p53-Mediated Apoptosis and Genomic Instability Diseases

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Mutations in several DExH-containing DNA helicases, including *XPD*, *XPB*, *WRN*, and *BLM*, are associated with rare familial cancer syndromes characterized by genomic instability and cancer susceptibility. Known cellular activities of these helicases include DNA replication, repair, recombination, and/or transcription. The p53 tumor suppressor is a regulator of cellular responses to stress, and is biochemically involved in the induction of cell-cycle arrest, apoptosis and DNA repair, all of which contribute to maintenance of genomic integrity. Physical and functional interactions of p53 with DExH-containing DNA helicases have been described. We propose that such interactions could be compromised in inherited disorders and contribute to their cancer susceptibility. In particular, the role of DNA helicases in p53-mediated apoptotic pathways is reviewed.

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Since the tumor suppressor gene product p53 was first discovered two decades ago (1, 2), it has gained unique notoriety as a guardian of the genome for its role as a gatekeeper that controls cell growth and death after DNA damage (3–6). Considerable effort has been invested in deciphering the specific biochemical pathways in which p53 is involved, particularly since *p53* is mutated in over half of all human cancers (7, 8). The p53 mutational spectrum in human tumors has prompted hypotheses concerning the etiology of human cancer and the functional domains of the p53 protein (7, 9). Furthermore, the familial cancer syndrome, Li-Fraumeni (LFS), has been linked to germline *p53* mutations (10) and to germline mutations in genes that are part of the p53 pathway, such as *hCHK2* (11). Such mutations have been correlated directly with genomic instability in the form of chromosomal aberrations, infidelity of centrosome duplication, and aneuploidy, which may enhance the rate of accumulation of secondary mutations (including loss of a remaining wild-type *p53* allele) and thus contribute to the development of cancer (12).

It is well established that p53 is a central regulator of cellular responses to stress (13–15), leading to reversible or permanent cell-cycle arrest, DNA repair, or apoptosis. There is a low level of latent p53 present in normal cells, but upon a variety of stresses involving DNA-damaging and non-DNA-damaging agents (such as radiation, chemotherapeutic drugs, ribonucleotide depletion, hypoxia, and oncogene expression) p53 is post-transcriptionally modified by kinases and acetylases and accumulates in the nucleus. Recent reports have uncovered a novel type of modification that involves conjugation to the ubiquitin-like protein SUMO-1 (16, 17). Upon its stabilization and subsequent activation, p53 directs the sequence-specific transcriptional activation of target genes, including *mdm2*, a specific antagonist of p53 activity (18), $p21^{Cip1/WAF-1/Sdi1}$ ($p21^{WAF-1}$), a cyclin-dependent kinase inhibitor (19–21), *GADD45*, a gene associated with growth suppression and DNA repair (22, 23), *bax*, whose product dimerizes with Bcl-2 and prevents Bcl-2 from blocking apoptosis (24), *NOXA*, a BH3-only member of the Bcl-2 family and putative mediator of apoptosis induced through p53 (25), and *p53R2*, a novel ribonucleotide reductase involved in the p53 checkpoint for repair of damaged DNA (26). Active p53 can also function as a transcriptional repressor, without known DNA sequence specificity, and possibly through interaction with histone deacetylases (27). Recently, by using novel technologies such as SAGE and cDNA microarray hybridization, many additional genes that are transcriptionally regulated by p53 have been identified (28, 29). The sequence-specific transcriptional activation function of p53 is thought to play a major role

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in p53-mediated pathways, partly because most *p53* mutations in human cancers are missense and map to the central portion or DNA-binding domain of the protein, leading to its loss of function as a transcription factor. However, naturally occurring mutations in the COOH-terminal portion of p53 can affect its cell growth inhibitory and apoptotic activities while leaving an intact DNA-binding domain (30). These results and those of others (31–33) indicate that the transactivation-independent activities of p53 also contribute to its cellular functions, including tumor suppression. In addition, a variety of proteins bind to p53 (34). In this regard, several human DNA helicases form complexes with p53 in vivo and in vitro. The functional consequences of such interactions will be the focus of this brief review.

