Chapter

The Role p53 Protein in DNA Repair

Bakhanashvili Mary

Abstract

The tumor suppressor p53 protein controls cell cycle and plays a vital role in preserving DNA integrity. p53 is activated by varied stress signals and the distribution of p53 between the different subcellular compartments depends on the cellular stress milieu. DNA repair pathways protect cells from damage that can lead to DNA breaks. The multi-functional p53 protein promotes DNA repair both directly and indirectly through multiple mechanisms; it accomplishes multi-compartmental functions by either numerous p53-controlled proteins or by its inherent biochemical activities. Accumulating evidence supports the contribution of p53 in the maintenance of the genomic integrity and in various steps of the DNA damage response, through its translocation into nucleus and mitochondria. p53 may also be utilized by viral polymerases in cytoplasm to maintain genomic integrity of viruses, thus expanding the role of p53 as a 'guardian of the genome'. We summarize recent findings highlighting roles of p53 in DNA repair.

Keywords: p53, DNA repair, mitochondrial DNA, viral DNA

1. Introduction

Humans are persistently exposed to various chemical and physical agents that have the potential to damage genomic DNA, such as, irradiation (IR), ultraviolet (UV) light, reactive oxygen species (ROS), et cetera [1]. The integrity and survival of a cell is critically dependent on genome stability and mammalian cells have established multiple pathways to repair different types of target DNA lesions to safeguard the genome from deleterious consequences of various kinds of stresses [2]. The significance of the DNA repair in the protection of genomic stability is highlighted by the fact that many proteins/factors involved have been preserved through evolution [3].

DNA damage, induced by endogenous and exogenous agents, is a common event and must undergo a variety of DNA damage repair in order to ensure the faithful transfer of genetic information during cell division [3]. Four main DNA polymerases are involved with nuclear DNA replication: DNA polymerase α, β, δ and ε [1] (**Figure 1**). DNA repair pathways, which are also recognized as guardians of the genome, protect cells from numerous damages leading to DNA breaks [4]. Failure to restore DNA lesions or inappropriate repair of DNA damage give rise to genomic instability, which is a hallmark of cancer. Remarkably, mild and massive DNA damage are differentially integrated into the cellular signaling networks and, in consequence, provoke different cell fate decisions. After mild damage, the cellular response is cell cycle arrest, DNA repair, and cell survival, whereas severe damage,

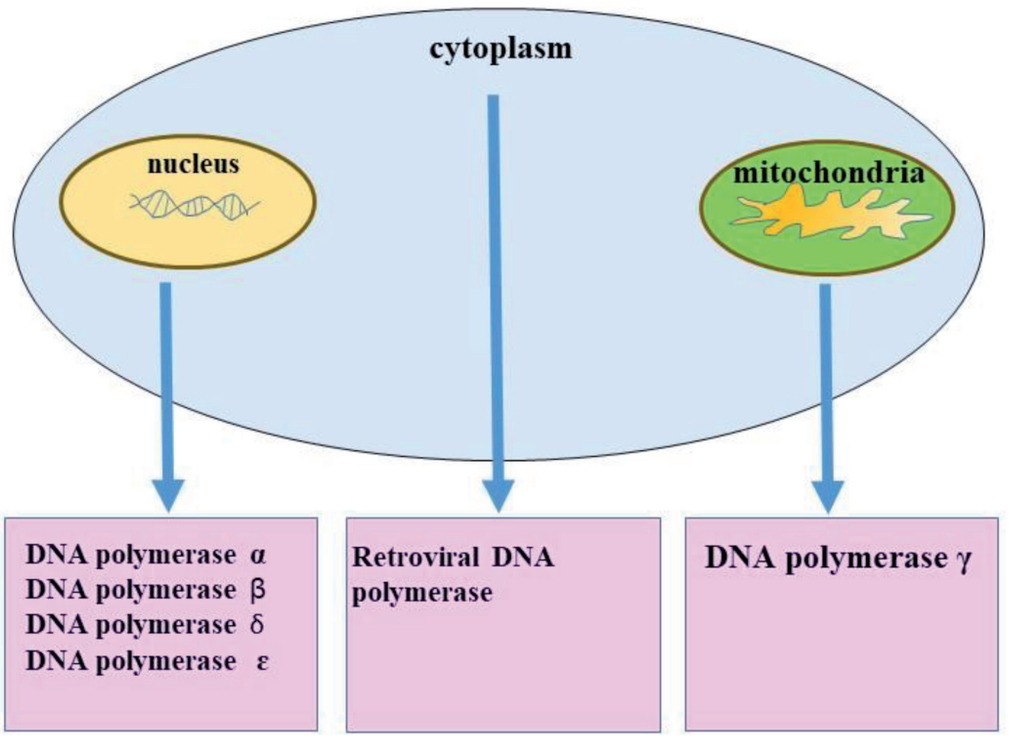

Figure 1.
Sub-cellular localization of eukaryotic and retroviral DNA polymerases.

drives the cell death response. The inability of the DNA damage response (DDR) to repair following endogenous and exogenous insults can lead to (i) an accumulation of errors in genomic DNA, (ii) subsequent malignant transformation, (iii) cancer progression and (iv) further impairment of the DNA repair capacity. DNA repair mechanisms comprise the detection and deletion (excision) of the lesion, the rejoining of DNA ends and the restoration of the complementary sequence based on a DNA template.

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]. The presence of a "mutator phenotype" could increase the acquisition of alterations that could lead to enhanced drug resistance limiting the effectiveness of anti-cancer drug treatment.

Viral infection is characterized by the high genetic variability found in virus populations [6]. This phenomenon is attributed to the inaccuracy of the replication machinery that is unique to the viral life cycle. Virulence, pathogenesis and the ability to develop effective antiretroviral drugs and vaccines are largely dependent on genetic diversity in viruses [7]. Retroviruses are RNA viruses that replicate through a DNA intermediate in a process catalyzed by the viral reverse transcriptase (RT) in cytoplasm (**Figure 1**) [7]. Human immunodeficiency virus type 1 (HIV-1), the etiological agent of AIDS, exhibits exceptionally high mutation frequencies [8]. 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 proofreading activity. A strong mutator phenotype is also observed for herpes viral DNA polymerase mutants with reduced intrinsic 3′ → 5′ exonuclease activity [9].

Mitochondrial DNA (mtDNA) alterations have been associated with various human diseases with impaired mitochondrial function [10]. Mitochondrial DNA polymerase γ (pol γ) is responsible for replication of mtDNA and is implicated in all repair processes (**Figure 1**) [11]. Mitochondrial DNA is prone to mutations, since it is localized near the inner mitochondrial membrane in which reactive oxygen

species are generated. Additionally, mtDNA lacks histone protection and the highly efficient DNA repair mechanisms [12]. The mutation rate of mtDNA is estimated to be about 20–100-fold higher than that of nuclear DNA [13]. The mutagenic mechanisms were shown to be replication errors caused by mis insertion (as a result of a dNTP excess), or decreased proofreading efficiency [14, 15].

