functions in transcription.

Interestingly, p53 protein in cytoplasmic extracts of MCF-7 cells displays a relatively high level of 3′→ 5′ exonuclease activity in comparison to nuclear lysates of LCC2 cells [55]. The biochemical difference between the p53 in nuclear and cytoplasmic compartments raises questions whether nuclear p53 loses exonuclease function of cytoplasmic p53 or acquires an additional functions (*e.g.* efficient sequence-specific DNA binding and transactivation). The disparity in expression of p53 exonuclease activity may be attributable to the different post-transcriptional events: a)post-translational modifications (*e.g*. phosphorylation, acetylation) may regulate the ability of p53 to serve as an exonuclease in the nucleus and in the cytoplasm; b) The alteration of p53 protein conformation from mutant (in cytoplasm) to wild-type (in nucleus) may be responsible for low level of exonuclease activity in nucleus [71]. c) the interaction of p53 with other proteins and/or DNA polymerases may affect on expression its various biochemical activities.

5.3. p53 exonuclease activity in mitochondria

Mitochondrial DNA mutations can arise from different sources, including errors made by pol γ, the enzyme that replicates the mitochondrial genome. The mitochondrial pol γ belongs to a family A DNA polymerase, and as observed for other family A DNA polymerases, this enzyme excises the terminal nucleotide at a much slower rate than observed for the potent 3′→5′ exonuclease-proficient T4 DNA polymerase [72]. The mutagenic mechanisms were shown to be replication errors caused by incorporation of wrong nucleotide (as a result of a dNTP excess), or decreased proofreading efficiency. Furthermore, a potentially important source of replication infidelity is damage due to reactive oxygen species. Among several known oxidized dNTPs, one that is particularly common and potentially highly mutagenic is 8-oxo-7,8-dihydro-2'-deoxyguanosine (8-oxodG) [73]. Incorrect 8-oxo-dGTP-A base pairing can lead to A-T to C-G transversions if the incorporated 8-oxo-dGMP escapes proofreading and any subsequent repair. pol γ, was demonstrated to stably misincorporate 8-oxo-dGTP opposite template adenine in a complete DNA synthesis reaction *in vitro* [74]. Low-

fidelity DNA synthesis in mitochondria was observed despite the presence of the intact proofreading exonuclease, thus indicating that the 8-oxo-GMP-A mismatch was not efficiently proofread.

A certain fraction of p53 translocates to mitochondria. Mitochondrial localization of p53 was observed in both stressed and non-stressed cells [75,76], where p53 was shown to physically and functionally interact with both, the mtDNA and pol γ in response to mtDNA damage induced by exogenous and endogenous insults [77]. p53 is localized in mitochondria to the inside face of the inner membrane i.e, in matrix, the compartment in which mtDNA is located [57,77]. The functional cooperation of p53 and pol γ during DNA replication was studied using the mitochondrial fraction of p53-null H1299 cells, as the source of pol γ [57]. p53 affected the accuracy of DNA replication by promoting excision of misincorporated nucleotides which increased in the presence of either added recombinant wild-type p53, or endogenous p53 provided by the cytosolic extracts from H1299 cells over-expressing wild-type p53, but not from cells expressing the exonuclease-deficient mutant p53–R175H. Endogenous p53 in mitochondrial extracts of HCT116 (p53+/+) cells had increased exonuclease activity compared with that from HCT116(p53-/-) cells and adding exogenous p53 complemented the HCT116(p53-/-) mitochondrial extract mediated mispair excision. Furthermore, nucleotide misincorporation was reduced in the mitochondrial extracts of HCT116 (p53+/+) cells compared with that of HCT116(p53-/-) cells. Irradiation-induced mitochondrial translocation of endogenous p53 in HCT116(p53+/+) cells correlated with the enhancement of error-correction activities. This evidence strongly supports a direct role of p53 in mitochondria providing exonuclease activity for DNA repair required for error-repair pathway [57]. Therefore, p53 not only serves as guardian of the nuclear genome but also of the mitochondrial genome.

p53 interacts physically with mtDNA and pol γ in response to mtDNA damage induced by endogenous insults including oxidative stress. The intrinsic exonuclease activity of pol γ does not efficiently proofread 8-oxodG misinserted opposite adenine [78]. Once 8-oxodGMP is incorporated opposite adenine by pol γ it is preferentially extended rather than excised, which increases its mutagenic potential. Interestingly, human mitochondrial single-stranded DNA binding protein (HmtSSB) was identified as a novel protein-binding partner of p53 in mitochondria. HmtSSB enhances intrinsic 3'→5' exonuclease activity of p53, particularly in hydrolysing 8-oxodG present at 3'-end of DNA, suggesting that p53 is directly involved in DNA repair within mitochondria during oxidative stress.

5.4. p53 exonucleolytic proofreading may affect the mutation spectra of DNA polymerase

The accuracy of DNA synthesis reflects complex interactions between the parameters of the catalytic "triad" involved in DNA polymerization: DNA polymerase, the nature of the mispair and proofreading exonucleases (fidelity–enhancing accessory component) [1,22]. DNA polymerase catalyzed both, misinsertion and mismatch extension reactions and the extent of proofreading depend on the type of the mispair, and the influence of surrounding sequences of the template. Various cellular and viral DNA polymerases share common pattern of mispair formation and extension: namely, purine-pyrimidine mispair (*e.g.* A:C mispair) is easily

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inserted and more efficiently extended than the purine-purine (*e.g.* A:A or A:G mispair) or pyrimidine-pyrimidine mispair (e.g. C:C or C:T) [79,80]. Thus, the general trend of mispair extension is A:C>A:A>A:G. Interestingly, p53 displays variation in excision of mismatched base pairs; the protein exhibits preferential excision of purine-purine transversion mispairs (*e.g.* A:A, A:G) over purine-pyrimidine transition mispairs (*e.g.* A:C, G:T) [49]. Apparently, the variances in the extension and excision spectrum generated are different for these two reactions. The mispair excision pattern (A:G>A:A>A:C) detected with p53 is an interesting observation with respect to the contribution of proofreading to fidelity; it is compatible with the mispair extension specificity obtained with this particular sequence studied.

The importance of the mispair extension efficiency as a fidelity parameter was illustrated by the fact that an increased forward polymerization capacity for transition A:C mispair, as compared to transversion A:G mispair, overcomes the ability of p53 exonuclease activity in cytoplasm to excise nucleotide mispairs under the similar exonuclease to polymerase ratios [56]. Indeed, the purine-pyrimidine mispair A:C (the most easily formed and extended) is less efficiently excised and the purine-purine A:A and A:G mispairs (less efficiently formed and extended), are rather efficiently excised. Therefore, it is conceivable that the structural feature that make the mismatched terminus a poor substrate for elongation (polymerization) is a good substrate for degradation (exonucleolysis) [81].

Remarkably, p53 exonuclease displays the same pattern of mispair excision specificity with RNA/DNA substrate observed with DNA/DNA template-primer [50]. The mispair excision pattern obtained with identical RNA and DNA sequences indicates that the p53 exonuclease activity for different mismatches is dependent upon the nature of the mispair. The same relative order obtained during replication in extracts and in reconstituted reaction, demonstrates the reproducibility of the observations, thus indicating that this specificity reflects the proofreading potential of human replication apparatus.

