[Cell Cycle 6:9, 1006-1010, 1 May 2007]; ©2007 Landes Bioscience

Extra View

p53: Guardian of the Genome and Policeman of the Oncogenes

Alejo Efeyan

Manuel Serrano*

Tumor Suppression Group; Spanish National Cancer Research Center (CNIO); Madrid, Spain

*Correspondence to: Manuel Serrano; Spanish National Cancer Research Center (CNIO); 3 Melchor Fernandez Almagro Street; Madrid 28029 Spain; Tel.: +34.91.732.8032; Fax: +34.91.732.8028; Email: mserrano@cnio.es

Original manuscript submitted: 03/28/07
Manuscript accepted: 03/28/07

Previously published online as a *Cell Cycle* E-publication:
http://www.landesbioscience.com/journals/cc/abstract.php?id=4211

KEY WORDS

p53, ARF, tumor suppression, DNA damage, oncogenes

ACKNOWLEDGEMENTS

A.E. is supported by a fellowship from the Spanish Ministry of Education and Science (MEC). Research at M.S. laboratory is funded by the CNIO and by grants from the MEC and from the European Union (INTACT, PROTEOMAGE).

ABSTRACT

The process of malignant transformation universally entails genetic damage and oncogenic signaling, two stresses that are signaled to p53 through different genetic pathways. Based on this, it is possible to distinguish two jobs for p53: "guardian of the genome" that consists in sensing and reacting to DNA damage through the ATM/ATR and Chk1/Chk2 kinases, and "policeman of the oncogenes" that, correspondingly, consists in responding to oncogenic signaling through the p53-stabilizing protein ARF. Contrary to expectation, recent genetic evidence in mice indicates that the response of p53 to DNA damage has little or no impact on cancer protection. In contrast, ARF-dependent activation of p53 is critical for p53-mediated tumor suppression. Here, we discuss the mechanistic implications of these observations and their relevance for cancer therapy.

The p53 gene is the most frequently mutated gene in human cancer: about 50% of all human cancers have lost p53 or express an inactive, mutant, p53.[1] More than twenty years of intense investigation have produced a progressively sharper picture of why this protein is so important for cancer protection. In brief, p53 is a master transcription factor that under normal conditions is functionally inactive due to its rapid degradation by the ubiquitin ligase MDM2. However, upon the infliction of almost literally any cellular stress, MDM2-driven degradation is halted, and p53 accumulates and gains full competence in transcriptional activation.[2] The p53 transcriptional program includes the activation of a number of cell cycle inhibitors and pro-apoptotic proteins, which results in apoptosis or irreversible proliferative arrest, the latter also known as senescence.[3,4] Among the various cellular stresses present during malignant transformation, two have been particularly well studied in relation to p53 due to their universal occurrence in cancer, namely, DNA damage and oncogenic signaling.

THE GUARDIAN OF THE GENOME

DNA damage was the first type of stress found to activate p53 and, based on this, p53 has been widely regarded as "the guardian of the genome".[5] Extensive characterization of the signaling routes that connect DNA damage with p53 have identified a cascade of Ser/Thr kinases that includes ATM, ATR, Chk1 and Chk2, which phosphorylate p53.[6-9] This signaling cascade is permanently activated in human cancer, suggesting that the cancerous state is intrinsically associated to the generation of DNA damage.[10,11] The constitutive DNA damage present in cancer cells is thought to emanate primarily from the strong generation of reactive oxygen species,[12] as well as, from the aberrant firing of DNA replication origins.[13,14] Recent characterization of mice genetically manipulated with a knocked-in p53 that cannot be phosphorylated at two of the main residues targeted by ATM/ATR/Chk1/Chk2, namely, Ser18 and Ser23 (Ser15 and Ser20 in human p53), indicates an important role of these phosphorylation sites in some, but not all, the DNA damage induced and p53-dependent responses.[15] In agreement with this, mice carrying p53S18A/S23A alleles are tumor prone,[15] although this phenotype is considerably milder than in the case of p53-null mice.[16,17] These data suggest that the activation of p53 in response to DNA damage occurs through multiple pathways, which in addition to the well-established kinase cascade of ATM/ATR/Chk1/Chk2, probably include other kinases such as p38, JNK/SAPK and c-Abl.[18-24] Regarding human cancer, the available information gathered from the analysis of (epi)genetic aberrations indicates that the aforementioned DNA damage signaling kinases are not, in general, significant targets of (epi)genetic inactivation.[25-27] The only exception to this is found in hematological

malignancies, which present a high incidence of mutations in ATM (13–40% depending on the particular type of malignancy).[28] In line with this, a recent large-scale sequencing effort of 210 diverse human cancers has identified ATM among the three most frequently mutated kinases (5% incidence).[29]

Based on the above genetic evidence, it can be concluded that DNA damage is conveyed to p53 through multiple redundant pathways in which many transducers participate, but none of them plays a critical role and, therefore, alteration of a single component does not have a significant impact on p53 function.