Thus, in various compartments of the cell, enhanced DNA replication fidelity is a vital activity for the preservation of genomic stability for many organisms.

2. DNA repair

Genomic integrity of the cell is crucial for the successful transmission of genetic information to the offspring and its survival [16]. DNA is constantly being damaged. Essentially, DNA lesions can occur in two major ways, affecting either a single-stranded break (SSB) or double-stranded (DSB) or mono-adducts and inter-strand crosslinks, respectively. To combat this, eukaryotes have developed complex DNA damage repair (DDR) pathways (**Figure 2**). The active pathways for DNA repair are base excision repair (BER), nucleotide excision repair (NER), and mismatch repair MMR for SSB repair, whereas homologous recombination (HR) and non-homologous end-joining (NHEJ) for DSB repair [16]. Nucleotide excision repair (NER) removes a variety of helix-distorting lesions such as typically induced by UV irradiation, whereas base excision repair (BER) targets oxidative base modifications. Mismatch repair (MMR) scans for nucleotides that have been erroneously inserted during replication. The most deleterious types of damage in DNA are DSBs that are typically induced by IR and resolved either by NHEJ or by HR, whereas RECQ helicases assume various roles in genome maintenance during recombination repair and replication.

A low fidelity of DNA synthesis in various compartments of the cell by main replicative DNA polymerases leads to genomic instability (mutator phenotype) [17]. The errors produced during DNA synthesis could result from three fidelity

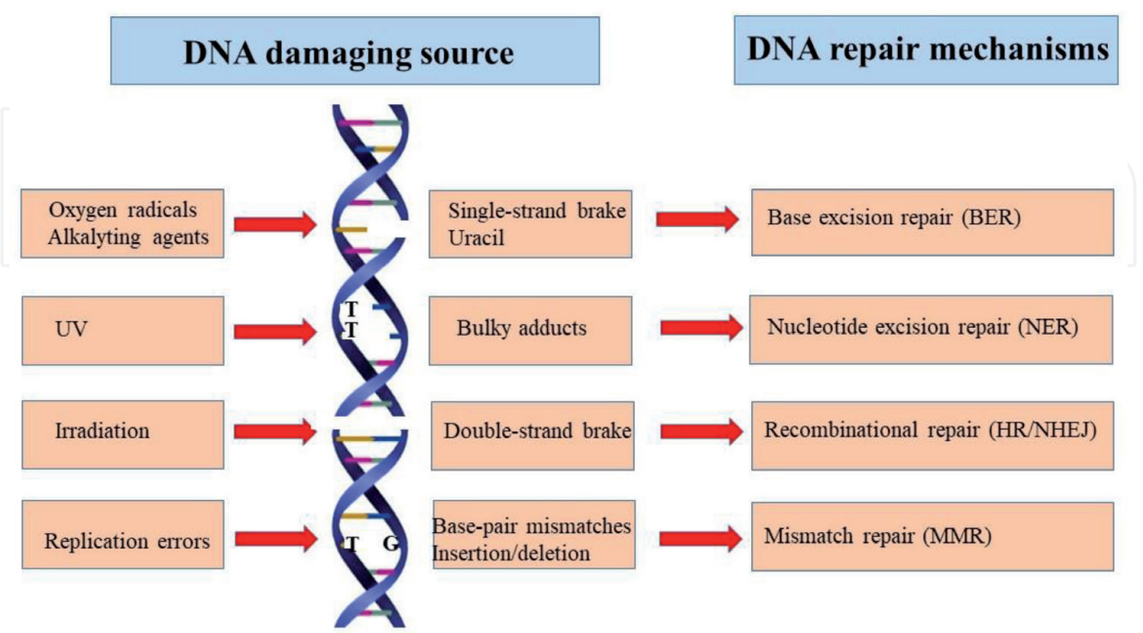

Figure 2.
DNA damage and repair mechanisms. Various DNA damaging agents cause a range of DNA lesions with different outcomes at both the genomic and cellular levels. Each are corrected by a specific DNA repair mechanism, namely, base-excision repair (BER), nucleotide excision repair (NER), homologous recombination (HR)/non-homologous end-joining (NHEJ) or mismatch repair (MMR).

Biochemical properties of cellular DNA polymerases			
	Function	**3′ → 5′ exonuclease**	**Proofreading**
Nuclear DNA polymerases			
α	primase	no	no
β	repair	no	no
δ	Lagging DNA synthesis, repair	yes	yes
ε	Leading DNA synthesis, repair	yes	yes
Mitochondrial DNA polymerase			
γ	DNA synthesis	yes	yes
Retroviral DNA polymerase			
HIV-1 RT	DNA synthesis	no	no

Table 1.
Biochemical properties of eukaryotic and retroviral DNA polymerases.

determining processes: a) nucleotide misinsertion into the nascent DNA, b) lack of exonucleolytic proofreading activity, that is, the mechanism to identify and excise incorrect nucleotide incorporated during DNA synthesis, and c) extension of mismatched 3′-termini of DNA (**Table 1**) [18].

Incorrectly repaired DNA lesions can lead to mutations, genomic instability, changes in the regulation of cellular functions, progression of cancer and premature aging. Cells can repair the large variety of DNA lesions through a variety of sophisticated DNA-repair machineries, recognizing and activating battery of proteins/factors for the repair of damaged DNA. DNA replication is a complex process influenced by numerous proteins/factors. The most important part of the DNA damage response is the activation of tumor repressor p53 protein [18].

3. Tumor suppressor p53 protein and DNA repair

The p53 represents a major factor for the maintenance of genome stability and for the suppression of cancer [19, 20]. The p53 protein is commonly referred to as the "*guardian of the genome*" due to its activities directed at maintaining genomic stability through the repair of damaged DNA [19]. Mutations in p53 are the most frequent molecular alterations detected in all human cancers [21]. Approximately 50% of human tumors harbor p53mutations while the remaining malignancies expressing wtp53 display functional inactivation of the p53 pathway [22]. The loss of the functional p53 may be responsible for genetic variability and the development of cancer [22]. Mutations in p53 result in a loss of its physiological function, accompanied by the accumulation of a novel gain-of function protein [23].