Among the base substitution mutations, 80% are transitions and 20% are transversions [13]. An interesting observation is that external proofreading activity in the replication apparatus may preferentially correct some of the misincorporated beses to reduce the rates of transversions. p53 may affect the mutation spectra of DNA polymerase (*e.g.* HIV-1 RT) by acting as an external proofreader [56]. Indeed, HIV-1 RT gains significant benefit from proofreading with A:G mispair (about 15-fold decrease in A:G mispair extension) as compared with A:C mipair (about 2.8-fold decrease), since the enzyme has difficulty extending from this particular mispair. Furthermore, the low mispair extension capacity implies that DNA polymerase has a substantially higher probability of dissociation from the transversion mispairs. Dissociation would prevent mutation fixation, because the mispairs would be subject to removal by the external p53 proofreading activity. Thus, base substitutions that produce transversions may be decreased in the presence of p53, indicating that the mutation spectra might be generated through the actions of RT (DNA polymerase) and cytoplasmic p53 (exonuclease).

The mutational spectra and error rates during DNA synthesis probably depends on the composition and position of mispair, since each position provides a new set of protein-DNA contacts. There is the possibility that neighboring nucleotide sequence may influence recognition of the altered geometry of the mismatch by the enzyme/protein responsible for the

proofreading or/and proofreading efficiency. The fact that p53 binds mismatch in the two different sequence contexts tested, indicates that the recognition and binding of 3'-terminally mismatched DNA substrates by p53 might be independent of the sequence context. Since formation of exonuclease complexes requires "melting" of the terminal three base pairs at the primer end, the nature of mismatch at the primer end and the A+T- or G+C-richness of the primer terminus affect the rate for formation of exonuclease complexes. It has been proposed that high A-T content of the primer terminus compared with high G-C content increases excision rates by assisting the strand separation process. Hence, a comprehensive study of various DNA substrates are needed to determine the effect of local sequence context on the substrate specificity of the p53 exonuclease and whether p53 could take advantage of A+T richness to prepare duplex DNA for the hydrolysis reaction.

6. Intermolecular pathway of proofreading by p53 exonuclease

Following the incorporation of wrong nucleotide the DNA polymerase stalling and the kinetic delay allows error correction by intramolecular or/and intermolecular pathway [3]. The intramolecular pathway entails "movement" of the primer end from the polymerase to the intrinsic exonuclease active site (without dissociating from the DNA). In this way, DNA polymerase functions as a "self-correcting" enzyme that removes its own polymerization errors as it moves along the DNA. The intermolecular proofreading may occur when misinsertion is followed by polymerase dissociation from the mismatched template-primer, leaving the 3′ terminal mispair accessible to the external exonuclease for binding and error correction. In both cases, the efficiency of editing misinserted nucleotides by a 3′→ 5′ exonuclease would be directly dependent on the DNA polymerase capacity to extend from a misincorporated nucleotide.

Polymerase dissociation at a mispair is an important consideration for proofreading for both exonuclease-deficient and exonuclease-proficient polymerases, thus allowing error correction by a separate 3′→ 5′ exonuclease. The formation of exonuclease complex with the primer end of the mismatched DNA participates in error correction during DNA synthesis [42]. A functional interaction between the p53 exonuclease and DNA polymerase activities was observed. The 3'-terminal mismatched DNA binding and exonuclease activities of p53 are implicated in the recognition and excision step of mismatch repair. It is conceivable that the binding of p53 to mismatched DNA and preferential excision of mismatched nucleotides may be a relevant event in the biological function of the protein in DNA repair. The experiments in which DNA polymerases, either exonuclease-deficient (*e.g.* HIV-1 RT) or exonuclease-proficient (*e.g.* pol γ) were tested for the extension of preformed 3′- terminally mispaired substrates in the presence of p53 (conditions that mimic a situation of intermolecular editing), points to a mechanism of mismatch correction prior to polymerization [56,57]. Under DNA replication conditions the un-extended 3'-terminal mismatched DNA produced following misincorporation, dissociated from the DNA polymerase and was recognized by p53 (Fig.2). Upon excision of the mispair, p53 exonuclease dissociates and the corrected pri-

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mer could be transferred to the polymerase and undergo a rebinding process by the DNA polymerase with a subsequent DNA polymerization.

It is important to note, that DNA polymerase could gain enormous benefit from proofreading even from a relatively weak exonuclease, if the polymerase has difficulty extending from a particular mispair [20]. Exonuclease has a dramatic impact on the accuracy of polymerase by preventing the occurrence of base substitutions during continues DNA replication. All that is required is discrimination against extension from a mispair within the polymerase active site.

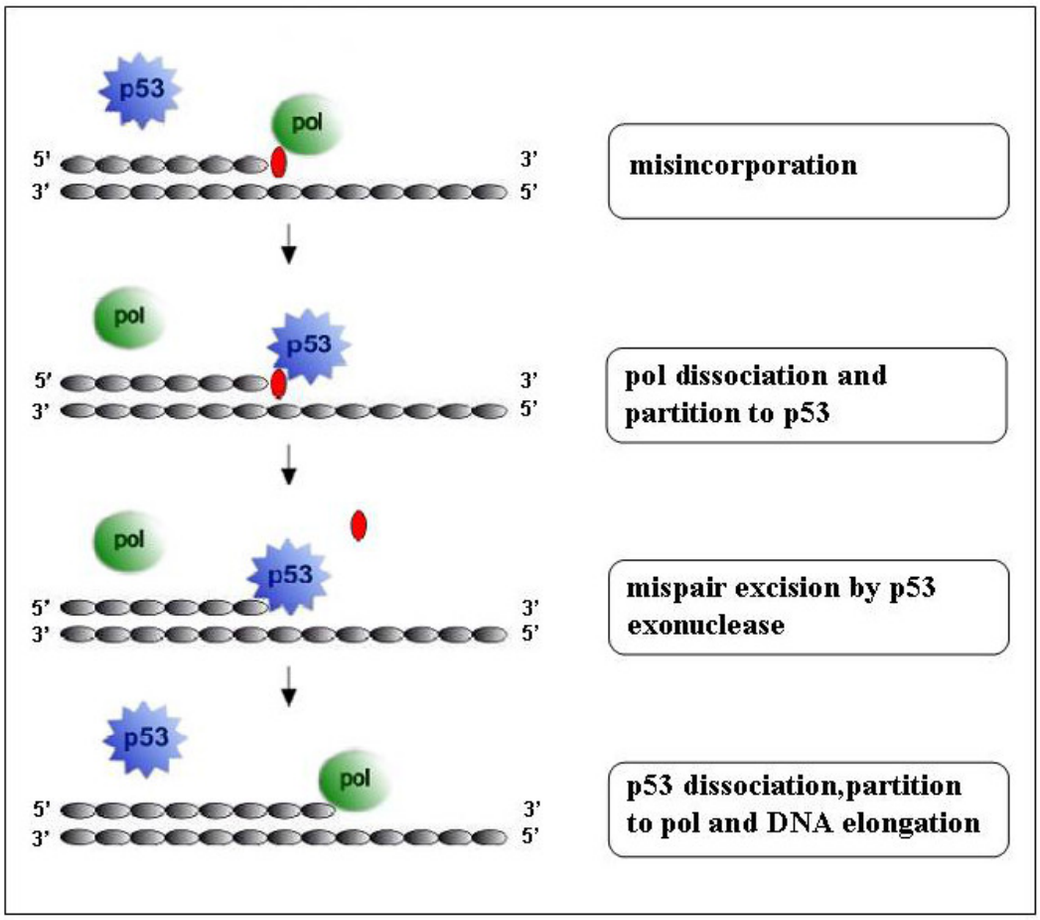

Figure 2. Model for error-correction by p53. The incorporation of wrong nucleotide ( ) into DNA results in DNA polymerase (pol) dissociation from the template-primer, leaving the 3'-terminal mispair accessible to the p53. Upon excision of the mispair, the p53 dissociates thus allowing the DNA polymerase to re-associate with the correct 3'-terminus and resume DNA synthesis.