PHYSICAL AND FUNCTIONAL INTERACTIONS OF p53 WITH DExH-CONTAINING DNA HELICASES

The first DNA helicase found to be associated with p53 was the SV40 large T-antigen, which prompted the discovery of the p53 molecule itself (1, 2). It was later reported that wild-type, but not mutant, p53 inhibited the unwinding activity of SV40 T antigen (35). A number of cellular DNA helicases have been associated with some rare familial cancer syndromes characterized by genomic instability and cancer susceptibility reminiscent of LFS (36). Taken together, these observations led us to the hypothesis that there could be functions of p53 regulated by its physical and functional interactions with DExH-containing DNA helicases, and that such functions could be compromised in inherited disorders and contribute to their cancer susceptibility.

NER-associated helicases: XPD and XPB

Nucleotide excision repair (NER) is the major pathway for repair of bulky DNA adducts and lesions caused by ultraviolet (UV) irradiation (37). Mutations in genes associated with NER have been found in the rare human syndrome Xeroderma pigmentosum (XP). UV sensitivity and slow repair of UV damage characterize cells from individuals with this disease and lead to lesions and cancers in sunlight-exposed areas of the skin. The genes complementing the NER defect in two of the seven XP complementation groups, namely *XPD* and *XPB*, encode DExH-containing DNA helicases (38, 39). Both helicases are found as subunits of the transcription/repair complex TFIIH, and they are essential when mammalian NER is reconstituted in vitro with purified components (40). Cockayne syndrome (CS) and Trichothiodystrophy (TTD) are rare inherited disorders which are distinct from XP, but surprisingly show, in some cases, mutations in the *XPB* or *XPD* helicases. Although cells from CS and TTD individuals have increased UV sensitivity, the patients are not cancer-prone. A subset of the CS cases is caused by mutation of the *CSB* gene. CSB is also a DExH-containing putative DNA helicase that is involved in the transcription-coupled repair function of NER, and possibly in transcription elongation (41).

In testing the hypothesis that p53 may modulate DNA helicase activities as part of its stress-activated responses, our laboratory was the first to show that the carboxyl-terminal domain of p53 binds to XPD, XPB, and CSB and that wild-type, but not mutant, p53 inhibits the helicase activity of XPD and XPB (42). Furthermore, LFS fibroblasts, which contain a mutated p53, show a reduced efficiency of NER (42). These findings have been confirmed and extended by others to show that expression of wild-type p53 can enhance NER in p53-null fibroblasts (43–45). In addition, overexpression of the p53 carboxyl-terminus can cause metaphase fragility at the same loci as loss of CSB, possibly through inhibition of CSB function as a transcription elongation factor (41). Thus, p53 can modulate the activities of these helicases, as well as the functions associated with them, including DNA repair. Base excision repair (BER), a DNA repair pathway specialized in the excision of single damaged-base residues, is also enhanced by p53 (46), and Ref-1/APE1, a multi-functional protein that serves as the abasic (A/P) endonuclease in BER, can regulate transactivation and the apoptotic functions of p53 (47). In addition to contributing to the repair of DNA damage, p53 can also trigger cell-cycle checkpoints or induce cell death by apoptosis to avoid the fixation of mutations that may arise from unrepaired DNA damage. Increased expression of wild-type, but not mutant, p53 via microinjection of a CMV-driven expression vector can induce apoptosis efficiently in normal fibroblasts. Using this technique, we showed that the induction of p53-dependent apoptosis is attenuated in XPD and XPB (but not TTD) fibroblasts, and can be rescued by wild-type *XPD* or *XPB*, respectively (33). Interestingly, p53 retains its ability to transactivate endogenous $p21^{WAF\text{-}1}$ in XPD and XPB cells, and the carboxyl-terminus of p53 is sufficient to induce apoptosis in normal human fibroblasts (33). These results indicate that a direct protein–protein interaction involving the carboxyl-terminus of p53 and the DNA helicases could contribute to a p53-mediated apoptotic pathway. Presumably, impairment of p53-mediated apoptosis in human syndromes characterized by helicase deficiencies enhances genomic instability and mutagenesis.