Under normal conditions within the cell, p53 is maintained at low levels by the E3 Ubiquitin ligase MDM2, mediating p53 proteasomal degradation [23]. In response to exposure to various endogenous and exogenous stress signals (such as DNA damage, oncogene activation, hypoxia, and nutrient depletion), the protein is stabilized and functionally activated by a series of post-translational modifications (*e.g.,* phosphorylation, acetylation) resulting in p53 accumulation at nuclear and

extra-nuclear sites [21, 24]. Activated p53 is a pleiotropic regulator and, as a transcription factor, binds to specific DNA sequences thereby regulating the expression of plethora of target genes controlling proliferation, senescence, DNA repair, and cell death. p53 is involved in diverse cellular processes including cell cycle arrest (thus preventing the replication of damaged DNA allowing time for the cells to repair DNA), apoptosis (for eliminating cells that contained excessive and irreparable damaged DNA), or DNA-damage repair (**Figure 3**) [20, 23]. These processes together protect the organism from genetically unstable cells that drive cancer.

p53 exhibits the functional heterogeneity in its basal (non-induced) state and under various p53 inducible circumstances [20]. Increasing evidences suggest various "non-transcriptional functions" of p53, that can contribute to tumor suppressor activity [25]. p53 may modulate DNA repair through processes, which are independent of its transactivation function. p53 is actively transported between the nucleus and cytoplasm. Furthermore, p53 translocate to mitochondria [26]. p53 can directly interact with DNA repair related cellular factors [27]. The origin, duration, intensity of the stress signals, the interaction with other cellular or viral proteins, and stress-mediated subcellular localization of p53 determines the outcome of the p53 response, namely, its pro- or anti-survival functions [28]. p53 protein executes multi-compartmental functions in the cell by either numerous p53-regulated proteins or by its intrinsic biochemical activities [28].

3.1 p53 and DNA repair in nucleus

The functioning of the eukaryotic genome relies on effective and accurate DNA replication and repair [2]. DNA replication in the nucleus of eukaryotic cells employs DNA polymerases (pols) α, β, δ, and ϵ, that are the key enzymes required to maintain the integrity of the genome under all these circumstances [1, 3]. However, the maintenance of genomic integrity is complicated by the fact that the genome is persistently challenged by a variety of endogenous and exogenous DNA-damaging factors [4]. DNA lesion can block DNA replication, which can lead to double-strand breaks (DSB) or alter base coding potential, leading to mutations. The accumulation of damage in DNA can affect gene expression leading to the malfunction of many cellular processes [4]. Various DNA repair systems operate in cells to remove DNA lesions, and several proteins are known to be the key components of these repair systems.

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 [25]. p53 mediating various activities are correlated with the levels of the p53 protein in the cells [27, 29]. The non-genotoxic stress may include a long-lasting, moderate accumulation of p53 in nucleus. Conversely, acute genotoxic stress may induce rapid and transient accumulation of very high levels of p53 with preferential activation of target genes involved in apoptosis [29]. There is a possibility that both transcriptional and transcription-independent pathways act in synergy thereby amplifying the potency of involvement of p53 in DNA repair.

p53 localized in cell nuclei in response to replication stress actively participate in various processes of DNA repair and DNA recombination via its ability to interact with components of the repair and recombination machinery and by its various biochemical activities [30, 31]. Both *in vitro* and *in vivo* data suggest an intricate relationship between the biochemical activities of p53 in DNA replication and recombination. The notion that p53 plays a role in DNA repair pathways *in vivo* is supported by the observation that p53 knockout mice exhibit an increase in chromosomal abnormalities and a deficiency in global genomic repair [32]. p53 is

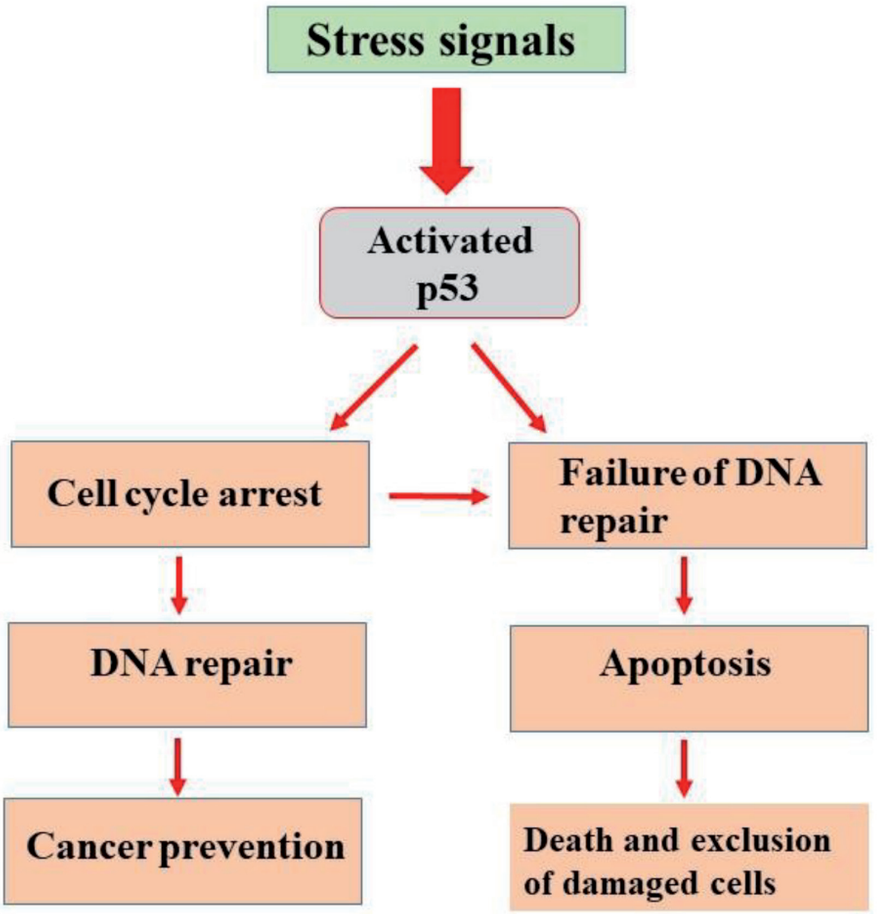

Figure 3.
In response to various endogenous and exogenous stress signals, the activated p53 arrests the cell cycle until the DNA damage is repaired thereby preventing the cancer. If the DNA damage cannot be repaired apoptosis occurs for eliminating cells that contained excessive and irreparable damaged DNA.

involved in almost all nuclear DNA repair pathways including BER, NER, MMR, NHEJ and HR [32]. The transcription-independent functions play a prominent role as a facilitator of DNA repair by halting the cell cycle to allow time for the repair machineries to restore genome stability [25].

The C-terminal 30 amino acids of p53 were shown to recognize several DNA damage-related structures.