THE POLICEMAN OF THE ONCOGENES

Among the many and varied stimuli that have been reported to activate p53, oncogenic signaling[30] has gained much attention because, as DNA damage, is also universally present in cancer. Therefore, analogous to the title of "guardian of the genome", we can also assign to p53 the function of "policeman of the oncogenes". Oncogenic signaling activates p53 through ARF,[31-33] which, in turn, interacts with MDM2 inhibiting its p53-ubiquitin ligase activity. In this manner, ARF-dependent stabilization of p53 results in a dramatic increase in p53 activity. Many transcription factors activate ARF in response to oncogenic signaling,[34,35] most notably DMP1.[36,37] Mice lacking ARF have a remarkable tumor-prone phenotype,[38,39] although not as severe as p53-deficient mice,[16,17] and there is good genetic evidence in mice supporting the relevance of the ARF/MDM2/p53 axis in tumor suppression.[40] Importantly, mice deficient in ARF present a normal DNA damage response, indistinguishable from ARF-proficient mice.[38,39] Regarding human cancer, the analysis of (epi)genetic alterations indicate that ARF is indeed inactivated with an extraordinary high frequency (~30%).[34] However, inactivation of ARF almost invariably occurs in combination with the loss of p16INK4a, thus generating an ambiguity about which is the key targeted tumor suppressor. In this regard, it should be mentioned the existence of germline point mutations that inactivate ARF alone (i.e., sparing p16INK4a) in human kindreds predisposed to cancer.[41,42] Nonetheless, the number of germline mutations that inactivate p16INK4a only (i.e., sparing ARF) outnumbers by a factor of ~20 those that inactivate ARF alone.[43] Together, currently available evidence indicates that ARF is an important upstream regulator of p53, whose lack of activity has a significant impact on cancer.

EVALUATING THE IMPORTANCE OF THE TWO JOBS OF p53

An important concept emerging from the previous studies is that the DNA damage and the oncogenic signaling pathways are, for the most part, independent: DNA damage is communicated to p53 through the kinases ATM/ATR/Chk1/Chk2, as well as the JNK, p38, and c-Abl pathways; whereas oncogenic signaling is communicated to p53 through ARF. Moreover, the two types of signals may occur separately in time. For example, in experimental tumors, the infliction of a strong DNA damage can be the initiator event that mutates oncogenes and tumor suppressors, and this is followed by a period of proliferation that selects additional oncogenic mutations (Fig. 1). An important issue that had remained speculative is the relative importance of these activating signals, namely, DNA damage and oncogenic signaling, for the tumor suppressive activity of p53. Recently, the groups of Gerard Evan and ours have shown that p53 is completely unable to protect from cancer when its ability to sense oncogenic signaling is ablated due to the absence of ARF and despite an efficient response to DNA damage.[44,45] These results were obtained employing two different genetic approaches in mice, and examining spontaneous tumors, as well as, tumors induced by DNA damaging agents.

Our experimental approach was based on the super-p53 mice previously generated in our laboratory.[46] These animals have an additional genomic copy of p53 that retains the same features as the endogenous p53 alleles. Consequently, these mice possess three copies of p53 instead of the normal diploid gene dosage. This is a modest change in p53 total activity (i.e., an increment of ~50%) that, nonetheless, is sufficient to provide a clear and robust enhancement in p53-mediated responses, including cancer protection.[46] To evaluate the relevance of oncogenic signaling, we generated compound super-p53/ARF-null mice. As anticipated by the lack of involvement of ARF in DNA damage response, all the p53-mediated responses to DNA damage were enhanced in super-p53 mice, regardless of the presence or absence of ARF. In contrast, oncogenic signaling-mediated activation of p53 was completely lost in the absence of ARF.[44] The key result was obtained when cancer susceptibility was examined in super-p53/ARF-null mice. Contrary to our expectation and despite having an enhanced response to DNA damage, super-p53/ARF-null mice were equally sensitive to cancer as normal-p53/ARF-null mice. This occurred both in chemically-induced tumors and in spontaneous tumors. These results indicate that the DNA damage response of p53 is of minor importance for cancer protection compared to the ARF-dependent activation of p53.