In further support of the physiological relevance of p53-helicase binding, induction of apoptosis through activation of endogenous p53 by DNA damage leads to functional interactions with DNA helicases. XPD lymphoblastoid cell lines (LCLs) exhibit a reduced and delayed apoptotic response after treatment with doxorubicin, a DNA-damaging agent frequently used in cancer therapy and capable of inducing apoptosis by a p53-dependent pathway (48). We confirmed the dependence of dox-

orubicin-induced apoptosis on p53 stabilization in LCLs through the expression of the HPV16 E6 oncoprotein, which hastens p53 degradation by the ubiquitin system, and prevents caspase activation and apoptosis after drug treatment. The accumulation of p53 was observed concurrently at time-points soon after doxorubicin treatment in normal and XPD LCLs, indicating that the molecular basis for the delayed response to doxorubicin lies in factors downstream of p53 stabilization. In addition, receptor-mediated apoptosis is unaffected in XPD LCLs. It has been suggested that XPD mutations can affect TFIIH-directed gene transcription (49). However, recent studies on the biochemistry of transcription by TFIIH have uncovered a critical enzymatic role for XPB in transcription initiation and promoter escape and a structural role for XPD in promoter escape (50), and indicated that XPD activity may not be required for transcription (51). Consistently, expression of the p53 target genes $p21^{WAF-1}$, *GADD45* and *bax* was up-regulated similarly by doxorubicin in normal and XPD LCLs, and no XPD genotype-specific differences were observed in the transcription of the apoptosis effector genes Flice, FasL, Fas, Fadd, DR3, FAP, FAF, TRAIL, TNFRp55, TRADD, RIP, Granzyme B, Mch2, ICErel-III, ICH-1, Mch3, ICE, or ICELap6 (48). A more extensive analysis is now being conducted by cDNA microarray expression hybridization, using the NCI Human Oncochip developed at the NCI Advanced Technology Center, to analyze the effect of XPD and XPB mutations in gene transcription after DNA damage. Our initial results indicate that XPD mutations do not affect gene transcription overall, either in basal or drug-challenged conditions, and further support the hypothesis that a transactivation-independent p53-mediated apoptotic pathway is deficient in XPD mutants (Robles, unpublished).

RecQ family helicases: WRN and BLM

A distinct subfamily within the DExH-containing DNA helicases is that constituted by *E. coli* RECQ and its homologues. Several human homologues of *RECQ* have been cloned, and germline mutations in three of them, namely *WRN*, *BLM* and *RECQ4*, are responsible for the clinically distinct cancer susceptibility diseases Werner syndrome (WS), Bloom syndrome (BS) and Rothmund-Thomson syndrome (RTS), respectively. Genomic instability is a common feature found in cells from these diseases. This is manifested as a high rate of sister chromatid exchanges in BS cells as well as acquired chromosomal mosaicism in WS and RTS cells (52).

Recently, we reported that, through its carboxyl-terminus, p53 can bind to WRN in vivo and in vitro (53). This finding has been confirmed and extended to show that p53 binds to the carboxyl-terminus of WRN, and that WRN is a co-activator of p53-mediated transcription (54). Similar to our findings with XPD and XPB fibroblasts, we showed that p53-mediated apoptosis is attenuated in WS fibroblasts, as well (53). Primary fibroblasts from WS individuals display a characteristic premature ageing/senescence phenotype in culture (55) that can interfere potentially with the induction of apoptosis. For this reason, only early passage WS fibroblasts were used for p53 microinjection experiments, and the percentage of senescent cells was confirmed by staining for senescence-associated β-galactosidase activity (56). Time-course studies showed a persistent reduction in the percentage of p53-mediated apoptosis in WS fibroblasts when compared with normal fibroblasts. However, both types of fibroblasts were equally sensitive to p53-independent induction of apoptosis by the microinjection of a caspase-1 expression vector or by treatment with staurosporine. Furthermore, co-microinjection of a wild-type *WRN* expression vector, but not a wild-type *XPD* expression vector, restored the sensitivity of WS fibroblasts to p53-mediated apoptosis. These data provide genetic evidence that WRN contributes to p53-mediated apoptosis independently of other DNA helicase(s) (53).