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 ssDNA and dsDNA ends [33, 34], (b) p53 catalyzes DNA and RNA strand transfer and promotes the annealing of complementary DNA and RNA single-strands [35, 36], (c) p53 binds insertion/deletion mismatches and bulges [37], (d) p53 binds to three-stranded heteroduplex joints and four-stranded Holliday junction DNA structures with localization specifically at the junction, suggesting that p53 directly participates in recombination repair [38], (e) it can bind DNA in a non-sequence-specific manner [39], (f) p53 exhibits a Mg2+ dependent 3′ → 5′ exonuclease activity [40–43].

Noticeably, the same central region within p53, where tumorigenic mutations are clustered, recognizes DNA sequence specifically, is required for junction-specific binding of heteroduplex joints and is necessary and sufficient for the 3′ → 5′ exonuclease activity on DNA [28]. In addition to p53's biochemical activities, numerous reports on physical and functional protein interactions further strengthened the proposal of a direct role of p53 in BER, NER, and DSB repair.

a. Oxidative DNA damage is largely repaired by the BER pathway. p53 might directly facilitate BER mainly via association with BER components. Wtp53 directly enhanced BER activity measured both *in vitro* and *in vivo* [44]. Genotoxic stress induced a p53-dependent modulation in BER activity throughout the cell cycle. The idea that p53 is directly involved in BER is supported by various studies: BER activity in cell extracts correlates with levels of purified wtp53 [29], the ability of p53 to augment BER activity is correlated with its ability to interact directly both with AP endonuclease and with DNA Polymerase β [27, 45]. Hot-spot tumor-derived p53 mutants do not significantly enhance BER, supporting the possibility that the stimulatory effect of wtp53 may contribute to its ability to suppress tumorigenesis. Based on these results, p53 stabilization of the DNA pol β–AP-DNA complex is likely to be the mechanism underlying the stimulation of BER by p53 [27].

   The cellular response depends on the dose of genotoxic agent introduced to the cells. Increasing doses of genotoxic agents cause the accumulation of activated p53 that determines the onset of BER or apoptosis. Low doses of DNA damaging agent resulted in the enhancement of p53-dependent BER activity whereas high levels induced different p53 post-translational modifications that down regulate BER pathway and instead provoked an apoptotic response [29]. The quantitative changes in p53 protein level were associated with qualitative changes in p53 phosphorylation status. In all, this may indicate that increasing doses of genotoxic agents cause the accumulation of activated p53 that determines the onset of BER or apoptosis.

b. NER is an important DNA repair process that detects and eliminates lesions including both chemical alteration and structural distortion of the DNA helix (*e.g.*, photoproducts induced by UV irradiation and other bulky lesions) [25]. The NER pathway retains two damage detection pathways: Transcription Coupled Repair (TC-NER) and Global Genome Repair (GG-NER), depending on the mode of damage recognition in the entire genome versus actively transcribed regions [46–48]. TC-NER detects and removes transcription blocking lesions in transcribed sections of the genome; triggered when a lesion inhibits transcription elongation by RNA polymerase II, thereby preventing cell death. GGR-NER detects lesions across the whole genome, including non-transcribed regions. Upon lesion detection by either the TC or GG arm, repair proceeds via a final common pathway [25]. The role of p53 in promoting GG-NER is more consistent compared to p53 function in TC-NER. p53 facilitates NER by promoting lesion recognition or detection by recruiting the p300 histone acetylase to damage sites, which acetylates the histone H3, leading to global chromatin relaxation and increased lesion accessibility making an additional contribution to the maintenance of genome stability [46–47].

   Pathogenic mutations in the GG components XPC and DDB2 (XPE) result in xeroderma pigmentosum (XP) a disease characterized by increased UV-sensitivity and skin cancer incidence [46]. Conversely, mutation in TC genes result in Cockayne's syndrome that is characterized by neurological abnormalities but no increase in skin cancer incidence. Some NER proteins, particularly the GG damage recognition proteins, can decide a cell's fate by triggering the initiation of the repair pathway or by signaling apoptosis [46]. Therefore, if the GG pathway is defective, neither DNA repair nor apoptosis occurs, resulting in a cancer cell containing high levels of UV-induced mutations that does not

undergo apoptosis. How this non-transcriptional function of p53 contributes to tumor suppression is unclear.

c. DNA mismatch repair (MMR) is an important DNA repair pathway, which facilitates removal of incorrect nucleotides incorporated during replication. p53 facilitates excision of incorrect nucleotides produced from the error prone nature of DNA polymerases and misincorporation of the incorrect base [25]. Mismatched bases can be either a G/T or A/C pair. To initiate MMR a nick in the DNA either 5′ or 3′ to the mismatch must occur. Proteins that bind the mismatch in humans are *E. coli* MutS homologs. MSH2 is a major component of the MMR MSH2-MSH6 complex and is a known to be transcriptionally upregulated by p53 following UV [49]. In vitro studies established that the MSH2–MSH6 complex can enhance the binding of p53 to DNA substrates with topological distortions, and this activity depends on the phosphorylation state of p53(S392) [50, 51]. Connections between p53 and MMR have been made in various systems demonstrating a role for MMR proteins in influencing p53-related processes. p53 and MMR proteins can function synergistically in mice, as Msh2−/− p53−/− females arrested as embryos and they quickly developed tumors relative to the single-mutant animals [52]. p53 signaling was shown to be suppressed in MSH2-deficient cells [53]. While these p53-dependent mechanisms have been linked to MMR regulation, MSH2 has been implicated in a variety of repair pathways and it is necessary to determine if p53 function is pertinent. An interesting notion is that, p53 interacts with and transcriptionally regulates its gene target in MMR. Further studies are needed to define if p53 transcription-dependent and independent functions work alongside in MMR or whether these functions are separate and dependent on the cellular insult or pathway choice.

d. 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 [54]. Excision of incorrectly polymerized nucleotides by exonucleases is an imperious mechanism diminishing the errors during DNA polymerization [55]. Certain organisms with a deficiency of exonucleolytic proofreading, have an increased susceptibility to cancer, especially under conditions of stress. Because the misincorporation of non-complementary dNTPs during DNA replication represents a chief mechanism of gene mutation [56], the removal of the wrong nucleotides from DNA is critical for genomic stability. The intrinsic limited accuracy of DNA polymerases and the imbalance of intracellular dNTP pools are the two most important factors responsible for DNA replication errors [57, 58]. The proofreading for such replication errors by the 3′ → 5′ exonuclease activity associated with the DNA replication machinery is extremely important in reduction of the occurrence of mutations. Interestingly, the mammalian DNA pol α, an enzyme considered to be responsible for the lagging strand replication [59], lacks the 3′ → 5′ exonuclease proof-reading activity and is prone to making replication errors [60].

