—Review—

p53 Biological Network: At the Crossroads of the Cellular-Stress Response Pathway and Molecular Carcinogenesis

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Abstract

p53 as a key molecular node in the stress response pathway, including inflammation. p53 is involved in several critical pathways including cell cycle arrest, apoptosis, DNA repair, and cellular senescence, which are essential for normal cellular homeostasis and maintaining genome integrity. The alteration of the *TP53* gene or posttranslational modification in the p53 protein can alter its response to cellular stress. The molecular archaeology of the *TP53* mutation spectrum generates hypotheses concerning the etiology and molecular pathogenesis of human cancer. The spectrum of somatic mutations in the *TP53* gene implicates environmental carcinogens, and both endogenous agents and processes in the etiology of human cancer.

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Key words: p53, nitric oxide, inflammation, cancer

Brief History

p53 was first discovered about 25 years ago as a 53kD protein bound to the hexameric DNA helicase, Simian Virus (SV-40) large-T antigen Lane et al.[1]/ Linzer et al.[2]. Earlier reviews have extensively described the intriguing history of p53 Harris[3]/Oren et al.[4]. Briefly, the gene encoding p53 (*TP53*), cloned from neoplastic rodent and human cells, was initially described as an oncogene with weak oncogenic properties. However, it was later realized in the late 1980's that original *TP53* cDNA clones obtained from human or mouse tumor cell lines contained a missense mutation and researchers were studying missense mutant forms of *TP53* rather than a wild-type (WT) gene. Further studies indicated that wild-type *TP53* suppresses neoplastic transformation of rodent fibroblasts *in vivo* and the growth of rodent and human cancer cells *in vitro* and *in vivo*. The history of *TP53* took a critical turn, when researchers discovered that it is mutated frequently in a variety of human cancers and its mutation spectrum provides insight into molecular carcinogenesis (reviewed in Levine et al.[5]/Hollstein et al.[6]/Greenblatt et al.[7]). The discovery, that *TP53* mutation is the most common genetic alteration in human cancer, lead to the studies describing the multiple function of WT p53, critical for maintaining the genetic stability and cellular homeostasis Hofseth et al.[8]/Vogelstein et al.[9] **(Fig. 1)**.

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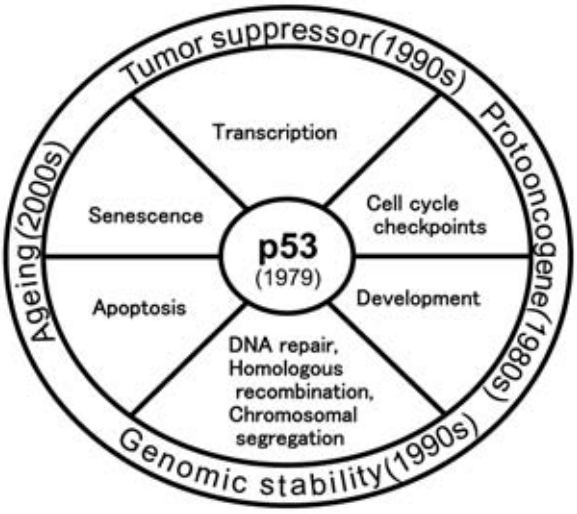

Fig. 1 Diagrammatic illustration of the history of p53 functions since its discovery in 1979. *TP53* was first described as a protooncogene and later as a tumor suppressor gene. Subsequent advancement in the studies of p53 functions recognized its role in maintaining and guarding the genomic integrity. As shown, p53 is involved in transcription, cell cycle, apoptosis, senescence, DNA repair and development.

TP53 Mutation in Human Cancer: Molecular Archaeology

More than 20,000 mutations in the *TP53* gene have accrued in IARC (International Agency for Research on Cancer) *TP53* mutation database and it is readily available for public use (http://www-p53.iarc.fr/index.html). In contrast to other tumor suppressor genes, e.g., *APC*, *BRCA1*, and *ATM*, where the most frequent types of mutations include nonsense mutations, deletions, and insertions, *TP53* shows an unusual spectrum of mutations. *TP53* predominantly shows missense mutations, in which the encoded protein contains amino acid substitutions. The missense mutation not only abrogates the tumor suppressive function, but also leads to the gain of oncogenic function by changing the repertoire of genes whose expression are controlled by this transcription factor Lane et al.[10]/Dittmer et al.[11]/Hsiao et al.[12].