BS fibroblasts are also resistant to apoptosis induced by ectopic expression of p53, and the introduction of wild-type *BLM* restores them to normal sensitivity (Wang et al., submitted for publication). LCLs derived from BS donors are resistant to either γ-radiation or doxorubicin-induced cell killing, both of which are largely dependent on p53 (48, 57), and sensitivity can be restored by stable expression of wild-type *BLM*. In contrast, BS cells have a normal *Fas*-mediated apoptosis, normal accumulation of p53, normal induction of p53-responsive genes $p21^{WAF-1}$, *GADD45* and *bax*, and normal G1-S and G2-M cell-cycle arrest in response to DNA damage (Wang et al., submitted for publication). The carboxyl-terminus of p53 mediates its binding to BLM in vitro and stabilization of p53 by γ-radiation enhances such binding in vivo (Wang et al., submitted for publication).

MECHANISTIC INSIGHTS

Currently, we are focusing on the identification of the pathway(s) that bring together p53 and the DExH-containing DNA helicases. There are several possible approaches to elucidating these interactions. Not only is p53 involved in cellular processes other than apoptosis, but also XPB, XPD, WRN and BLM each participate in specific aspects of replication, repair, recombination, and/or transcription. One possible mechanism of p53 and BLM interaction in the induction of apoptosis is starting to emerge. BLM localizes in nuclear foci at promyelocytic leukemia (PML) nuclear bodies (NBs) (58, 59), structures that also contain PML, hypophosphorylated Rb, SUMO-1, p53, and other proteins (60, 61). PML has been implicated in several apoptotic pathways (62, 63). Cells from LFS patients (carrying *p53* germline mutations) and LCLs without functional p53 have a decreased accumulation of BLM in NBs, whereas isogenic lines with functional p53

Table 1

Members of the DExH-containing DNA helicase superfamily and their physical and functional association with p53

DNA helicase	Human disorder	Helicase activity	Cancer proneness	p53-mediated		
				Apoptosis	Inhibition of helicase activity	Binding to helicase
XPB	Xeroderma pigmentosum, group B	3′→5′	Yes	Reduced	Yes	Yes
XPD	Xeroderma pigmentosum, group D	5′→3′	Yes	Reduced	Yes	Yes
CSB	Cockayne syndrome	No	No	Normal **	ND	Yes
RECQ2/BLM	Bloom syndrome	3′→5′	Yes	Reduced	Yes **	Yes
RECQ3/WRN	Werner syndrome	3′→5′	Yes	Reduced	Yes **	Yes
RECQ4/RTS	Rothmund-Thomson syndrome	ND	Yes	ND	ND	ND

ND = not done.
** Unpublished data.

exhibit normal accumulation. These results indicate that the localization of BLM to NBs may be dependent on its binding to p53 in such a way, that p53 may control intracellular trafficking of BLM to the NBs (Wang et al., submitted for publication). Further studies need to be done to confirm this novel function of p53 and to clarify how BLM and other DNA helicases may cooperate to induce apoptosis.

CONCLUSION

The remarkable similarities between the different DExH-containing DNA helicases in their functional and physical interactions with p53 lead us to speculate that a common defect in the p53-dependent apoptosis pathway may contribute to the cancer predisposition associated with mutations in such helicases, including XPB, XPD, WRN and BLM (see Table 1). Our primary hypothesis is that DNA damage triggers p53 activation as part of the cellular stress response directed towards the preservation of genomic integrity. Signal transduction pathways and enzymatic activities downstream of p53 can be dependent on p53-mediated gene transcription and/or protein–protein interactions involving, for instance, DNA helicases. In this model, p53 may act as a scaffolding protein, sensing specific types of DNA damage and alternative DNA structures (e.g., Holliday junction and tetraplex DNA), and participating in the assembly of repair or apoptotic complexes at the site of a lesion. Site-specific post-translational modifications of p53, such as phosphorylation or acetylation may dictate the ultimate cellular response to the damage. Modified p53 may be released from a repair complex(es), thus enabling the repair of potentially mutagenic lesions, as well as activating a cell-cycle arrest response through gene transcription. In cases of more severe damage or in certain cell types, p53 may remain bound to those complexes and, possibly through the inhibition of associated helicase activities, halt attempts to repair damaged DNA, and thus favor apoptosis (see Fig. 1).