Three steps, base selection, exonucleolytic proofreading, and DNA elongation, ensure the high fidelity of DNA replication. wtp53 exhibits an intrinsic 3′ → 5′ exonuclease activity. wtp53, co-located with the DNA replication machinery [61], specifically interacts with pol α and has been shown to preferentially eliminate mismatched nucleotides from DNA with its 3′ → 5′ exonuclease activity, thereby enhancing the DNA replication fidelity of pol α *in vitro* [41].

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 [62]. The examination of the rates of HU-induced mutations in H1299 (p53-null) and H460 (wtp53) cells discovered substantially augmented mutation rates in H1299 cells. Furthermore, the HU-induced mutation frequency was significantly reduced by introduction of wtp53 expression vector into the p53-null H1299 cells. Thus, wtp53 expression was associated with a reduction of mutations caused by replication errors under the stress of dNTP pool imbalance [62].

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 [63]. 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, representing that the p53 exonuclease from the p53/pol-prim complex was indispensable 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 [63].

e. DSBs are the most severe type of DNA damage, and these DSBs generated at the replication fork are repaired by two principal repair pathways: homology-based repair (HR) and non-homologous end-joining (NHEJ) [25, 31]. Furthermore, replication blocking lesions such as bulky adducts are subject to HR repair, thereby rescuing the replication fork. HR is considered the most error-free pathway, because sister chromatids are the preferred template, however, it can also produce genetic instability upon up- or down-regulation [25].

Depending on the type and quality of the DSB repair pathway involved, the repair process may end up with deletions, loss of heterozygosity, and chromosomal translocations which may accelerate the multistep process of tumorigenesis. p53 can control HR *in vitro* by specific recognition of the heteroduplex intermediates, and *in vivo* by modulating the functions of different HR-specific proteins [38, 64]. Numerous groups detected that wtp53 represses HR on both extra-chromosomal and intra-chromosomal DNA substrates by at least one to two orders of magnitude [31]. Conversely, inactivation of p53 by mutation or complex formation by viral proteins increased HR by several orders of magnitude. Importantly, experiments with p53 mutants revealed severe HR inhibitory defects for all tested hotspot mutants. Mutant p53s which are known to reduce or even abolish p53's transcriptional transactivation and cell cycle regulatory capacity, did not significantly affect HR inhibition [65, 66]. These discoveries confirmed that p53 activities in transcriptional transactivation and checkpoint control are separable from its functions in homology-based DSB repair and provided undoubted proof for a direct role of p53 in HR [67].

p53 prevents the accumulation of DSBs at stalled-replication forks induced by UV or hydroxyurea (HU) treatment. When DNA replication is blocked, p53 becomes phosphorylated on serine 15 and associates with key enzymes of HR such as, Rad51, and Rad54 [68, 69]. Notably, during replication arrest p53 remains inactive in transcriptional transactivation, further supporting the direct

involvement in HR regulatory functions unrelated to transcriptional transactivation activities.

p53 preferentially represses HR between certain mispaired DNA sequences. p53 specifically recognizes preformed heteroduplex joints structurally resembling early recombination intermediates, when comprising these mispairings [68]. p53 is able to attack DNA by 3′–5′ exonuclease activity principally during Rad51-mediated strand transfer and to display a DNA substrate preference for heteroduplex recombination intermediates with a further enhancement of the exonucleolytic activity for mispaired as compared to correctly paired heteroduplex DNA [38].

Highlighting the significance of p53 DNA interactions in the regulation of strand exchange events, p53 inhibits branch migration of Holliday junctions (HJs) [25, 31]. p53 recognizes this HJs -like structure and controls the generation and branch migration of the replication fork as well as its resolution, to prevent error-prone DSB repair and to cause replication pausing until the DNA lesion is repaired.

f. Mammalian cells repair the majority of double-strand breaks by NHEJ [69, 70] which is regarded as principally inaccurate process. The role of p53 in NHEJ remains unclear. p53 has an inhibitory effect on error-prone NHEJ but not error-free NHEJ [71], thereby suppressing genomic instability arising from low-fidelity repair. Remarkably, after the exposure to IR, DSB rejoining increases with loss of wtp53function. Inhibition of in vitro end-joining was observed with the oncogenic mutant p53(175H), whereas the phosphorylation-mimicking mutant p53(15D) failed to inhibit, thereby providing evidence for possible role of phosphorylated p53 in the regulation of NHEJ [72].

Various *in vitro* and *in vivo* studies have shown that p53 can rejoin or ligate compatible ends of DNA with DSBs [68, 70]. Evidently, p53 has several genetic interactions with components of the NHEJ pathway that are exhibited by downstream effects on cellular survival and cell-cycle control or effects on DNA repair. The molecular mechanisms of these interactions remain unresolved.

3.2 p53 and DNA repair in cytoplasm

Under normal conditions a basal pool of p53 is retained intra-cellular, with the distribution of p53 between the different subcellular compartments dependent on the cellular stress milieu [28]. Indeed, wtp53 occurs in cytoplasm in a subset of human tumor cells such as breast cancers, colon cancers and neuroblastoma [73–75]. Shuttling between nucleus and cytoplasm not only regulates protein localization, but also often impacts on protein function.

p53, localized in the cytoplasmic lysates of non-stressed p53-proficient cell lines [e.g. LCC2, HCT116 (p53+/+)] exerts an inherent 3′ → 5′ exonuclease activity displaying identical biochemical functions characteristic for recombinant wtp53 [76, 77]: 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 substrate, 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 excises nucleotides from nucleic acid substrates independently from DNA polymerase, 6) it fulfills the requirements for proofreading function; acts coordinately with the exonuclease-deficient viral DNA polymerases.

Viruses exploits their cellular host for their successful replication, they utilize cell proteins for multiple purposes during their intracellular replication [78]. Since viral infection evokes cellular stress, the infected cells harbor stabilized activated p53 and manipulate p53's guardian role. Interestingly, increased p53 levels have been noted following infection of cells with various viruses including retrovirus-human immunodeficiency virus [79], which exhibits exceptionally high genetic variability [6], due to the low fidelity of the replication apparatus that is exclusive to the retroviral life cycle.

Reverse transcriptase (RT) of HIV-1 is responsible for the conversion of the viral genomic ssRNA into the proviral DNA in the cytoplasm [7]. The lack of intrinsic 3′ → 5′ exonuclease activity, the formation of 3′-mispaired DNA and the subsequent extension of this DNA were shown to be determinants for the low fidelity of HIV-1 RT [80]. p53 can proofread for HIV-1 RT, increasing the fidelity of DNA synthesis by excising incorrectly polymerized nucleotides from RNA/DNA and DNA/DNA temple-primers in the direct exonuclease assay, when first binding to a 3′-terminus and during ongoing DNA synthesis *in vitro* with both template-primers [76]. The role of p53 in proofreading is two-fold: to excise preexisting 3′-terminal mismatches and to prevent the extension of 3′-mismatched primer ends by the polymerase [76]. p53 with its inherent exoribonuclease activity and excision of mispairs, has a potential to serve as an external trans-acting proofreader, providing the host-derived repair mechanism in cytoplasm.