In the case of Evan´s work, they activated p53 temporally at two different times during tumor development, either concomitantly to the tumor initiating DNA damage, or days after, when DNA

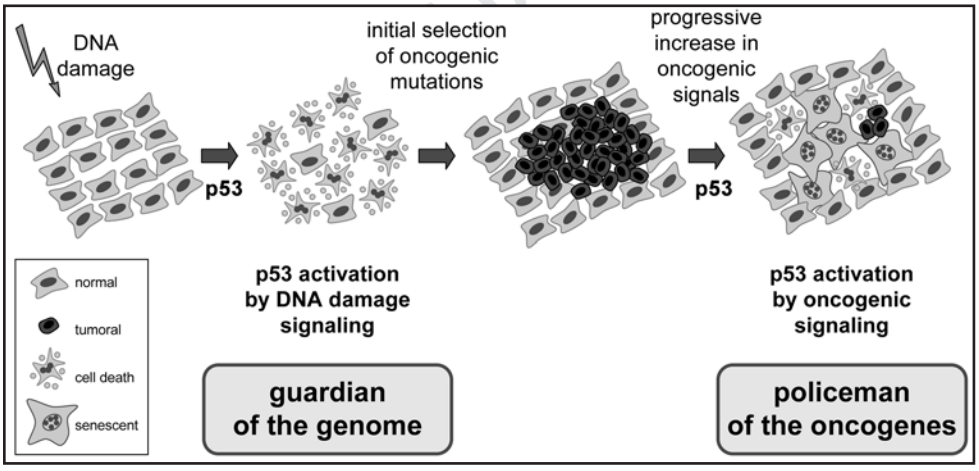

Figure 1. During tumor progression p53 is activated by DNA damage signaling and by oncogenic signaling. Cancer is initiated by a DNA damage insult that, in general, results in cell death. Surviving, but damaged, cells may carry oncogenic mutations and can generate an incipient tumor. Oncogenic signaling may increase through the accumulation of additional alterations. If the cells of the incipient tumor retain ARF and p53, oncogenic signaling results in cell death or senescence. Hypothetical survivor cells may continue on the road to full malignancy.

damage had faded, and malignant cells were emerging. Temporal activation of p53 was made possible thanks to mice carrying a knock-in chimeric p53 fused to a domain of the estrogen receptor (p53-ER).[47] In this context, p53-ER is functionally null in the absence of 4-hydroxy-tamoxifen (4-OHT), but this can be switched to a full competent p53 protein when 4-OHT is present.[47] The most unexpected result was the observation that the presence of functional p53 during the time of DNA damage infliction, i.e., the tumor initiator, did not have any detectable effect on tumor development. However, they found that switching on p53 eight days after tumor initiation, when DNA damage was no longer detectable, had a surprisingly potent protective effect against tumor formation. Furthermore, they demonstrated that this tumor protective activity of p53 was absolutely dependent on the presence of ARF, because no protection was achieved when the same experiment was performed in ARF-null mice.[45]

Together, these two works[44,45] compellingly indicate that the critical job of p53 to provide cancer protection is to function as "the policeman of the oncogenes", rather than as "the guardian of the genome".

EXPLAINING THE PARADOX

The proposal that p53 protects from cancer primarily by responding to oncogenic signaling, and not to DNA damage, seems at odds with the well-established role of p53 in DNA damage response. This apparent paradox requires a mechanistic explanation and, for this, it is convenient to discuss first what is the impact of p53 in determining the fate of severely damaged cells. It is important to bear in mind that DNA damage not only triggers p53-mediated apoptosis, but also other forms of cell death that may indeed be favored by the absence of p53, such as mitotic catastrophe[48] (Fig. 2A). In fact, it is now evident that the response of solid tumors to radio/chemotherapy does not correlate with p53 status, neither in tumor-derived cell lines nor in cancer patients.[48,49] In the light of these evidences, and together with our recent observations,[44,45] it seems that the activation of p53 by DNA damage may serve of little additional protection against cancer because most of the damaged cells, having or lacking p53, will be ultimately eliminated either by p53-dependent apoptosis or by p53-independent cell death.