7. Hallmarks of proofreading and p53 exonuclease activity

Two variables might affect the efficiency of excision from the mispair [1]. First, one hallmark of proofreading is the "next-nucleotide effect". Increased proofreading at the expense of

DNA replication is observed at low concentrations of dNTPs, a condition which prevents error production during replication *in vivo* by antimutator DNA polymerases. The enhancement of the extent of polymerizing activity at the expense of proofreading activity can be achieved by the presence of high concentrations of dNTPs and dNTP pool imbalances; both conditions are mutagenic. Increasing the concentration of the next correct nucleotide to be incorporated following the mispair enhances the probability of mismatch extension, thereby decreasing proofreading efficiency. Increased polymerizing activity reduces proofreading even in the presence of a fully functional exonuclease activity. Since a decrease in accuracy of DNA synthesis with increasing next correct dNTP concentration is a well-established phenomena of proofreading, the observed dependence of fidelity of DNA synthesis by exonuclease-deficient DNA polymerase *e.g.* HIV-1 RT on next nucleotide concentration, implies that the 3′→ 5′ exonuclease of p53 in cytoplasm might be effective in eliminating polymerase-catalyzed base-substitution errors [56]. This effect supports a coordinated action of the p53-exonuclease in cytoplasm with HIV-1 RT during DNA synthesis.

Second, the polymerase/exonuclease ratio serves as an important enzymatic "marker' of polymerase fidelity [1]. Exonucleolytic proofreading is a major determinant of replication fidelity. The balance between the DNA polymerizing and 3′→ 5′ exonuclease reactions usually affects the overall accuracy of DNA synthesis to ensure optimal DNA replication efficiency and to prevent excessive DNA degradation of correctly synthesized DNA. The high ratio of exonuclease to polymerase at the constant dNTP concentrations may increase the fidelity of DNA synthesis.

Cellular responses to DNA damage include repair processes that act coordinately prior to, during and after DNA replication, to maintain genomic stability. The accuracy of DNA synthesis might respond to alterations in composition of replication complex. p53 function may be regulated by controlling where the protein is in the cell. Various stress conditions may trigger distinct signaling pathways in controlling p53 nucleo–cytoplasmic-mitochondrial translocation, thus contributing to heterogeneity of p53-dependent responses. The identification of the p53 protein in cytoplasm or in mitochondria that may enhance the fidelity of DNA polymerase suggests that the accuracy of DNA synthesis by the enzyme may respond to alterations in composition of replication complex. Most probably, p53 in nucleus or cytoplasm or mitochondria might have a transient interaction with replication complex. Therefore, the DNA synthesis in each compartment may be dynamic process with p53 component binding and dissociating the DNA polymerization complex during dsDNA synthesis, thus affecting the polymerase/exonuclease (p53) ratio. The change in the ratio of DNA polymerase vs exonuclease (p53) could be achieved through a reduction in polymerization efficiency of DNA polymerase due to mutations, or from over-expression of p53, or through p53 gene induction (increase in p53 concentration) or p53 targeting (increase in local nuclear or cytoplasmic or mitochondrial concentration). p53 is able to excise 3'-terminal nucleotides during the ongoing DNA synthesis *i.e.* coupled with DNA polymerization and following direct binding to template-primer *i.e.* independent of DNA polymerase, thus increasing the potency of involvement of the protein during the DNA replication by acting as an external proofreader in each cellular compartment. Consequently, the presence of p53 in nucleus/

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cytoplasm/mitochondria, by carrying these properties, may be relevant to the accuracy of DNA synthesis by various DNA polymerases.

8. Excision of nucleoside analogs from DNA by p53 protein

Many nucleoside analogs (NAs), potent anti-cancer and antiviral drug compounds, include a variety of purine and pyrimidine nucleoside derivatives which may compete with physiological nucleosides. Nucleoside analogs, clinically active in cancer chemotherapy (*e.g*. Ara-C, in the treatment of hematological malignancies, or gemcitabine-dFdC, against a variety of solid tumors) and in treatment of virus infections (*e.g.* 3′-azido-2,3,-deoxythymidine-AZT, 2,3-dideoxycitidine-ddC, inhibitors of HIV-1 RT), are incorporated into DNA and cause cell death or inhibition of viral replication [82,83]. These drugs are intracellularly converted to the active analog trophosphates, which are then incorporated into replicating DNA. The incorporated NA, structurally mimicing a mismatched nucleotide at the 3'-terminus, blocks further extension of the nascent strand (chain termination) and causes stalling of replication forks with higher probability to the dissociation of the enzyme from template-primer. The high toxicity of dideoxynucleotide compounds may be caused by high rates of incorporation of the NA into mtDNA and the persistence of these analogs in mtDNA due to inefficient excision. Analysis of the processes involved in the removal of NAs and repair of stalled forks is important to better understand the mechanisms that spare toxicity to these drugs.

Proofreading exonuclease activity is capable of removing wrong nucleotides from DNA, providing a mechanism that potentially causes drug resistance. In general, the amount of NAs presented at the DNA termini depends on the efficiency of the incorporation of the compounds by DNA polymerases and on the rate of excision by 3′→5′ exonucleases [83]. The excision of the incorporated NA from the 3′-end of DNA by exonucleases may decrease their potential for chain termination and may be viewed as a potential cellular mechanism of resistance to anti-viral drugs or anti-cancer NAs. The role of p53 exonuclease in maintaining genomic stability in mammalian cells is particularly relevant with respect to the development of anticancer and antiviral therapies.

Many anticancer agents induce cellular cytotoxicity by causing DNA damage. Cells developed several repair mechanisms to facilitate the excision of incorporated NAs. The cytotoxic activity of gemcitabine (2'2'-difluorodeoxycitidine, dFdC) was strongly correlated with the amount of dFdCMP incorporated into cellular DNA. Interestingly, dFdCTP incorporation by human DNA polymerase α results in “masked termination” of DNA synthesis, where following a single dFdCTP incorporation into DNA, the primer is extended by only one additional dNTP before polymerization is inhibited [84]. The p53 protein recognizes dFdCMP-DNA in whole cells, as evidenced by the fact that p53 protein rapidly accumulated in the nuclei of the gemcitabine treated ML-1 cells [85]. Although, the excision of the dFdCMP at the penultimate position from the 3′-end of the DNA was slower than the excision of matched or mismatched nucleotides in whole cells with wtp53 (ML-1) and not detectable in CEM cells harboring mutant p53. ML-1 cells were more sensitive to the

cytotoxic effect of the drugs compared to the p53-null or mutant cells. Transfection of p53-null cells with wild-type p53 expression vector enhanced the sensitivity of the cells to gemcitabine. Taken together, these authors concluded that recognition of the incorporated NAs in DNA by wild-type p53 did not confer resistance to gemcitabine, but may have facilitated the apoptotic cell death process. It was reported that treatment with gemcitabine resulted in an increased production of DNA-dependent protein kinase (DNA-PK) and p53 complex in nucleus, that interacts with the gemcitabine-containing DNA [86]. DNA-PK and p53 sensor complex may serve as a mechanism to activate the pro-apoptosis function of p53. Apparently, the prolonged existence of the NA-stalled DNA end induced the kinase activity, which subsequently phosphorylated p53 and activated the downstream pathways leading to apoptosis.