Why study the *TP53* mutation spectrum? The *TP53* gene is well suited for mutational spectrum analysis for several reasons. *TP53* mutations occur in about 50% of human cancers, and so far, more than 20,000 entries have accrued in the database. The analysis of this database can provide statistically valid conclusions. The modest size of the p53 gene (11 exons, 393 amino acids) permits study of the entire coding region, and it is highly conserved in vertebrates, allowing the extrapolation of data from animal models Soussi et al.[13]. Point mutations that alter p53 function are distributed over a large region of the molecule, especially in the hydrophobic midportion Hollstein et al.[6]/Levine et al.[5]/Greenblatt et al.[14], where many base substitutions alter p53 conformation and sequence-specific transactivation activity; thus the correlation between distinct mutants and functional changes are possible.

Based on evidence from mutational spectra analyses in human cancers, a molecular linkage can be established between a specific cancer and exposure to a particular carcinogen and is well exemplified in liver, skin and lung cancers. The most prominent mutation in liver tumors, from patients living in areas with high aflatoxin B_1 exposure, is a G to T transversion at the third nucleotide of codon 249, which changes an amino acid arginine to serine Hsu et al.[15]/Bressac et al.[16]/Soini et al.[17]. A dose-dependent relationship between dietary aflatoxin B_1 intake and codon 249^{ser} p53 mutations is observed in hepatocellular carcinoma from Asia, Africa and North America (reviewed in Harris[18]). A positive correlation has been reported between the mutation load of codon 249^{ser} mutant cells in nontumorous liver and dietary AFB_1 exposure Aguilar et al.[19]. Kirk et al., reported the presence of 249^{ser} p53 mutation in the plasma of aflatoxin B_1 (AFB_1)-exposed patients with HCC and a few noncancerous cases with cirrhosis from the Gambia Kirk et al.[20]. Exposure to AFB_1 and hepatitis B virus infection produced a multiplicative effect on the risk of developing HCC in the Gambian population Kirk et al.[21]. Furthermore, the treatment of human liver cells with AFB_1 produces 249^{ser} mutation *in vitro* Aguilar et al.[22]/Mace et al.[23]. The detection of 249^{ser} p53 mutations in plasma DNA provides the possibility of early detection of HCC in high-risk populations.