In contrast to the above, the presence or absence of p53 may have radically opposite consequences on the fate of cells under oncogenic signaling (Fig. 2B). Upon oncogenic signaling, murine cells containing ARF and p53 undergo apoptosis or senescence, whereas cells lacking either ARF or p53 become neoplastically transformed.[30,31] It is thought that at some point during the evolution of incipient tumor cells, oncogenic signaling reaches a threshold of intensity that activates the expression of ARF, with the ensuing activation of p53 followed by apoptosis or senescence.[50,51] In the event that some tumor cells had acquired mutations that impair the p53 response, these cells would continue proliferating allowing tumor progression.

In summary, current evidence suggests that the status of p53 may have little impact on the fate of DNA damaged cells (Fig. 2A), whereas it may have a radical impact on the fate of cells under oncogenic signaling (Fig. 2B). This is a plausible rationale to explain why the response of p53 against DNA damage has no impact on the subsequent development of cancer in mice, whereas the ARF-dependent response of p53 to oncogenic signaling is critical for cancer protection.[44,45]

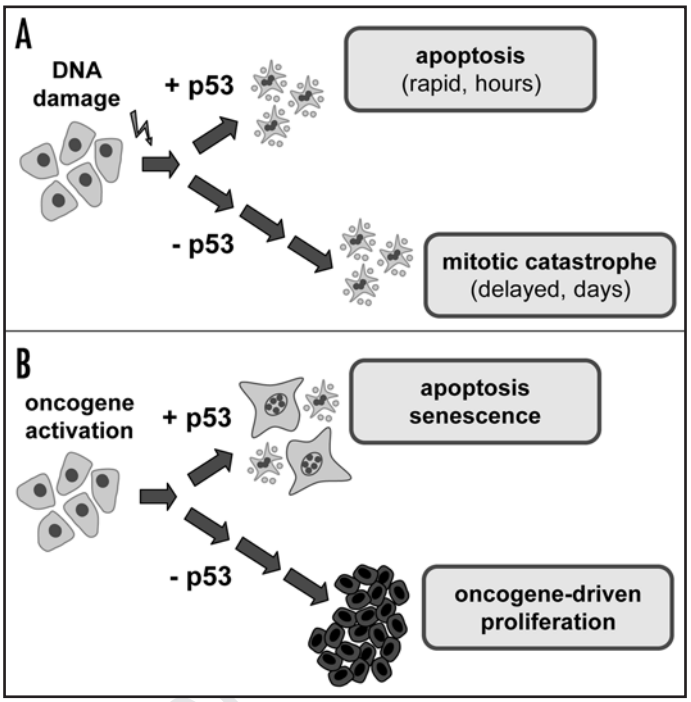

Figure 2. Differential impact of p53 on cellular fate upon DNA damage or upon oncogenic signaling. In response to DNA damage, cells with p53 undergo cell death by apoptosis, but cells lacking p53 undergo aberrant mitosis that also leads to cell death by mitotic catastrophe (A). In response to oncogenic signaling, cells with p53 are eliminated through apoptosis or senescence, whereas cells lacking p53 proliferate under oncogenic govern (B).

COMPLETING THE PUZZLE

A number of caveats still remain to be addressed, such as how many kinases transduce DNA damage to p53, the relative importance of each of them, and their degree of redundancy. Regarding ARF, its role in communicating oncogenic signaling is solidly established in mice, but this is far from clear in the case of human cells. Studies with human fibroblasts have not been conclusive because in some fibroblast cell lines ARF is expressed and responds to oncogenic stress,[52] whereas in other cell lines the expression of ARF is barely detectable and does not respond to oncogenic stress.[53,54] Furthermore, in the case of human epithelial cells, it appears that ARF is not induced by oncogenic stress.[55,56] Additional studies are still necessary to firmly establish whether ARF plays a significant role in the activation of p53 by oncogenic signaling in human cells. Moreover, the implication of ARF in human cancer is still ambiguous due to the fact that in most cases, inactivation of ARF is accompanied by the concomitant inactivation of p16INK4a (see above).

The fact that DNA damage and oncogenic signaling are relayed to p53 through separate routes does not mean a complete absence of inter-connection. In particular, oncogenic signaling may generate DNA damage through the elevation of reactive oxygen species (ROS) and through the aberrant firing of replication origins.[13,14,57-60] Finally, some studies using in vitro cultured cells have observed that ARF may enhance DNA damage signaling,[61-63] although, as mentioned above, experiments using ARF-deficient mice do not support an important implication of ARF in DNA damage.