Remarkably, p53 present in complex with DNA-PK exhibited 3′→5′ exonuclease activity with mismatched DNA, however the active p53 was unable of excising efficiently the incorporated drug from NA-DNA construct containing gemcitabine at the penultimate site and a matched pair at the 3′-end [86]. It should be noted, that the specific effects of gemcitabine exposure appeared to vary depending on the duration of treatment and upon the cell line. The drug-induced apoptosis were further compared in two lines derived from the MCF-7 cells: MN-1 cells with wild-type p53 and MDD2 cells containing mutant p53 [87]. The MDD2 cells were significantly more resistant to gemcitabine induced cytotoxicity than the MN-1 cells. Unexpectedly, MDD2 cells accumulated more gemcitabine than MN-1 cells, with higher incorporation into nucleic acids. The activation of gemcitabine to its phosphorylated form was similar in both cell lines and it was suggested that the absence of 3′→5′ exonuclease activity in the mutant p53 cell line accounted for the enhanced incorporation into nucleic acids. The presence of a dysfunctional p53, presumably, allows the cells that accumulate DNA damage to continue proliferating. It should be pointed out, that wild-type p53 in ML-1 cells removed the purine nucleoside analog fludarabine (F-ara-A) more efficiently than gemcitabine [85]. Further studies are needed to assess the role of p53 in cellular response to various anti-cancer purine and pyrimidine NA-induced DNA damage.

HIV-1 RT readily utilizes many NAs and the incorporation of nucleoside RT inhibitors (NRTIs) into the 3′-end of viral DNA leads to chain termination of viral DNA synthesis in cytoplasm [88]. The ability of p53 exonuclease activity to excise NA from DNA was studied. A decrease in incorporation of the NA (*e.g.* ddTTP or ddATP) into DNA by HIV-1 RT was shown during both RNA-dependent and DNA-dependent DNA polymerization reactions in the presence of either purified recombinant p53 or endogenous protein provided by cytoplasmic fraction of LCC2 cells [89]. Furthermore, p53 in the cytoplasm was able to excise the incorporated 3′-terminal NAs, although less efficiently than the matched or mismatched nucleotides; longer incubation times were required for excision of the terminally incorporated analogs. In control experiments, no reduction in incorporation of either ddTTP or ddATP was observed in the presence of cytoplasmic fraction of H1299 (p53-null) cells. These data suggest that p53 in cytoplasm may act as an external proofreader for NA incorporation and confer cellular resistance mechanism to the anti-viral compounds.

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Acquired mitochondrial toxicity occurs as a consequence of incorporation of anti-cancer or anti-viral NA into mtDNA and/or inhibition of mtDNA replication [90,91]. NRTIs, in addition to the target viral polymerase in cytoplasm (antiviral activity), can be incorporated into a mtDNA by pol γ, leading to termination of mtDNA synthesis and mitochondrial dysfunction (host toxicity). Mitochondrial toxicity may be caused by termination of the growing nascent DNA strand after incorporation of the NRTIs into mtDNA or by inhibition of pol γ exonucleolytic proofreading [90,91]. DNA synthesis/repair proceeding in nucleus-free mitochondria, relies upon a preassembled DNA replication machinery of pol γ and multiple proteins to maintain mtDNA integrity. p53 in mitochondria may functionally interact with pol γ, thus providing a proofreading function during mtDNA replication for excision of NAs [92]. Indeed, increased excision of the incorporated NAs from DNA was detected with H1299mit in the presence of recombinant or endogenous wild-type p53 but not exonuclease-deficient mutant p53-R175H: Mitochondrion-localized elevation of p53 following the IR-stress stimuli correlates with the low incorporation of NA. The fact that p53 localizes to the mitochondria and interacts with mtDNA and pol γ, taken together with observations that the presence of p53 (provided by recombinant or endogenous p53) reduces the amount of incorporation of NA in H1299mit, suggests that p53 may potentially participate in NA excision. p53 in mitochondria probably have a transient interaction with replication complex; the DNA synthesis may be dynamic process with p53 component binding and dissociating the polymerization complex during DNA synthesis, thus affecting the polymerase (pol γ)/exonuclease(p53) ratio. Consequently, the decrease in the ratio of pol γ/p53 due to the increase in local p53 concentration in mitochondria, may enhance the proofreading efficiency and excision of NA by external p53. Knowledge of the mechanism of inhibition of pol γ may be utilized to obtain selectivity for HIV-1 RT over pol γ. The removal of the incorporated NRTI by p53 exonuclease, indicates that the presence of the cellular component-p53 in mitochondria may be important in defining the cytotoxicity of NRTIs toward mitochondrial replication, thus affecting risk-benefit approach (NRTI toxicity versus viral inhibition).

Although dFdC is not a chain terminator, the extension of a dFdCMP-terminated primer is 25-fold slower than the extension of a canonical DNA primer in mitochondria. Moreover, the primer 3′-dFdCMP was excised with a 50-fold slower rate than the matched 3′-dCMP. Given that mtDNA repair is limited and inefficient [93], persistence of dFdCMP within mtDNA is predicted to be likely. The toxicological profile of gemcitabine resembles that of many other anti-viral nucleoside analogs and frequently mimics the symptoms of heritable mitochondrial defects. The mitochondria may be able to remove chain-terminating nucleoside analogs and resume normal mtDNA replication, but nucleoside analogs that do not chain terminate, and therefore can become part of the mitochondrial genome, may exert long term toxicity [85]. pol γ was able to extend a DNA primer containing 3′-dFdCMP although with decreased nucleotide incorporation efficiency at the first two downstream positions. p53 is able to remove the incorporated anti-cancer drug arabinosylcytosine (Ara-C) (pyrimidine analog) from DNA incorporated by pol γ in mitochondrial fraction of p53-null cells [92]. The binding and removal of chemically active anti-cancer and anti-viral NAs from DNA by p53 may lead to either drug resistance or activation of p53 pro-apoptotic functions (Fig.3).