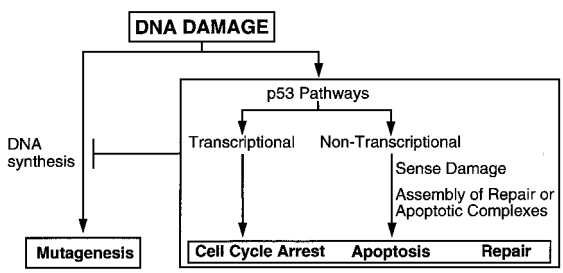

Fig. 1. p53-mediated cellular responses to DNA damage. DNA damage triggers p53 activation as part of the cellular stress response directed towards the preservation of genomic integrity. Signal transduction pathways and enzymatic activities downstream of p53 can be dependent on p53-mediated gene transcription and/or protein-protein interactions.

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REFERENCES

1. Lane DP, Crawford LV. T antigen is bound to a host protein in SV40-transformed cells. Nature 1979; 278: 261–3.
2. Linzer DI, Levine AJ. Characterization of a 54K dalton cellular SV40 tumor antigen present in SV40-transformed cells and uninfected embryonal carcinoma cells. Cell 1979; 17: 43–52.
3. Lane DP. Cancer. p53, guardian of the genome. Nature 1992; 358: 15–6.
4. Levine AJ. p53, the cellular gatekeeper for growth and division. Cell 1997; 88: 323–31.
5. Oren M. Regulation of the p53 tumor suppressor protein. J Biol Chem 1999; 274: 36031–4.
6. Prives C. Signaling to p53: breaking the MDM2-p53 circuit. Cell 1998; 95: 5–8.