CLINICAL RELEVANCE

The realization that oncogenic signaling could be more important than DNA damage signaling for the tumor suppressive activity of p53 is of relevance for the therapeutic approaches targeting p53. It can be asserted that essentially every solid cancer lacks a normal p53 response; in approximately half of the tumors this is due to alterations in p53 itself, whereas in the other half of cancers there are alterations in p53 regulators. Among these alterations, ARF silencing and MDM2 overexpression are the most common and relieve selective pressure against the elimination or mutation of p53 itself.[1] This type of tumors retaining potentially proficient, but dampened, p53 should be, in principle, susceptible to drugs that selectively activate p53. Small molecules such as Nutlin[64-66] and RITA[67] impair MDM2 binding to p53 and, thus, stabilize p53. The critical role of ARF in p53-mediated cancer protection[44,45] supports the concept that these drugs may constitute an excellent therapeutic approach, because Nutlin and RITA act similarly to ARF by disrupting the MDM2/p53 interaction. Moreover, recent data obtained with mouse models have shown that p53 reactivation in experimental and spontaneous tumors provokes a dramatic p53-mediated tumor response.[68,69] In some tumor types, reactivation of p53 resulted in apoptosis, whereas in other tumor types p53 produced widespread senescence followed by clearance of the senescent cells by the innate immune system and tumor regression.[68,69] It seems that the time is close for effective p53-based cancer therapies.