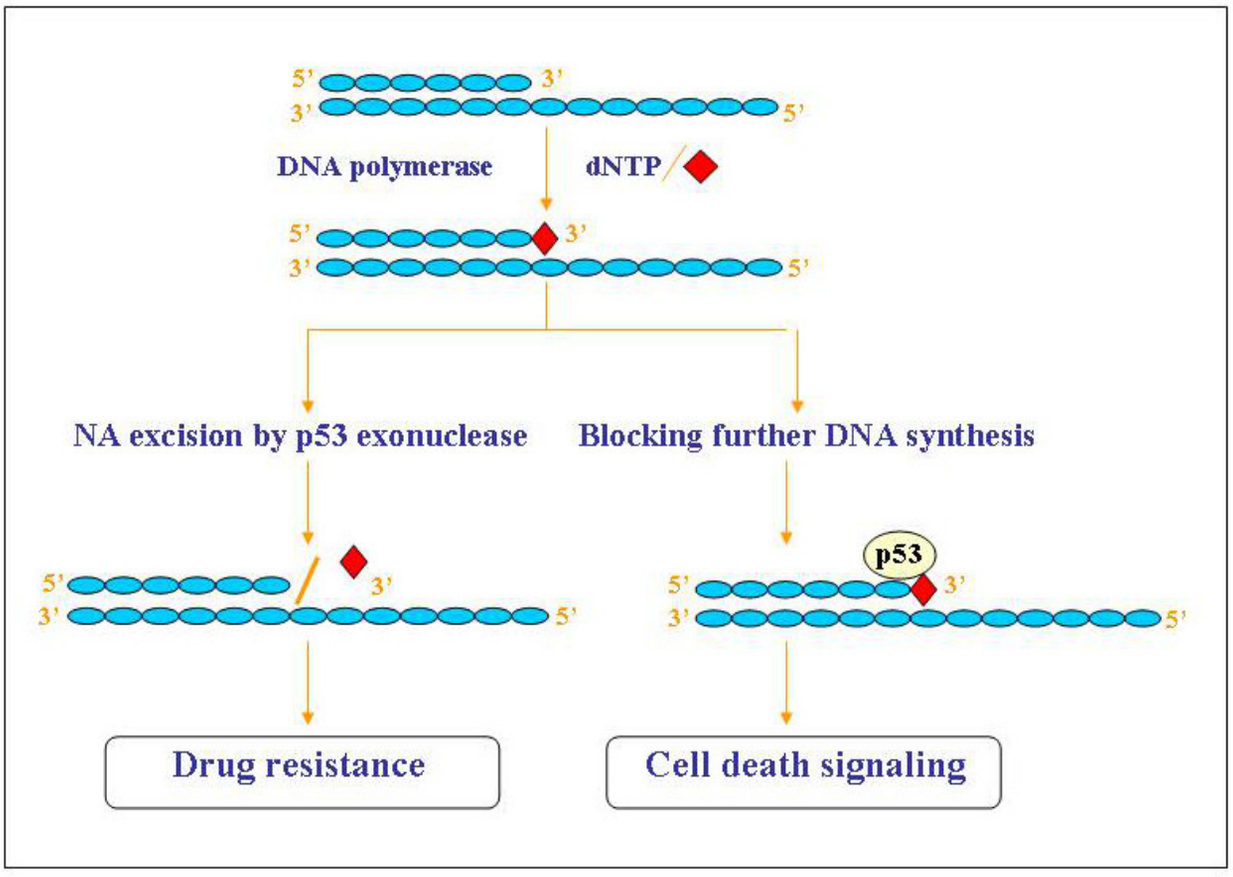

Figure 3. The potential functions of p53 in response to nucleoside analog-induced DNA damage. The p53 protein, following the recognition and preferential binding to the drug-containing DNA could display two different functions: the removal of the incorporated NA from DNA, thus conferring the resistance to the drugs, or may serve as a mechanism to activate the pro-apoptosis function of p53 and trigger the cell death program.

p53 is a multifunctional protein with positive and negative effects. In general, drug resistance that occurs in cancer chemotherapy and antiviral therapy is a negative event that will decrease the efficacy of the treatment. The behavior of p53 exonuclease probably depends on the sub-cellular localization of the p53, local concentration, nature of NA (purine, pyrimidine), position of the NA (3'-terminal NA, analog residue at the penultimate position and nature of the subsequent correct nucleotide) and on the local DNA sequence composition. The recognition and removal of NA from drug-containing DNAs by p53 exonuclease activity in various compartments of the cell may play a role in decreasing drug activity, leading to various biological outcomes: 1)the excision of the incorporated NA from DNA in nucleus may confer resistance to the drugs (negative effect) [85]; 2)the removal of the NA by p53 from DNA incorporated by HIV-1 RT in cytoplasm may confer resistance to the drugs by non-viral mechanism (negative effect) [89] and 3)the excision of NAs from mitochondrial DNA may decrease the potential for chain termination and host toxicity (positive effect) [92]. Apparently, the presence of p53 in mitochondria may be important, since the excision of the mispair and NA by p53 is favorite event for mitochondrial function.

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9. Conclusions and perspectives

Nature has devised multiple strategies to safeguard the genetic information and developed intricate repair mechanisms and pathways to reverse an array of different DNA lesions, including mismatches. An accessory proofreading exonuclease would be critical for the removal of the mispairs and therefore, for the maintenance of genomic integrity. The high incidence of mutations may be due to misinsertion and proofreading deficiency of DNA polymerases [65]. Mammalian cells have evolved several repair mechanisms for the maintenance of genomic integrity to prevent the fixation of genetic damage induced by endogenous and exogenous mutagens [3]. Cells may have several 3′→5′ exonucleases to preserve genomic integrity during DNA synthesis. Under conditions where the activity of one exonuclease is inactivated, the function of another exonuclease might be important for correcting errors produced during DNA replication. p53 was shown to be an example of accessory protein that may enhance the fidelity of DNA synthesis by exonuclease-deficient DNA polymerase, *e.g.* HIV-1 RT [56] and exonuclease-proficient DNA polymerase, *e.g.* pol γ [57] in various compartments of the cell: nucleus, cytoplasm and mitochondria. The preferential excision of mismatched nucleotides from the replicating DNA strand by p53, implies that this cellular error-correction pathway may compensate for a lack of effective proofreading of DNA polymerase induced replication errors. In addition, the proofreading activity of p53 may limit the tranversion mutations, indicating that p53 may affect the mutation spectra of DNA polymerase by acting as an external proofreader. The mutagenic capacity of a low fidelity DNA polymerase will be decreased through increase in exonuclease concentration or exonuclease targeting (increase in local p53 concentration).

p53 plays a pivotal role in the regulation of cell fate determination in response to a variety of cellular stresses. p53 may exert the functional heterogeneity in its non-induced and in its activated state. Furthermore, p53 is able to elicit a spectrum of different biological effective pathways in nucleus, cytoplasm and mitochondria. The increase of p53 protein levels will increases the amount of p53 with a 3′→5′ exonuclease activity. Hence, it is of interest to elucidate 3′→ 5′ exonuclease activity nucleus, cytoplasm and mitochondria of the cells with activated p53 induced by drug treatments (in the absence of DNA damage) or following UV irradiation (in the presence of DNA damage).

The role of p53 is particularly relevant with respect to the development of anticancer and antiviral therapies. The potency of NAs is dependent upon their incorporation at the 3′ ends of replicating DNA. However, clinical drug resistance limits the efficacy of these compounds. Cells have evolved several repair mechanisms to facilitate the excision of misincorporated nucleotides or nucleoside analogs. Uncovering the mechanisms, which are responsible for DNA repair of NA-induced DNA damage will have therapeutic value. The stress induced activation of p53 that occurs during cancer chemotherapy has negative and positive effects. The p53 protein is able to remove incorporated NA. Therapeutic strategies based on p53 are particularly interesting because they exploit the cancer cell's intrinsic genome instability and predisposition to cell death-apoptosis. p53 may remove incorporated therapeutic NAs from DNA or trigger apoptosis. The knowledge regarding functions of p53

in genome integrity and cancer evolution may facilitate drug screening and better design of therapeutic approaches.