3.3 p53 and DNA repair in mitochondria

DNA polymerase (pol) γ is the sole DNA polymerase that is responsible for replication and repair of mtDNA [81]. It is well established that defects in mtDNA replication lead to mitochondrial dysfunction and disease [56, 60]. Mutations in mtDNA can arise from exogenous sources, from endogenous oxidative stress, or as spontaneous errors of replication during either DNA synthesis or repair events [82]. Mitochondrial DNA is replicated by DNA polymerase γ in concert with replisome accessory proteins such as the mitochondrial DNA helicase, single-stranded DNA binding protein, topoisomerase, the multifunctional mitochondrial transcription factor A (TFAM) with important roles in mtDNA replication and initiating factors.

A high frequency of mutations within mtDNA, resulting in mitochondrial dysfunctions, is an important source of various diseases including cancer and human aging [81, 82]. To verify mtDNA integrity, cells hold various DNA damage response pathway(s) comprising mtDNA replication/repair preservation programs that either preclude or repair damage [83]. The mutagenic mechanisms were shown to be replication errors formed by either pol γ during DNA synthesis by incorporation of incorrect nucleotide or produced due to the presence of unbalanced dNTP concentrations, or by diminished proofreading efficiency. MtDNA is not protected by histones and mtDNA repair is ineffective [81]. Furthermore, a potentially important source of replication infidelity is damage due to ROS. pol γ, was demonstrated to stably misincorporate highly mutagenic 8-oxo-7,8-dihydro-2′-deoxyguanosine (8-oxodG) opposite template adenine in a complete DNA synthesis reaction *in vitro* [84].

Because of the susceptibility of mtDNA to oxidative damage and replication errors, it is vital to protect mtDNA genomic stability to preserve health. Mitochondrial localization of p53 was observed in non-stressed and stressed cells [26]. Mitochondrial p53 (mit-p53) levels are proportional to total p53 levels, and the majority of p53 was present inside the intra-mitochondrial compartment-matrix, in which mtDNA is located [85]. The mit-p53 physically and functionally interacts with both, mtDNA and pol γ [86].

Notably, with the exception of NER, components of these nuclear DNA repair pathways are also shared in mtDNA maintenance. Several studies illustrated the participation of p53 in mtDNA repair:

a. 53 enhances mitochondrial BER (mtBER) through direct interaction with the repair complex in mouse liver and cancer cells [87]. p53 modulates mtBER through the stimulation of the nucleotide incorporation step.

b. p53 interacts physically with human mtSSB (HmtSSB) *in vitro* via its transactivation domain and is proficient of hydrolyzing the 8-oxodG present at the 3′-end of DNA, a well-known marker of oxidative stress [88].

c. Intra-mitochondrial p53 provides an error-repair proofreading function for pol γ by excision of misincorporated nucleotides [89]. The p53 in mitochondria may affect the accuracy of DNA synthesis by acting as an external proofreader, thus reducing the production of polymerization errors.

4. Removal of nucleoside analogs from DNA by p53 protein

In addition to having a critical role in preservation of genome integrity, alterations in the expression, and function of DNA repair proteins are a major facilitator of tumor responses to chemo- and radiotherapy, commonly functioning by inducing DNA damage in tumor cells. 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, inhibitors of HIV-1 RT), are incorporated into DNA and cause cell death or inhibition of viral replication [90, 91]. These drugs are intracellularly converted to the active analog triphosphates, compete with physiological nucleosides and are then inserted into replicating DNA. The incorporated NA, structurally mimicking 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 [91]. Furthermore, the high toxicity of NA compounds may be caused by high rates of incorporation of the NA into DNA and their persistence in DNA due to inefficient excision. Removal of drugs by 3′ → 5′ exonuclease activity intrinsic to DNA polymerase or by external proofreading activity of external polymerases or proteins is presumably a potential cellular mechanism of resistance to anti-viral drugs or anti-cancer drugs.

The cytotoxic activity of gemcitabine (2′2’-difluorodeoxycitidine, dFdC) was strongly correlated with the amount of dFdCMP incorporated into cellular DNA [92]. 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 [93]. Although, the excision of the dFdCMP from the 3′-end of the DNA was slower than the excision of 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. The recognition of the incorporated NAs in DNA by wtp53 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 [93, 94]. 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 3′-end [94]. Notably, the specific effects of gemcitabine exposure appeared to vary depending on the duration of treatment and upon the cell line.

It should be pointed out, that wtp53 in ML-1 cells removed the purine nucleoside analog fludarabine (F-ara-A) more efficiently than gemcitabine [93]. 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, 95]. p53 protein in the cytoplasm excises the incorporated NAs during both RNA-dependent and DNA-dependent DNA polymerization reactions, although less efficiently than the mismatched nucleotides; longer incubation times were required for excision of the terminally incorporated analogs [96]. The 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.

Pol γ is unique among the cellular replicative DNA polymerases as it is sensitive to inhibition by nucleoside analogue reverse transcriptase inhibitors (NRTIs) used in the treatment of HIV, which can cause an induced mitochondrial toxicity [97]. Acquired mitochondrial toxicity occurs as a consequence of incorporation of NA into mtDNA or inhibition of mtDNA replication or both. A terminally incorporated NA may be removed by p53 in mitochondria [97]. The removal of the incorporated NA by p53 exonuclease, indicates that the presence of the cellular component-p53 in mitochondria may be important in defining the cytotoxicity of NAs toward mitochondrial replication, thus affecting risk–benefit approach (NA toxicity versus viral inhibition) [98, 99]. Apparently, the presence of p53 in mitochondria may be important, as the excision of the mispair and NA by p53 is favorable event for mitochondrial function.

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 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) [93]; 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) [96] and 3)the excision of NAs from mitochondrial DNA may decrease the potential for chain termination and host toxicity (positive effect) [97].