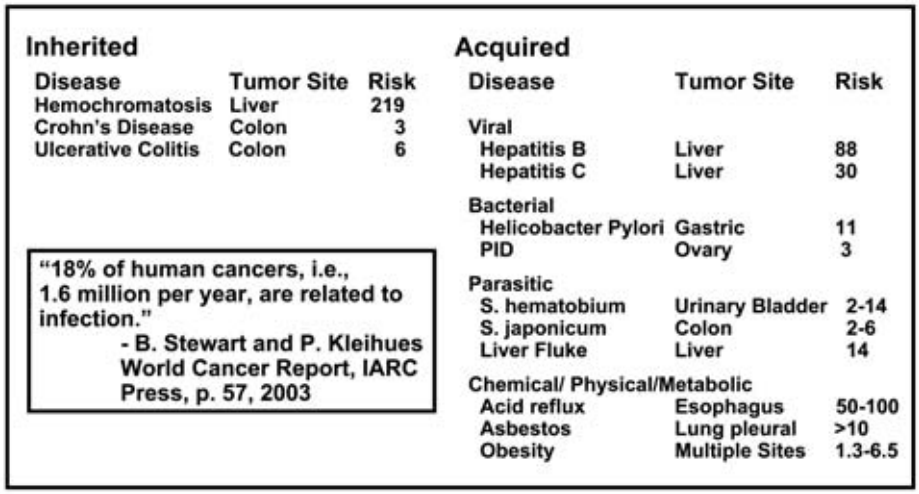

Fig. 2 Chronic inflammation and infection can increase the risk of cancer. Cancer-prone chronic inflammatory diseases can be either inherited e.g., hemochromatosis, ulcerative colitis, Crohn's disease, or acquired through infection by virus, e.g., Hepatitis B or Hepatitis C; bacteria, e.g., Helicobacter *pylori*; parasites, e.g., Schistosoma *hematobium* or Schistosoma *japonicum*; or can be caused by chemical or physical exposure and deregulation of metabolic processes.

Nitric oxide, p53 and Cancer

Chronic inflammation can increase the risk of cancer (**Fig. 2**). Nitric oxide ($NO^{\bullet}$) is a critical mediator of inflammation and is involved in the regulation of tumorigenesis (reviewed in Hussain et al.[24], **Fig. 3**). It is important to recognize that $NO^{\bullet}$ involves a complex chemistry and is extensively reviewed elsewhere Beckman et al.[25]/Hofseth et al.[26]. The ultimate effect of $NO^{\bullet}$ depends on its quantity, redox status of the cells, cell types and the presence of metals (reviewed in Hussain et al.[24]). Use of a highly sensitive assay for determining the load of *Tp53* mutations before the clonal-expansion of mutated cells in cancer-prone oxyradical overload diseases can identify individuals with an increased cancer risk and provide linkage between exposure to reactive oxygen and nitrogen species, and cancer (reviewed in Hussain et al.[24]). Noncancerous patients with oxyradical overload diseases, e.g., ulcerative colitis, hemochromatosis and Wilson disease showed an increased p53 mutation load and enhanced NOS2 expression prior to the development of cancer Hussain et al.[27]/Hussain et al.[28]/Hussain et al.[29]. These findings are consistent with the hypothesis that the generation of reactive species, for example, oxygen and nitrogen species, and aldehydes, induce a high frequency of p53 mutations in oxyradical overload disease that may contribute to the increased risk of cancer.

Our investigation of primary human colon tumors establishes a strong positive relationship between the presence of NOS2 in tumors and the frequency of G: C to A: T transitions at CpG sites. These mutations also are common in lymphoid, esophageal, head and neck, stomach, brain and breast cancers Hollstein et al.[6]/Levine et al.[5]/Greenblatt et al.[7]. Increased NOS2 expression has been demonstrated in four of these cancers Thomsen et al.[30]/Ellie et al.[31]/Ambs et al.[32]/Gallo et al.[33]. Tumor-associated $NO^{\bullet}$ production may modify DNA directly, or may inhibit DNA repair activities Wink et al.[34], such as the recently described human thymine-DNA glycosylase, which has been shown to repair G: T mismatches at CpG sites Sibghat-Ullah et al.[35]. Because $NO^{\bullet}$ production also induces p53 accumulation Messmer et al.[36]/Forrester et al.[37], the resulting growth inhibition can provide an additional strong selection pressure for mutant p53. $NO^{\bullet}$ may, therefore, act as both an endogenous initiator and promoter in human colon carcinogenesis, and specific inhibitors of NOS2, as demonstrated in an animal tumor model Thomsen et al.[38], may have