10. Future directions

The functional interaction between p53 and DNA polymerase may have important consequences for the maintenance of genomic integrity and pose significant challenges to the development of p53-targeting cancer therapies. Mutant p53 can be classified as a loss-of-function or gain-of-function protein depending on the type of mutation [27,28]. Characterization of exonuclease-deficient H115N mutant p53 revealed that although exonuclease-mutant H115N p53 can induce cell cycle arrest more efficiently than wild-type p53, its ability to produce apoptosis in DNA damaged cells is markedly impaired [60]. Does exonuclease-mutant p53 promote mismatch genetic instabilities? What is the ultimate phenotypic result of this genomic instability? Is it truly contributing to the increased proliferation, seen in tumors of mutp53 mice, and can these results be extended to human tumors? In order to answer these questions, more studies must be conducted on the biology of various mutant p53's and their interaction with the factors involved in DNA repair and apoptosis. Characterizing the instability phenotype of cells after perturbing these interactions will lead to a better understanding of the main causes of mutant p53-mediated genomic instabilities, which might also be point mutant-specific. p53 have a dual role in response to therapy, as exonuclease that by excision of incorporated anti-cancer drugs may confer resistance to drugs or as mediator of cell death induced by chemotherapy [85]. These features could serve as a template for the development of p53-targeting cancer therapies.

A major focus in the future would be to characterize the cellular and biological functions of p53 in mitochondria in response to various stresses. There are many missing points about the biological roles of p53 in mitochondria that still remain to be identified. How p53 can be imported into mitochondria? Whether p53 determines the percent of mutated mtDNA (heteroplasmy in a cell)? Uncovering the mechanisms by which pol γ-mediated mtDNA mutations and depletion are manifested in tissues in the absence and presence of p53 is the next step in understanding causes for mtDNA –related diseases. Understanding how p53 can be imported into mitochondria, will be important and could contribute towards the design of new therapies for cancer and other diseases.

The control of the viral mutation rate could be a viable anti-retroviral strategy. Still more work needs to be done in order to understand the molecular mechanisms involved in controlling fidelity not only at a molecular level (*i.e.*, intrinsic RT fidelity), but also related to the cytoplasmic p53 protein that can modulate the viral mutation rate and affect the incorporation of NRTIs into viral DNA. New understandings of the sub-cellular localization of p53, its role in the fidelity of proviral DNA synthesis in cytoplasm and drug resistance, therefore, may have broad implications for cellular and molecular biology as well as medicine. It may form the basis for new strategies in targeted antiviral therapy that focus on the sub-cellular context of p53 in cells.

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Depletion and mutation of mitochondrial DNA during chronic NRTI therapy may lead to cellular respiratory dysfunction and release of reactive oxidative species, resulting in cellular damage [91]. Future NRTIs should provide higher specificity for HIV-RT and lower incorporation by pol γ to minimize mitochondrial toxicity. Whether the effective targeting of p53 in mitochondria may result in decrease of mitochondrial toxicity in response to conventional anti-viral therapies? Further studies are needed to elucidate if p53, by error-correction functions in mitochondria, can decrease mitochondrial toxicity.

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References

[1] Echols H, Goodman MF. (1991) Fidelity mechanisms in DNA replication. Ann. Rev. Biochem. 60: 477-511.

[2] Reha-Krantz. (2010) DNA polymerase proofreading: Multiple roles maintain genome stability. BBA 1804: 1049-1063.

[3] McElhinny SAN, Pavlov Y, Kunkek T. (2006) Evidence for extrinsic exonucleolytic proofreading. Cell Cycle 5: 958-962.

[4] Shevelev IV, Hubscher U. (2002) The 3′→5′ exonucleases. Nat.Rev. 3: 1-12.

[5] Jackson AL, Loeb LA. (1998) The mutation rate and cancer. Genetics 148: 1483-1490.

[6] Copeland WC, Ponamarev MV, Nguyen D, Kunkel TA, Longley MJ. (2003)Mutations in DNA polymerase gamma cause error-prone DNA synthesis in human disorders. Acta Biochim Pol. 50:155-167.

[7] Kaguni LS. (2004) DNA polymerase γ, the mitochondrial replicase. Ann. Rev. Biochem. 73: 293-320.

[8] Singh KK. (2004) Mitochondria damage checkpoint in apoptosis and genome stability. FEMS Yeast Res.5: 127-132.

[9] Pesole G, Gissi C, De Chirico A, Saccone C. (1999) Nucleotide substitution rate of mammalian mitochondrial genomes. J Mol Evol 48: 427-434.

[10] Johnson A, Johnson K. (2001a) Fidelity of nucleotide incorporation by human mitochondrial DNA polymerase. J Biol. Chem. 276: 38090-38106.

[11] Johnson A, Johnson K. (2001b) Exonuclease proofreading by human mitochondrial DNA polymerase. J Biol. Chem. 276: 38097-38107.

[12] Trifunovic A, Wredenberg A, Falkrnberg M, Spelbrink JN, Rovio AT, Bruder CE, et al. (2004) Somatic mtDNA mutations cause aging phenotypes without affecting reactive oxygen species production. Nature 429: 417-423.

[13] Svarovskaya ES, Cheslock SR, Zhang W, Hu W, Pathak VK (2003) Retrovira mutation rates and reverse transcriptase fidelity. Front. Biosci.8: d117-d134.

[14] Katz R, Skalka AM. (1990) Generation of diversity in retroviruses. Ann. Rev. Genet. 24: 409-445.

[15] Menéndez-Arias L. (2009) Mutation rates and intrinsic fidelity of retroviral reverse transcriptases. Viruses. 1: 1137–1165.

[16] Perrino FW, Preston BD, Sandell LL, Loeb LA. (1989) Extension of mismatched 3′ termini of DNA is a major determinant of the infidelity of human immunodeficiency virus type 1 reverse transcriptase. Proc. Natl. Acad. Sci. USA 86: 8343-8347.

[17] Bakhanashvili M, Hizi A (1992) Fidelity of the reverse transcriptase of human immunodeficiency virus type. FEBS Lett. 306: 151-156.

[18] Bakhanashvili M, Hizi A. (1993) Fidelity of DNA synthesis exhibited in vitro by the reverse transcriptase of the lentivirus equine infectious anemia virus. Biochemistry 32: 7559-7567.

[19] Tian W, Hwang YT, Hwang CBC. (2008) The enhanced DNA replication fidelity of a mutant herpes simplex virus type 1 DNA polymerase is mediated by an improved nucleotide selectivity and reduced mismatch extension ability. J Virology 82: 8937-8941.

[20] Kunkel T. (1988) Exonucleolytic proofreading. Cell 53: 837-840.

[21] Brutlag D, Kornberg A. (1972) Enzymatic synthesis of deoxyribonucleic acid 36. A proofreading function for the 3′ leads to 5′ exonuclease activity in deoxyribonucleic acid polymerases. J. Biol. Chem. 247: 241-248.

[22] Hubscher U, Maga G, Spadari S. (2002) Eukaryotic DNA polymerases. Ann. Rev. Biochem. 71: 133-163.

[23] Joyce C.M. (1989) How DNA travels between the separate polymerase and 3′→5′ exonuclease sites of DNA polymerase I (Klenow fragment). J. Biol. Chem. 264: 858-866.

[24] Perrino FW, Loeb LA. (1990) Hydrolysis of 3′ -terminal mispairs in vitro by the 3′→5′exonuclease of DNA polymerase δ permits subsequent extension by DNA polymerase α. Biochemistry 29: 5226-5231.

[25] Maki H, Kornberg A. (1987) Proofreading by DNA polymerase III of Escherichia coli depends on cooperative interaction of the polymerase and exonuclease subunits. Proc. Natl. Acad. Sci. USA 84: 4389-4392.

[26] Albrechtsen N, Dornreiter L, Grosse F, Kim E, Wiesmuller L, Deppert W (1999) Maintenance of genomic integrity by p53: complementary roles for activated and non-activated p53. Oncogene 18: 7706-7717.