5. Excision of non-canonical nucleotides by p53 protein

The genome is constantly under attack from extrinsic and intrinsic damaging agents. Uracil (dU) mis-incorporation in DNA is an intrinsic factor resulting in genomic instability and DNA mutations. The excessive levels of genomic uracil in DNA can modify gene expression by interfering with promoter binding and transcription inhibition, can change transcriptional stalling, or induce DNA strand

breaks leading to apoptosis. The factors that influence uracil levels in DNA are cytosine deamination, de novo thymidylate (dTMP) biosynthesis, salvage dTMP biosynthesis, and DNA repair. Furthermore, mis-incorporation occurs when DNA polymerases incorporate dUTP into DNA, in place of dTTP, and the rate of mis-incorporation is believed to be determined by the intracellular dUTP:dTTP ratio [100, 101]. The enzyme deoxyuridine triphosphate nucleotidohydrolase (dUTPase), which facilitates the conversion of dUTP to dUMP further utilized by thymidylate synthase (TS) for synthesis of dTMP, avoids mis-incorporation of dU into DNA in nucleus by decreasing the dUTP/dTTP ratio [101]. The misincorporation of dU, as a result of accumulation of dUTP, plays a critical role in cytotoxicity mediated by TS inhibitors, such as the commonly used anticancer drug 5-fluorouracil (5-FU) [102]. DNA directed cytotoxicity of chemotherapeutic agents (e.g.5-FU) not only depends on accumulation of dUTP, but may also be determined by the efficiency of the DNA repair mechanisms (e.g. excision repair) which preclude the incidence of the mistake.

Pol γ in mitochondria is incapable to readily correct U:A mismatches [11]. HIV-1 RT in the cytoplasm of HIV-infected cells efficiently inserts the non-canonical dUTP into the proviral DNA and extends the dU-terminated DNA [103]. The misincorporation of dUTP leads to mutagenesis, and to down-regulation of viral gene expression [104].

Within the context of error-correction events, p53 as a DNA binding protein, contributes an external proofreading function; upon excision of the dU, the p53 dissociates, thus letting the transfer of the substrate with the correct 3′-terminus to DNA polymerase and renewal of DNA synthesis.

The biochemical data show that the procession of U:A and mismatched U:G lesions enhances in the presence of recombinant or endogenous cytoplasmic or mitochondrial p53 [105]. p53 in cytoplasm can participate through the intermolecular pathway in a dU-damage-associated repair mechanism by its ability to remove preformed 3′-terminal dUs, thus preventing further extension of 3' dU-terminated primer during DNA synthesis by HIV-1 RT. Similarly, p53 in mitochondria can function as an exonuclease/proofreader for pol γ by either decreasing the incorporation of non-canonical dUTP into DNA or by promoting the excision of incorporated dU from nascent DNA, thus expanding the spectrum of DNA damage sites exploited for proofreading as a trans-acting protein [106].

During genomic DNA replication another form of replication errors arises during the incorporation of nucleotides carrying the correct base, but the wrong sugar at substantial rates [107]. DNA polymerases often incorporate ribonucleoside triphosphates (rNTPs) into DNA because of the much higher concentration of rNTPs than that of dNTPs in the cellular nucleotide pool. Indeed, more than 10^6 rNMPs are incorporated during one round of replication of a mammalian genome [107]. Newly incorporated rNMPs destabilize DNA and pose a major threat to genome integrity due to their reactive 2'OH group. The inserted rNs are the most abundant non-canonical nucleotides in the genome. Failure of rN removal is associated with genome instability in the form of mutagenesis, replication stress, DNA breaks, and chromosomal rearrangements. The aberrant accumulation of rNs in the genome leads to human diseases including Aicardi–Goutières syndrome (AGS), the severe autoimmune disease, and tumorigenesis [108]. Mammalian cells have developed strategies to prevent persistent rN accumulation. In eukaryotes, rNs embedded into DNA are primarily repaired by RNase H2-initiated repair pathway. Ribonucleotide excision repair (RER) may be directly coupled to replication and results in rapid post-replicative repair of rNMPs [108]. Remarkably, exonuclease-proficient yeast and human DNA polymerases can proofread incorporated rNs, albeit inefficiently [107].

Recent studies have demonstrated the importance of p53 in 3′-terminal RER pathway through a functional collaboration with HIV-1 RT, acting in a coordinated manner to attain higher fidelity. p53, functioning as a trans-acting proofreader in cytoplasm, can decrease the stable incorporation of rNs, into DNA by HIV-1 RT [109]. p53 can influence events needed for RER by possessing the compatible biochemical properties: p53 is pertinent in the correction of replication errors produced by HIV-1 RT during distinct steps of rN incorporation through intermolecular pathway: by removal pre-existing 3′-terminal rN; by reducing rN incorporation; by preventing extension of a 3′ rN-terminated primer, by attenuating stable incorporation of rNs. Thus, p53, functioning as a trans-acting proofreader in cytoplasm, can decrease the stable incorporation of rNs.

The fact that p53 in cytoplasm can edit an incorrect sugar irrespective of the nature of base, expands the role of p53 as a proofreader in the repair of replication errors by removing both a base mismatch and an incorrect sugar.

6. Conclusions

Mammalian cells have evolved multiple strategies to safeguard the genetic information to prevent the fixation of genetic damage induced by endogenous and exogenous mutagens [16]. p53 protein plays a crucial 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 [16]. Remarkably, DNA repair transcription-independent functions of wtp53, contributing to tumor suppression, were found to protect cells from DNA damage independently of the transcription-mediated functions of p53 [25]. Thus, a more comprehensive understanding of how p53 transcription- independent functions are induced in response to a variety of cellular insults is vital. This report focuses on direct roles of p53 in DNA repair during DNA replication in various compartments of the cell. Apparently, p53 has more than one contributions to DNA replication fidelity, which could depend on sub-cellular localization of p53, on the type and incidence of replication obstacles, on the levels of p53 protein [28].

p53 is able to elicit a spectrum of different effective DNA repair pathways in nucleus, cytoplasm and mitochondria (**Figure 4**). Within the nucleus, p53 regulates different repair mechanisms, in response to endogenous and exogenous replicative stress*: e.g.* HR (by restricting excess recombination through interactions with Rad51), NER, BER, and MMR through interactions with relevant components of the respective pathways [25, 31].

In the cytoplasm, p53 may contribute effective proofreading for exonuclease-deficient DNA polymerases (*e.g.*HIV-1 RT) thereby correcting errors produced during DNA replication [110, 111]. Moreover, the proofreading activity of p53 may limit the transversion mutations, indicating that p53 may affect the mutation spectra of DNA polymerase by acting as an external proofreader [111]. Recent studies also show that cytoplasmic p53 possesses the potential to remove the incorporated non-canonical dUTP into DNA by HIV-1 RT through an intermolecular pathway [105]. Furthermore, p53, functioning as a trans-acting proofreader, can decrease the stable incorporation of rNs [109]. The data implies that p53 excises incorrect sugar in addition to base mispairs, thereby expanding the role of p53 in the repair of replication errors.