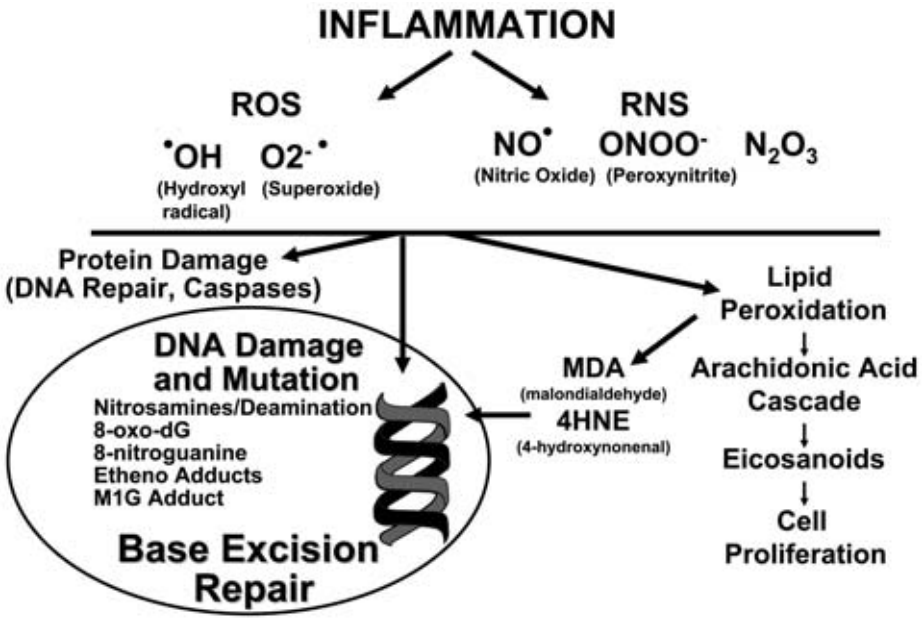

Fig. 3 Inflammation triggers a complex response involving the generation of free radicals that damage critical cellular components. The reactive oxygen or nitrogen species produced during inflammation can either directly damage DNA and modify proteins or can generate reactive aldehydes, e.g., malondialdehyde (MDA) and 4-hydroxynonenal (HNE) by initiating and enhancing lipid peroxidation. These reactive aldehydes can produce exocyclic adducts like pyrimodo [1,2-alpha]purin-10(3H)one (M1G) and ethenoadducts. These adducts can generate missense mutations in the target genes including *TP53*. The reactive species, including NO• can also cause posttranslational modification in proteins involved in DNA repair and apoptosis.

chemopreventive potential in human colorectal cancer. In addition to inducing mutations in genes, NO• can also cause global DNA damage to activate the anticarcinogenic p53 stress response pathway through posttranslational modifications Hofseth et al.[39], leading to the transcriptional transrepression of NOS2 Forrester et al.[37]/Ambs et al.[40] and transcriptional transactivation of specific genes Staib et al.[41].

Evidence from both *in vitro* and *in vivo* studies have established the existence of a feedback inhibitory loop between p53 and NOS2 Forrester et al.[37]/Ambs et al.[40]. *TP53* knockout mice produce a higher basal level of NO• when compared with WT p53 mice Ambs et al.[40]. A recent study, using mice deficient in both *TP53* and NOS2, provides evidence that p53 and NO• cooperatively regulate tumorigenesis Hussain et al.[42]. Lymphomas and leukemia developed more rapidly in TP53-/-NOS2-/- or TP53-/-NOS2+/-mice than in TP53-/-NOS2+/+ mice that were cross bred to be >99% C57BL6 background.

Structure-Function Relationship of p53

In the normal unstressed condition, p53 is maintained at a very low level by ubiquitine-mediated proteasomal degradation (reviewed in Woods et al.[43]). One of the key proteins in the regulation of p53 stability is MDM2, which is also a p53 transcriptional target, thus establishing a feedback loop Wu et al.[44]/Haupt et al.[45]. MDM2 interacts with the N-terminal region of p53 and functions as an ubiquitin ligase Fang et al.[46]/Honda et al.[47]. However, its temporary stabilization and functions are modulated by either mutations in *TP53* or posttranslational modification in a critical functional region of the protein (reviewed in Appella et al.[48]/Hussain et al.[49]). Because a majority of the missense mutations are in the sequence-specific DNA binding region of the protein, much attention has been paid to the transcription-transactivator