7. Hollstein M, Sidransky D, Vogelstein B, Harris CC. p53 mutations in human cancers. Science 1991; 253: 49–53.
8. Levine AJ, Momand J, Finlay CA. The p53 tumour suppressor gene. Nature 1991; 351: 453–6.
9. Hussain SP, Harris CC. Molecular epidemiology of human cancer: contribution of mutation spectra studies of tumor suppressor genes. Cancer Res 1998; 58: 4023–37.
10. Malkin D, Li FP, Strong LC, et al. Germ line p53 mutations in a familial syndrome of breast cancer, sarcomas, and other neoplasms. Science 1990; 250: 1233–8.
11. Bell DW, Varley JM, Szydlo TE, et al. Heterozygous germ line hCHK2 mutations in Li-Fraumeni syndrome. Science 1999; 286: 2528–31.
12. Boyle JM, Mitchell EL, Greaves MJ, et al. Chromosome instability is a predominant trait of fibroblasts from Li-Fraumeni families. Br J Cancer 1998; 77: 2181–92.
13. Vogelstein B, Kinzler KW. p53 function and dysfunction. Cell 1992; 70: 523–6.
14. Giaccia AJ, Kastan MB. The complexity of p53 modulation: emerging patterns from divergent signals. Genes Dev 1998; 12: 2973–83.
15. Ljungman M. Dial 9-1-1 for p53: mechanisms of p53 activation by cellular stress. Neoplasia 2000; 2: 208–25.
16. Rodriguez MS, Desterro JM, Lain S, Midgley CA, Lane DP, Hay RT. SUMO-1 modification activates the transcriptional response of p53. EMBO J 1999; 18: 6455–61.
17. Gostissa M, Hengstermann A, Fogal V, et al. Activation of p53 by conjugation to the ubiquitin-like protein SUMO-1. EMBO J 1999; 18: 6462–71.
18. Barak Y, Juven T, Haffner R, Oren M. mdm2 expression is induced by wild type p53 activity. EMBO J 1993; 12: 461–8.
19. Harper JW, Adami GR, Wei N, Keyomarsi K, Elledge SJ. The p21 cdk-interacting protein Cip1 is a potent inhibitor of G1 cyclin-dependent kinases. Cell 1993; 75: 805–16.
20. El-Deiry WS, Tokino T, Velculescu VE, et al. WAF1, a potential mediator of p53 tumor suppression. Cell 1993; 75: 817–25.
21. Noda A, Ning Y, Venable SF, Pereira-Smith OM, Smith JR. Cloning of senescent cell-derived inhibitors of DNA synthesis using an expression screen. Exp Cell Res 1994; 211: 90–8.
22. Smith ML, Chen IT, Zhan Q, et al. Interaction of the p53-regulated protein Gadd45 with proliferating cell nuclear antigen. Science 1994; 266: 1376–80.
23. Wang XW, Zhan Q, Coursen JD, et al. GADD45 induction of a G2/M cell cycle checkpoint. Proc Natl Acad Sci USA 1999; 96: 3706–11.
24. Miyashita T, Reed JC. Tumor suppressor p53 is a direct transcriptional activator of the human bax gene. Cell 1995; 80: 293–9.
25. Oda E, Ohki R, Murasawa H, et al. Noxa, a BH3-only member of the Bcl-2 family and candidate mediator of p53-induced apoptosis. Science 2000; 288: 1053–8.
26. Tanaka H, Arakawa H, Yamaguchi T, et al. A ribonucleotide reductase gene involved in a p53-dependent cell-cycle checkpoint for DNA damage. Nature 2000; 404: 42–9.
27. Murphy M, Ahn J, Walker KK, et al. Transcriptional repression by wild-type p53 utilizes histone deacetylases, mediated by interaction with mSin3a. Genes Dev 1999; 13: 2490–501.
28. Polyak K, Xia Y, Zweier JL, Kinzler KW, Vogelstein B. A model for p53-induced apoptosis. Nature 1997; 389: 300–5.
29. Zhao R, Gish K, Murphy M, et al. Analysis of p53-regulated gene expression patterns using oligonucleotide arrays. Genes Dev 2000; 14: 981–93.
30. Zhou X, Wang XW, Xu L, et al. COOH-terminal domain of p53 modulates p53-mediated transcriptional transactivation, cell growth, and apoptosis. Cancer Res 1999; 59: 843–8.
31. Caelles C, Helmberg A, Karin M. p53-dependent apoptosis in the absence of transcriptional activation of p53-target genes. Nature 1994; 370: 220–3.
32. Haupt Y, Rowan S, Shaulian E, Vousden KH, Oren M. Induction of apoptosis in HeLa cells by transactivation-deficient p53. Genes Dev 1995; 9: 2170–83.
33. Wang XW, Vermeulen W, Coursen JD, et al. The XPB and XPD helicases are components of the p53-mediated apoptosis pathway. Genes Dev 1996; 10: 1219–32.
34. Prives C, Hall PA. The p53 pathway. J Pathol 1999; 187: 112–26.
35. Friedman PN, Kern SE, Vogelstein B, Prives C. Wild-type, but not mutant, human p53 proteins inhibit the replication activities of simian virus 40 large tumor antigen. Proc Natl Acad Sci USA 1990; 87: 9275–9.
36. Ellis NA. DNA helicases in inherited human disorders. Curr Opin Genet Dev 1997; 7: 354–63.
37. de Boer J, Hoeijmakers JH. Nucleotide excision repair and human syndromes. Carcinogenesis 2000; 21: 453–60.
38. Weeda G, van Ham RC, Vermeulen W, Bootsma D, van der Eb AJ, Hoeijmakers JH. A presumed DNA helicase encoded by ERCC-3 is involved in the human repair disorders xeroderma pigmentosum and Cockayne's syndrome. Cell 1990; 62: 777–91.
39. Flejter WL, McDaniel LD, Johns D, Friedberg EC, Schultz RA. Correction of xeroderma pigmentosum complementation group D mutant cell phenotypes by chromosome and gene transfer: involvement of the human ERCC2 DNA repair gene. Proc Natl Acad Sci USA 1992; 89: 261–5.
40. Aboussekhra A, Biggerstaff M, Shivji MK, et al. Mammalian DNA nucleotide excision repair reconstituted with purified protein components. Cell 1995; 80: 859–68.
41. Yu A, Fan HY, Liao D, Bailey AD, Weiner AM. Activation of p53 or loss of the Cockayne syndrome group B repair protein causes metaphase fragility of human U1, U2, and 5S genes. Mol Cell 2000; 5: 801–10.
42. Wang XW, Yeh H, Schaeffer L, et al. p53 modulation of TFIIH-associated nucleotide excision repair activity. Nat Genet 1995; 10: 188–95.
43. Leveillard T, Andera L, Bissonnette N, et al. Functional interactions between p53 and the TFIIH complex are affected by tumour-associated mutations. EMBO J 1996; 15: 1615–24.
44. Ford JM, Hanawalt PC. Li-Fraumeni syndrome fibroblasts homozygous for p53 mutations are deficient in global DNA repair but exhibit normal transcription-coupled repair and enhanced UV-resistance. Proc Natl Acad Sci USA 1995; 92: 8876–80.
45. Ford JM, Hanawalt PC. Expression of wild-type p53 is required for efficient global genomic nucleotide excision repair in UV-irradiated human fibroblasts. J Biol Chem 1997; 272: 28073–80.
46. Offer H, Wolkowicz R, Matas D, Blumenstein S, Livneh Z, Rotter V. Direct involvement of p53 in the base excision repair pathway of the DNA repair machinery. FEBS Lett 1999; 450: 197–204.
47. Gaiddon C, Moorthy NC, Prives C. Ref-1 regulates the transactivation and pro-apoptotic functions of p53 in vivo. EMBO J 1999; 18: 5609–21.
48. Robles AI, Wang XW, Harris CC. Drug-induced apoptosis is delayed and reduced in XPD lymphoblastoid cell lines: possible role of TFIIH in p53-mediated apoptotic cell death. Oncogene 1999; 18: 4681–8.
49. Coin F, Bergmann E, Tremeau-Bravard A, Egly JM. Mutations in XPB and XPD helicases found in xeroderma pigmentosum patients impair the transcription function of TFIIH. EMBO J 1999; 18: 1357–66.