Within the mitochondria, various studies illustrated the participation of p53 in mtDNA repair in a variety of systems: a)p53 enhances BER through direct interaction with the repair complex in mouse liver and cancer cells [87]. b) Intramitochondrial p53 provides an error-repair proofreading function for pol γ by

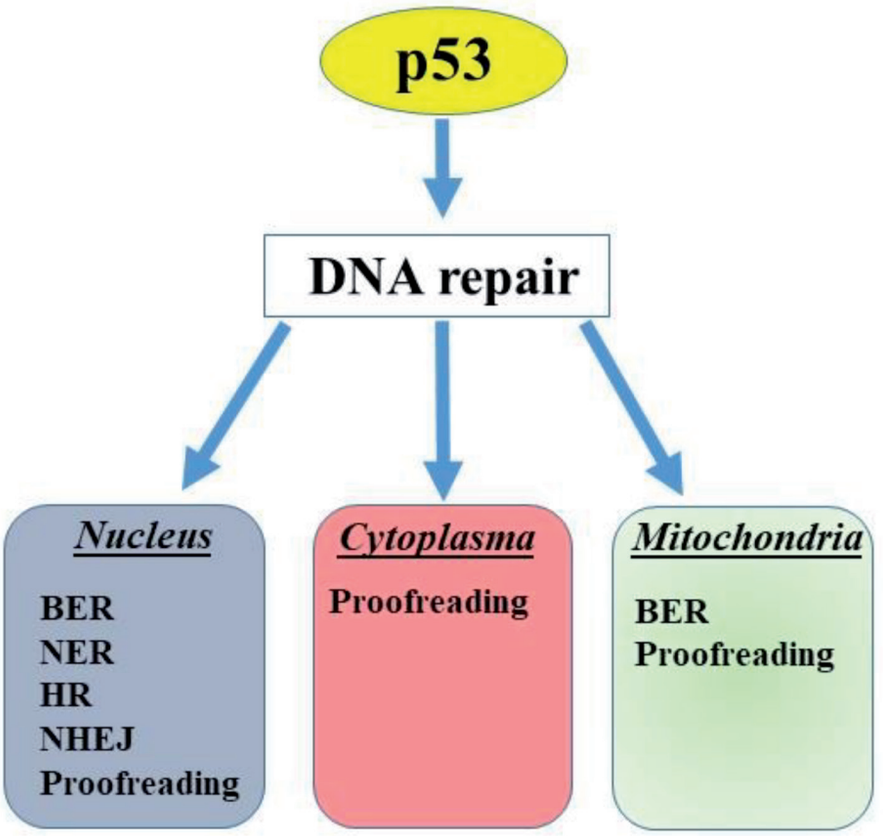

Figure 4.
p53 functions in DNA repair. p53 under both normal and stress conditions, can help cellular and viral DNA polymerases to promote the repair of DNA in various cellular compartments. The result of p53 activation depends on many variables, including the extent of the stress or damage. In this model, basal p53 activity or that induced by stress signals elicits the protector responses that support the repair of genotoxic damage by various pathways.

excision of misincorporated nucleotides [89]. c)p53 is proficient of hydrolyzing the 8-oxo-7,8-dihydro-2′-deoxy-guanosine (8-oxodG) present at the 3′-end of DNA, a well-known marker of oxidative stress [88]. d)p53 regulates mtDNA copy number, which may impact mitochondrial and cellular functions [112].

Therapeutic strategies based on p53 are particularly interesting because they exploit the cancer cell's intrinsic genome instability and predisposition to cell death-apoptosis [90, 91]. The role of p53 is predominantly relevant with respect to the development of anticancer and antiviral therapies. Removal of drugs by 3′ → 5′ exonuclease activity may also facilitate resistance to anti-cancer or anti-viral treatments. Clinical drug resistance limits the efficacy of these compounds. Uncovering the mechanisms, which are responsible for DNA repair of NA-induced DNA damage will have therapeutic value. The p53 protein is able to remove incorporated NA. The stress induced activation of p53 that occurs during anti-cancer or anti-viral therapy has negative and positive effects. p53 may remove incorporated therapeutic NAs from DNA or trigger apoptosis. More studies regarding functions of p53 in genome integrity and cancer evolution may facilitate drug screening and better design of therapeutic approaches.

7. Future directions

The functional interaction between p53 and DNA polymerase may have important consequences for the maintenance of genomic integrity and in the development

of p53- targeted clinical therapies. Further assessments are required to establish the role of p53 in DNA replication and the significance of these functions in various cellular compartments and treatment responses. Studies on the biology of various mutant p53 isoforms and their interaction with the factors involved in DNA repair and apoptosis, will be relevant to establish whether the direct involvement of p53 in DNA repair is a tumor suppressor function of this important anti-oncogene. 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 [113]. By utilizing various function-mutant p53 isoforms, more studies must be conducted on the biology of mutant p53 forms and their interaction with the factors involved in DNA repair and apoptosis, in order to recognize the molecular mechanisms that mediate p53-dependent control of DNA replication by cellular and viral DNA polymerases.

p53 has 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 [93]. p53, by removal of the incorporated NA, could confer a cellular resistance mechanism to the antiviral compounds. Finally, the excision of NAs from mitochondrial DNA may decrease the potential for chain termination and host toxicity. These features could serve as a template for the development of p53-targeting therapies.

The control of the viral mutation rate could be a practical anti-retroviral strategy. 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). It is important to further elucidate the molecular mechanisms involved in governing fidelity not only at a molecular level (*i.e.*, intrinsic RT fidelity), but also related to the cytoplasmic p53 protein that can control the viral mutation rate and can affect the incorporation of NAs 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, may create the basis for new strategies in targeted antiviral therapy that focus on the sub-cellular context of p53 in cells.

A major issue 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 links about the biological functions of mitochondrial p53 that are required to be investigated. Whether p53 defines the percent of mutated mtDNA (heteroplasmy in a cell)? Uncovering the mechanisms by which pol γ-mediated mtDNA mutations and depletion are manifested in cells in the absence and presence of p53 is significant step in understanding underlying causes for mtDNA–related diseases. Depletion and mutation of mtDNA may lead to cellular respiratory dysfunction and release of reactive oxidative species, resulting in cellular damage [99]. Future NAs should provide higher specificity for HIV-RT and lower incorporation by pol γ to diminish mitochondrial toxicity. Whether the effective targeting of p53 in mitochondria by error-correction functions, may result in decrease of mitochondrial toxicity in response to conventional anti-viral therapies? Understanding how p53 can be imported into mitochondria, will be important and could contribute toward the design of new therapies for various diseases.

Author details

Bakhanashvili Mary[1,2]

1 Infectious Diseases Unit, Sheba Medical Center, Tel-Hashomer, Israel

2 The Mina and Everard Goodman Faculty of Life Sciences, Bar-Ilan University, Ramat-Gan, Israel

*Address all correspondence to: bakhanus@yahoo.com

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