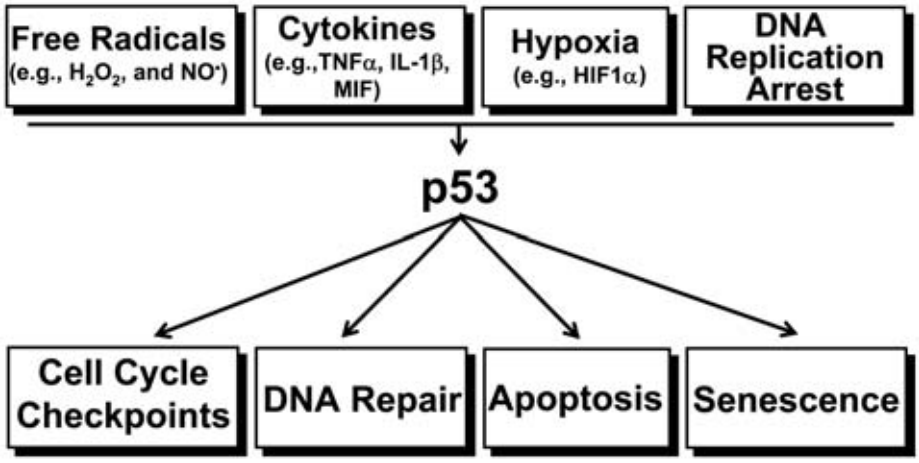

Fig. 4 Inflammatory stress activates the p53 pathway. p53 is at the crossroads of multiple cellular stress response pathways including inflammation. The inflammatory stress response is complex and well coordinated, which includes the release of a variety of cytokines, e.g., TNFα, IL-1B, MIF, and IFNγ leading to the generation of reactive oxygen and nitrogen species, activation of HIF1α and DNA replication arrest. Sensors of these stresses upstream of p53, for example, ATM (ataxia telangiectasia mutated) or ATR (ATM and RAD 3-related) kinase cascades, lead to the stabilization of p53. Following p53 stabilization several target genes are activated to protect cells from stress. These target genes are involved in many vital cellular functions e.g., cell cycle, DNA repair, apoptosis and senescence.

function of the p53. Other functional domains of p53 including those in the carboxy-terminus (COOH) region, however, can be altered due to the change in protein conformation Milner et al.[50] caused by a missense mutation in the sequence-specific DNA binding region. The positively charged COOH region contains the putative major nuclear localization signal (amino acids 316-325), the oligomerization domain (amino acids 319-360), and a DNA damage-binding domain (amino acids 318-393) Brain et al.[51]/ Wang et al.[52]/Wu et al.[53]/Bakalkin et al.[54]. Several posttranslational events have been reported to be involved in the stabilization of p53 in order to perform its designated function following stress Appella et al.[48]/Prives et al.[55]/Braithwaite et al.[56]/ Woods et al.[43]/Yee et al.[57]/Harris et al.[58]. These p53 posttranslational modifications include phosphorylation, mostly at the N-terminus and phosphorylation, acetylation and sumoylation at the C-terminus region. Several overlapping and specific posttranslational modifications occur, following a variety of stress signals that activate p53 functions (reviewed in Appella et al.[48]). The function-structure relationship revealed by the analysis of the p53 mutation spectrum Hollstein et al.[6]/Greenblatt et al.[7], its NMR and crystallographic three dimensional structure Cho et al.[59]/Clore et al.[60]/Jeffrey et al.[61], and functional studies of wild-type versus mutant p53 activity (reviewed in Vogelstein et al.[62]) have generated both hypothesis for further study and strategies for the development of rational cancer therapy.

p53 Functions

The most significant function of p53, as a tumor suppressor, emerged from the findings that mice, deficient in *TP53*, are susceptible to spontaneous tumorigenesis Donehower et al.[63] and patients with cancer-prone Li-Fraumeni's syndrome contained a germline mutation in *TP53* allele Malkin et al.[64]/ Srivastava et al.[65]. p53 is involved in several important cellular functions that are responsible for maintaining cellular homeostasis and is convincingly at the crossroads of the cellular responses to a variety of stresses caused either endogenously or by external exposure (reviewed in Harris[66]/Hofseth et al.[8]/Braithwaite et al.[56]/Woods et al.[43]/Harris et al.[58]/