[27] Oren M (1999) Regulation of the p53 tumor suppressor protein. J Biol. Chem. 274: 36031-36034.

[28] Vousden KH, Prives C (2009) Blinded by the Light: The Growing Complexity of p53. Cell 137: 413-431.

[29] Soussi T (1995) The p53 tumor suppressor gene: from molecular biology to clinical investigation. In Molecular genetics of cancer (Cowell, J.K., ed.), p135-178, Bios. Scientific, Oxford, UK.

[30] Taira N, Yoshoda K. (2012) Post-translational modifications of p53 tumor suppressor: determinants of its functional targets. Histol Histopathol. 27: 437-443.

[31] Levine AJ. (1997) P53, the cellular gatekeeper for growth and division. Cell 88: 323-331.

[32] Freed-Pastor WA, Prives C. (2012) Mutant p53: one name, many proteins. Genes. Dev. 26: 1268-1286.

[33] Zhou J, Ahn J, Wilson SH, Prives C. (2001) A role for p53 in base excision repair. EMBO J. 20: 914–923.

[34] Gaiddon C, Moorthy NC, Prives C. (1999)Ref-1 regulates the transactivation and pro-apoptotic functions of p53 in vivo. EMBO J. 18: 5609–5621.

[35] Linke SP, Sengupta S, Khabie N, Jeffries BA, Buchhop S, Miska S, et al. (2003) p53 interacts with hRAD51 and hRAD54, and directly modulates homologous recombination. Cancer Res. 63: 2596–2605.

[36] Yang Q, Zhang R, Wang XW, Spillare EA, Linke SP, Subramanian D, et al. (2002) The processing of Holliday junctions by BLM and WRN helicases is regulated by p53. J Biol. Chem. 277: 31980–31987.

[37] Kern SE, Kinzler KW, Baker SJ, Nigro JM, Rotter V, Levine AJ, Friedman P, Prives C. Vogelstein B. (1991) Mutant p53 binds DNA abnormally. Oncogene 6: 131-136.

[38] Steinmeyer K, Deppert W. (1988) DNA binding properties of murine p53. Oncogene 3: 501-507.

[39] Bakalkin G, Yakovleva T, Selivanova G, Magnusson KP, Szekely L, Kiseleva E, Klein G, Terenius L, Wiman KG. (1994) p53 binds single-stranded DNA endsand catalyzes DNA renaturation and strand transfer. Proc. Natl. Acad. Sci. USA 91: 413-417.

[40] Oberosler P, Hloch P, Rammsperger U, Stahl H. (1993) p53-catalyzed annealing of complementary single-stranded nucleic acids. EMBO J 12: 2389-2396.

[41] Lee S, Elenbaas B, Levine A, Griffith J (1995) p53 and its 14kDa C-terminal domain recognize primary DNA damage in the form of insertion/deletion mismatches. Cell 81: 1013-1020.

[42] Bakhanashvili M, Hizi A, Rahav G. (2010) The interaction of p53 with 3'-terminal mismatched DNA. Cell Cycle 9, 1380-1389.

[43] Hwang BJ, Ford J.M, Hanawalt PC, Chu G. (1999) Expression of the p48 xeroderma pigmentosum gene is p53-dependent and is involved in global genomic repair. Proc. Natl. Acad. Sci. USA, 96: 424-428.

[44] Dudenhoffer C, Rohaly G, Will K, Deppert W, Wiesmullar L. (1998) Specific mismatch recognition in heteroduplex intermediates by p53 suggests a role in fidelity control of homologous recombination. Mol.Cell.Biol. 18: 5332-5342.

[45] Offer H, Wolkowicz R, Matas D, Blumenstein S, Livneh Z, Rotter V. (1999) Direct involvement of p53 in the base excision repair pathway of the DNA repair machinery. FEBS Lett. 450: 197-204.

[46] Mummenbrauer T, Janus F, Muller B, Wiesmuller L, Deppert W, Gross F. (1996) p53 protein exhibits 3′→5′ exonuclease activity. Cell 85: 1089-1099.

[47] Huang P. (1998) Excision of mismatched nucleotides from DNA: a potential mechanism for enhancing DNA replication fidelity by the wild-type p53 protein. Oncogene 17: 261-270.

[48] Skalski V, Lin Z, Choi BY, Brown KR. (2000) Substrate specificity of the p53-associated 3′→5′ exonuclease. Oncogene 19: 3321-3329.

[49] Bakhanashvili M. (2001) Exonucleolytic proofreading by p53 protein. Eur.J Biochem. 268: 2047-2054.

[50] Bakhanashvili M. (2001) p53 enhances the fidelity of DNA synthesis by human immunodeficiency virus type 1 reverse transcriptase. Oncogene 20: 7635-7644.

[51] Bakhanashvili M, Gedelovich R, Grinberg S, Rahav G. (2008) Exonucleolytic degradation of RNA by the tumor suppression protein p53 in cytoplasm. J Molec. Medicine 86: 75-88.

[52] Grinberg S, Teiblum G, Rahav G, Bakhanashvili M. (2010) p53 in cytoplasm exerts 3′→5′ exonuclease activity with dsRNA. Cell cycle 9: 2442-2455.

[53] Melle C, Nasheuer H. (2002) Physical and functional interactions of the tumor suppressor protein p53 and DNA polymerase α-primase. Nucleic Acids Res. 30: 1493-1499.

[54] Ballal K, Zhang W, Mukhopadyay T, Huang P.(2002) Suppression of mismatched mutation by p53: a mechanism guarding genomic integrity. J. Mol. Med. 80: 25-32.

[55] Lilling G, Novitsky E, Sidi Y, Bakhanashvili M. (2003) p53-associated 3′→5′ exonuclease activity in nuclear and cytoplasmic compartments of the cells. Oncogene 22, 233-245.

[56] Bakhanashvili M, Novitsky E, Lilling G, Rahav G. (2004) p53 in cytoplasm may enhance the accuracy of DNA synthesis by human immunodeficiency virus type 1 reverse transcriptase. Oncogene 23: 6890-6899.

[57] Bakhanashvili M. Grinberg S, Bonda E, Simon AJ, Moshitch-Moshkovitz S, Rahav G. (2008) p53 in mitochondria enhances the accuracy of DNA synthesis. Cell Death Diff. 15: 1865-1874.

[58] Bakhanashvili M, Hizi A. (1996) The interaction of the reverse transcriptase of humanimmunodeficiency virus type 1 with 3′-terminally mispaired DNA. Arch. Bioch. Bioph. 334: 89-96.

[59] Janus F, Albrechtsen N, Knippschild U, Wiesmuller L, Grosse F, Deppert W. (1999) Different regulation of the p53 core domain activities 3′ to 5′ exonuclease and sequence-specific DNA binding. Mol. Cell. Biol. 19: 2155-2168.

[60] Ahn J, Poyurovsky MV, Baptiste N, Beckerman R, Cain C, Mattia M, et al. (2009) Dissection of the sequence-specific DNA binding and exonuclease activities reveals a superactive yet apoptotically impaired mutant p53 protein. Cell Cycle 8: 1603-1615.

[61] Cho Y, Gorina S, Jeffrey PD, Pavletich NP. (1994) Crystal structure of a p53 tumor suppressor-DNA complex: understanding tumorigenic mutations. Science 265: 346–355.

[62] Cox LS, Hupp T, Midgley CA, Lane DP (1995) A direct effect of activated human p53 on DNA replication. EMBO J 14: 2099-2105.