50. Bradsher J, Coin F, Egly JM. Distinct roles for the helicases of TFIIH in transcript initiation and promoter escape. J Biol Chem 2000; 275: 2532–8.
51. Winkler GS, Araujo SJ, Fiedler U, et al. TFIIH with inactive XPD helicase functions in transcription initiation but is defective in DNA repair. J Biol Chem 2000; 275: 4258–66.
52. Lindor NM, Furuichi Y, Kitao S, Shimamoto A, Arndt C, Jalal S. Rothmund-Thomson syndrome due to RECQ4 helicase mutations: report and clinical and molecular comparisons with Bloom syndrome and Werner syndrome. Am J Med Genet 2000; 90: 223–8.
53. Spillare EA, Robles AI, Wang XW, Shen JC, Schellenberg GD, Harris CC. p53-mediated apoptosis is attenuated in Werner syndrome cells. Genes Dev 1999; 13: 1355–60.
54. Blander G, Kipnis J, Leal JF, Yu CE, Schellenberg GD, Oren M. Physical and functional interaction between p53 and the Werner's syndrome protein. J Biol Chem 1999; 274: 29463–9.
55. Thweatt R, Goldstein S. Werner syndrome and biological ageing: a molecular genetic hypothesis. Bioessays 1993; 15: 421–6.
56. Dimri GP, Lee X, Basile G, et al. A biomarker that identifies senescent human cells in culture and in aging skin in vivo. Proc Natl Acad Sci USA 1995; 92: 9363–7.
57. Little JB, Nagasawa H, Keng PC, Yu Y, Li CY. Absence of radiation-induced G1 arrest in two closely related human lymphoblast cell lines that differ in p53 status. J Biol Chem 1995; 270: 11033–6.
58. Zhong S, Hu P, Ye TZ, Stan R, Ellis NA, Pandolfi PP. A role for PML and the nuclear body in genomic stability. Oncogene 1999; 18: 7941–7.
59. Yankiwski V, Marciniak RA, Guarente L, Neff NF. Nuclear structure in normal and Bloom syndrome cells. Proc Natl Acad Sci USA 2000; 97: 5214–9.
60. Hodges M, Tissot C, Howe K, Grimwade D, Freemont PS. Structure, organization, and dynamics of promyelocytic leukemia protein nuclear bodies. Am J Hum Genet 1998; 63: 297–304.
61. Pearson M, Carbone R, Sebastiani C, et al. PML regulates p53 acetylation and premature senescence induced by oncogenic Ras. Nature 2000; 406: 207–10.
62. Wang ZG, Ruggero D, Ronchetti S, et al. PML is essential for multiple apoptotic pathways. Nat Genet 1998; 20: 266–72.
63. Quignon F, De Bels F, Koken M, Feunteun J, Ameisen JC, de The H. PML induces a novel caspase-independent death process. Nat Genet 1998; 20: 259–65.