Lane et al.[67]/Vogelstein et al.[9]) **(Fig. 4)**. The widely studied p53-regulated responses include apoptosis, cell cycle arrest, DNA repair, recombination and senescence. p53 functions largely as a transcription factor Polyak et al.[68]/Yu et al.[69], however, it may also have transcriptionally independent functions Caelles et al.[70]/Haupt et al.[71]. The wide array of p53 responses following stress are accomplished by a well-coordinated network, which involves several negative and positive feedback loops (reviewed in Harris et al.[58]).

p53 and Apoptosis

The role of p53 in apoptosis is studied extensively and has been linked to its tumor suppressor activity (reviewed in Yee et al.[57]). In p53-null transgenic mice, tumor progression is correlated with a loss of apoptosis Parant et al.[72]. p53 transcriptionally transactivates or transrepresses many different genes to trigger apoptotic responses involving both extrinsic and intrinsic pathways Fridman et al.[73]. Among other factors, it is the balance between proapoptotic and anti-apoptotic signals that determines the threshold of apoptosis. The p53-mediated transactivation of apoptosis-related genes include proapoptotic Bcl-2 family members e.g., Bax, Puma, Noxa, and Bid, which leads to the mitochondrial membrane depolarization in the intrinsic pathway; apoptotic protease activating factor-1 (APAF-1), a major component of apoptosome; and Fas/CD95, death receptor 4 (DR4), and DR5, components of the extrinsic apoptotic pathways. A mechanism involving oxidative stress in p53-mediated apoptosis has been described following the transactivation of several redox-related genes by p53, referred to as p53-inducible genes (PIGs) Polyak et al.[68]. p53-mediated upregulation of the antioxidant enzyme, manganese superoxide dismutase (MnSOD), can also create an imbalance in antioxidant enzyme machinery leading to oxidative stress and apoptosis Hussain et al.[74]. In addition to the mechanism involving p 53-dependent transactivation of apoptotic genes, transcription-independent mechanisms have also been suggested in p53-mediated apoptosis Haupt et al.[75]/Yee et al.[57]. Recent evidence has suggested that p53 can act as a functional homologue of the BH3-only protein (reviewed in Yee et al.[57]). p53 can also directly bind to and inhibit the Bcl-XL and Bcl2 proteins, leading to the release of cytochrome C Mihara et al.[76] and the initiation of caspase cascade. Given the fact that different components aid in the p53-mediated apoptotic response, the question always remains i.e., which one of these components is the essential player? There is strong evidence suggesting PUMA as a critical component of p53-mediated apoptosis Jeffers et al.[77]/Chipuk et al.[78]. However, in other cell-types, NOXA seems to be equally significant Villunger et al.[79]. Based on the complexity of the apoptotic process and a large number of transcriptional and nontranscriptional downstream targets of p53, it would be appropriate to consider not only one, but also a set of components and their coordinated effects to be responsible for p53-mediated apoptosis in one or a class of cell types Yee et al.[57].

p53 and DNA Repair

Although a key player, based on available evidence, it can be argued that p53's role in inducing apoptosis does not completely suffice for its tumor suppressing function. Therefore, other p53 functions e.g., cell cycle arrest, maintenance of genomic stability, DNA repair and senescence can be of utmost significance in the tumor suppressor function. p53 modulates DNA repair processes that include nucleotide excision repair (NER), Base excision repair (BER), nonhomologous end-joining (NHEJ) and homologous recombination by both transactivation-dependent and -independent pathways and, therefore, is suggested as a molecular node among the up-stream signaling cascade and down-stream DNA repair and recombination pathways (reviewed in Sengupta et al.[80]). The loss of p53 reduces the repair of UV-induced DNA damage in human cells Wang et al.[81]/Smith et al.[82]/Ford et al.[83]. p53 regulates the transcription of $p48^{DDB2}$ and xeroderma pigmentosum complementation group C (XPC) Hwang et al.[84]/Adimoolam et al.[85]. $p48^{DDB2}$ is one of the two subunits of UV-damage DNA binding