[63] Syvaoja J, Suomensaari S, Nishida C, Goldsmith JS, Chui GS, Jain S, Linn S. (1990) DNA polymerases alpha, delta, and epsilon: three distinct enzymes from Hela cells. Proc. Natl Acad. Sci. USA 87: 6664-6668.

[64] Peng H, Chen G, Du M, Singh N,Isaacson PG, Pan L. (1996) Replication error phenotype and p53 gene mutation in lymphoma of mucosa associated lymphoid tissue. Am.J Pathol. 148: 643-648.

[65] Stoler DL, Chen N, Basik M, Kahlenberg M, Rodriguez-Bigas MS, Petrelli NJ, Anderson GR. (1999) The onset and extent of genomic instability in sporadic colorectal tumor progression. Proc. Natl. Acad. Sci. USA. 96: 15121-15126.

[66] Offer H, Milyavsky M, Erez N, Matus D, Zurer I, Harris CC, Rotter V. (2001) Structural and functional involvement of p53 in BER in vitro and in vivo. Oncogene 20: 581-589.

[67] Stenmark-Askmalm M, Stal O, Sullivan S, Ferraud L, Sun XF, Carstensen J, Nordenskjold B. (1994) Cellular accumulation of p53 protein: an independent prognostic factor in stage II breast cancer. Eur. J Cancer 30A: 175-180.

[68] Moll UM, LaOuglia M, Benard, Riou G. (1995) Wild-type p53 protein undergoes cytoplasmic sequestration in undifferentiated neuroblastomas but not in differentiated tumors. Proc. Natl. Acad. Sci. US, 92: 4407-4411.

[69] Bosari S, Viale G, Roncalli M, Graziani D, Borsani G, Lee AK, Coggi G. (1995) p53 gene mutations, p53 protein accumulation and compartmentalization in colorectal adenocarcinoma. Am. J Pathol. 147: 790-798.

[70] Sun XF, Cartensen J.M, Zhang H, Stal O, Wingren S, Hatschek T, Nordenskjold B. (1992) Prognostic significance of cytoplasmic p53 oncoprotein in colorectal adenocarcinoma. Lancet 340: 1369-1373.

[71] Gaitonde SV, Riley JR, Qiao D, Martinez JD. (2000) Conformational phenotype of p53 is linked to nuclear translocation. Oncogene 19: 4042-4049.

[72] Braithwaite DK, Ito J (1993) Compilation, alighnment and phylogenetic relationships of DNA Polymerases. Nucleic Acids Res. 21: 787-802.

[73] Loft S, Poulsen HE. (1999) Markers of oxidative damage to DNA: antioxidants and molecular damage. Methods Enzymol. 300: 166-184.

[74] Katafuchi A, Nohmi T. (2010) DNA polymerases involved in the incorporation of oxidized nucleotides into DNA: their efficiency and template base preference. Mutat Res. 703: 24-31.

[75] Marchenko ND, Zaika A, Moll UM. (2000) Death signal-induced localization of p53 protein to mitochondria. A potential role in apoptotic signaling. J. Biol. Chem. 275: 16202-16212.

[76] Mahyar-Roemer M, Fritzsche C, Wagner S, Laue M, Roemer K. (2004) Mitochondrial p53 levels parallel total p53 levels independent of stress response in human colorectal carcinoma and glioblastoma cells. Oncogene 23: 6226-6236.

[77] Achanta G, Sasaki R, Feng L, Carew JS, Lu W, Pelicano H, et al. (2005) Novel role of p53 in maintaining mitochondrial genetic stability through interaction with DNA pol γ. EMBO J 24: 3482-3492.

[78] Wong TS, Rajagopalan S, Townsley FM, Freund SM, Petrovich M, Loakes D, Fersht AR. (2009) Physical and functional interactions between human mitochondrial single-stranded DNA binding protein and tumor suppressor p53. Nucleic Acids Res. 37: 568-581.

[79] Mendelman LV, Petruska JS, Goodman MF. (2009) Base mispair extension kinetics. Comparison of DNA polymerase alpha and reverse transcriptase. J. Biol. Chem. 265:2338- 2346.

[80] Perrino FW, Loeb LA. (1989) Proofreading by the ε subunit of Escherichia coli DNA polymerase III increases the fidelity of calf thymus DNA polymerase α. Proc. Natl. Acad. Sci. USA 86: 3085-3088.

[81] Sloane DL, Goodman MF, Echols H. (1988) The fidelity of base selection by the polymerase subunit of DNA polymerase III holoenzyme. Nucleic Acid Res. 16: 6465-6475.

[82] Keating MJ. (1997) In: Nucleoside Analogs in Cancer Therapy. Cheson BD. Keating, Plunkett W. (eds). Marcel Dekker, Inc., New York, pp201-226.

[83] Sluis-Cremer N, Arion D, Parniak MA. (2000) Molecular mechanisms of HIV-1 resistance to nucleoside reverse transcriptase inhibitors (NRTIs). Cell. Mol. Life Sci. 57: 1408-1422.

[84] Zhou Y, Achanta G, Pelicano H, Gadhi V, Plunkett W, Huang P. (2002) Action of (E)-2′-Deoxy-2′-(fluoromethylene) cytidine on DNA metabolism: incorporation, excision and cellular response. Mol. Pharmacology 61: 222-229.

[85] Feng L, Achanta G, Pelicano H, Zhang W, Plunkett W, Huang P. (2000) Role of p53 in cellular response to anticancer nucleoside analog-induced DNA damage. Int. J Molec Medicine 5: 597-604.

[86] Achanta G, Pelicano H, Feng L, Plunkett W, Huang P.(2001) Interaction of p53 and DNA-PK in response to nucleoside analogues: potential role as a sensor complex for DNA damage. Cancer Res 61: 8723-8729.

[87] Galmarini CM, Clarke ML, Falette N, Puisieux A, Mackey JR, Dumontet C. (2002) Expression of a non-functional p53 affects the sensitivity of cancer cells to gemcitabine. Int. J Cancer. 97: 439-445.

[88] Sluis-Cremer N, Arion D, Parniak MA. (2000) Molecular mechanisms of HIV-1 resistance to nucleoside reverse transcriptase inhibitors (NRTIs). Cell. Mol. Life Sci. 57: 1408-1422.

[89] Bakhanashvili M, Novitsky E, Rubinstein E, Levy I, Rahav G. (2005) Excision of nucleoside analogs from DNA by p53 protein, a potential cellular mechanism of resistance to inhibitors of human immunodeficiency virus type 1 reverse transcriptase. Antimic. Agents and Chem. 49:1576-1579.

[90] Fowler JD, Brown JA, Johnson KA, Suo Z. (2008) Kinetic investigation of the inhibitory effect of gemcitabine on DNA polymerization catalyzed by human mitochondrial DNA polymerase. J Biol. Chem. 283: 15339-15348.

[91] Lewis W, Day BJ, Copeland WC. (2003) Mitochondrial toxicity of NRTI antiviral drugs: an integrated cellular perspectives. Nature Reviews 2: 812-822.

[92] Bakhanashvili M, Grinberg S, Bonda E, Rahav G. (2009) Excision of nucleoside analogs in mitochondria by p53 protein. AIDS 23: 779-788.

[93] Ewald B, Sampath D, Plunkett W. (2008) Nucleoside amalogs: molecular mechanisms signaling cell death, Oncogene 27: 6522-6537.

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