protein (UV-DDB), whereas, XPC is a part of the global genomic repair (GGR)-specific complex that identifies the altered base pairing. Furthermore, $p48^{DDB2}$ regulates the p53 level following UV-damage, and thereby, suggests the existence of a positive feedback loop Adimoolam et al.[86]. Wild-type p53, but not the mutant protein, facilitates the recruitment of XPC and the TFIIH complex to the UV-damaged sites Wang et al.[87]. In addition to the role of p53 in BER involving transcriptional transactivation of genes, it also participates in a transactivation-independent manner. p53 modulates helicase activity of TFIIH complex by binding to XPB and XPD Wang et al.[81]/Leveillard et al.[88], thereby affecting the NER.

There are convincing evidence suggesting the involvement of p53 in the regulation of homologous recombination (HR) (reviewed in Sengupta et al.[80]). An increased frequency of HR is reported in different developmental stages of mice lacking p53 Bishop et al.[89]. Expression of p53 mutants enhanced HR, while WT p53 reduced the frequency of HR Akyuz et al.[90]. p53-mediated regulation of HR can be independent of its activity as a transcription factor Dudenhoffer et al.[91]/Willers et al.[92]. p53 can physically bind to RAD51 and RAD54, major components of HR machinery, and controls the level of HR Sengupta et al.[93]/Linke et al.[94]. Mutation in the *Tp53* hotspot codon 273 reduces the capacity of p53 protein to bind with RAD51-DNA complexes Buchhop et al.[95]/Susse et al.[96]. p53 interaction with RAD51 plays a key role in presynaptic, synaptic as well as postsynaptic phases of HR (reviewed in Sengupta et al.[80]).

p53 and Senescence

Cellular senescence confers a permanent withdrawal from the cell cycle and can be induced in response to various stresses. These stimuli include DNA damage, oncogenic signals, dysfunctional telomeres and epigenetic changes in chromatin (reviewed in Campisi[97]). Senescence can contribute to the suppression of cancer, however, senescent cells can also stimulate the proliferation and progression of preneoplastic cells Campisi[98]/Green et al.[99]/Campisi[100]. Senescence can also produce aging-related pathology (reviewed in Campisi[97]). Cellular senescence is largely regulated by the p53 Wahl et al.[101] and p16/Rb Beausejour et al.[102] pathways. The p53 pathway can be used by several different stimuli for senescence including dysfunctional telomere and RAS mitogenic signals involving reactive oxygen species Itahana et al.[103]/d'Adda et al.[104]/Pearson et al.[105]/Serrano et al.[106]. Dependency of some of these stimuli of senescence on p53 pathways is shown by the reversal of senescent growth arrest with the loss of p53 function, however, the reversal is not achieved in all cell types and their resistance to reversal depended on p16 Beausejour et al.[102]. The p53-mediated pathway to senescence involves the transcription of p53-dependent genes including p21, whereas, Rb pathways involve p16 induction, followed by Rb activation and chromatin reorganization, causing the suppression of E2F target genes Campisi[97]. pRb-mediated senescence is irreversible and cannot be reversed by inactivating p53 or pRb.

Concluding Remarks

Over the course of evolution, mammalian cells have acquired an intricate network of protective mechanisms to safeguard the genomic integrity. One of the prominent molecules is p53, which has earned its title as "guardian of the genome" by its diverse involvement in processes critical for guarding and fixing the genomic integrity and cellular homeostasis Lane[107]. One of the serious consequences due to a failure in the safety networks is the development of cancer. The fact that the p53 pathway is defective in the majority of human cancers, underscores its importance in protecting the cells from genetic, biochemical and physiological dysregulation that can contribute to tumor development. The identification of stresses and the mechanisms responsible for the stabilization of p53 and the subsequent activation of p53-dependent downstream pathways have placed the p53 protein at the crossroads of cellular stress response pathways. The elucidation of the p53-mediated pathways involving growth arrest, apoptosis, DNA repair, senescence, and

differentiation provides numerous molecular targets for intervention and therapy.

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