References

1. Toledo F, Wahl GM. Regulating the p53 pathway: In vitro hypotheses, in vivo veritas. Nat Rev Cancer 2006; 6:909-23.
2. Hayon IL, Haupt Y. p53: An internal investigation. Cell Cycle 2002; 1:111-6.
3. Lowe SW, Cepero E, Evan G. Intrinsic tumour suppression. Nature 2004; 432:307-15.
4. Vousden KH, Lu X. Live or let die: The cell's response to p53. Nat Rev Cancer 2002; 2:594-604.
5. Lane DP. Cancer. p53, guardian of the genome. Nature 1992; 358:15-6.
6. Siliciano JD, Canman CE, Taya Y, Sakaguchi K, Appella E, Kastan MB. DNA damage induces phosphorylation of the amino terminus of p53. Genes Dev 1997; 11:3471-81.
7. Chao C, Saito S, Anderson CW, Appella E, Xu Y. Phosphorylation of murine p53 at ser-18 regulates the p53 responses to DNA damage. Proc Natl Acad Sci USA 2000; 97:11936-41.
8. Saito S, Goodarzi AA, Higashimoto Y, Noda Y, Lees-Miller SP, Appella E, Anderson CW. ATM mediates phosphorylation at multiple p53 sites, including Ser(46), in response to ionizing radiation. J Biol Chem 2002; 277:12491-4.
9. Kurz EU, Lees-Miller SP. DNA damage-induced activation of ATM and ATM-dependent signaling pathways. DNA Repair (Amst) 2004; 3:889-900.
10. Bartkova J, Horejsi Z, Koed K, Kramer A, Tort F, Zieger K, Guldberg P, Sehested M, Nesland JM, Lukas C, Orntoft T, Lukas J, Bartek J. DNA damage response as a candidate anti-cancer barrier in early human tumorigenesis. Nature 2005; 434:864-70.
11. Gorgoulis VG, Vassiliou LV, Karakaidos P, Zacharatos P, Kotsinas A, Liloglou T, Venere M, Ditullio Jr RA, Kastrinakis NG, Levy B, Kletsas D, Yoneta A, Herlyn M, Kittas C, Halazonetis TD. Activation of the DNA damage checkpoint and genomic instability in human precancerous lesions. Nature 2005; 434:907-13.
12. Jackson AL, Loeb LA. The contribution of endogenous sources of DNA damage to the multiple mutations in cancer. Mutat Res 2001; 477:7-21.
13. Bartkova J, Rezaei N, Liontos M, Karakaidos P, Kletsas D, Issaeva N, Vassiliou LV, Kolettas E, Niforou K, Zoumpourlis VC, Takaoka M, Nakagawa H, Tort F, Fugger K, Johansson F, Sehested M, Andersen CL, Dyrskjot L, Orntoft T, Lukas J, Kittas C, Helleday T, Halazonetis TD, Bartek J, Gorgoulis VG. Oncogene-induced senescence is part of the tumorigenesis barrier imposed by DNA damage checkpoints. Nature 2006; 444:633-7.
14. Di Micco R, Fumagalli M, Cicalese A, Piccinin S, Gasparini P, Luise C, Schurra C, Garre M, Nuciforo PG, Bensimon A, Maestro R, Pelicci PG, d'Adda di Fagagna F. Oncogene-induced senescence is a DNA damage response triggered by DNA hyper-replication. Nature 2006; 444:638-42.
15. Chao C, Herr D, Chun J, Xu Y. Ser18 and 23 phosphorylation is required for p53-dependent apoptosis and tumor suppression. Embo J 2006; 25:2615-22.
16. Donehower LA, Harvey M, Slagle BL, McArthur MJ, Montgomery Jr CA, Butel JS, Bradley A. Mice deficient for *p53* are developmentally normal but susceptible to spontaneous tumours. Nature 1992; 356:215-21.
17. Jacks T, Remington L, Williams BO, Schmitt EM, Halachmi S, Bronson RT, Weinberg RA. Tumor spectrum analysis in *p53*-mutant mice. Curr Biol 1994; 4:1-7.
18. Milne DM, Campbell LE, Campbell DG, Meek DW. p53 is phosphorylated in vitro and in vivo by an ultraviolet radiation-induced protein kinase characteristic of the c-Jun kinase, JNK1. J Biol Chem 1995; 270:5511-8.
19. Hu MC, Qiu WR, Wang YP. JNK1, JNK2 and JNK3 are p53 N-terminal serine 34 kinases. Oncogene 1997; 15:2277-87.
20. Fuchs SY, Adler V, Buschmann T, Yin Z, Wu X, Jones SN, Ronai Z. JNK targets p53 ubiquitination and degradation in nonstressed cells. Genes Dev 1998; 12:2658-63.
21. Fuchs SY, Adler V, Pincus MR, Ronai Z. MEKK1/JNK signaling stabilizes and activates p53. Proc Natl Acad Sci USA 1998; 95:10541-6.
22. Bulavin DV, Saito S, Hollander MC, Sakaguchi K, Anderson CW, Appella E, Fornace Jr AJ. Phosphorylation of human p53 by p38 kinase coordinates N-terminal phosphorylation and apoptosis in response to UV radiation. Embo J 1999; 18:6845-54.
23. Buschmann T, Potapova O, Bar-Shira A, Ivanov VN, Fuchs SY, Henderson S, Fried VA, Minamoto T, Alarcon-Vargas D, Pincus MR, Gaarde WA, Holbrook NJ, Shiloh Y, Ronai Z. Jun NH2-terminal kinase phosphorylation of p53 on Thr-81 is important for p53 stabilization and transcriptional activities in response to stress. Mol Cell Biol 2001; 21:2743-54.
24. Harris SL, Levine AJ. The p53 pathway: Positive and negative feedback loops. Oncogene 2005; 24:2899-908.
25. Ingvarsson S, Sigbjornsdottir BI, Huiping C, Hafsteinsdottir SH, Ragnarsson G, Barkardottir RB, Arason A, Egilsson V, Bergthorsson JT. Mutation analysis of the *CHK2* gene in breast carcinoma and other cancers. Breast Cancer Res 2002; 4:R4.
26. Bartek J, Lukas J. Chk1 and Chk2 kinases in checkpoint control and cancer. Cancer Cell 2003; 3:421-9.
27. Feng J, Yan J, Chen J, Schlake G, Jiang Z, Buzin CH, Sommer SS, Dritschilo A. Absence of somatic ATM missense mutations in 58 mammary carcinomas. Cancer Genet Cytogenet 2003; 145:179-82.
28. Gumy-Pause F, Wacker P, Sappino AP. ATM gene and lymphoid malignancies. Leukemia 2004; 18:238-42.
29. Greenman C, Stephens P, Smith R, Dalgliesh GL, Hunter C, Bignell G, Davies H, Teague J, Butler A, Stevens C, Edkins S, et al. Patterns of somatic mutation in human cancer genomes. Nature 2007; 446:153-8.
30. Serrano M, Lin AW, McCurrach ME, Beach D, Lowe SW. Oncogenic *ras* provokes premature cell senescence associated with accumulation of p53 and p16INK4a. Cell 1997; 88:593-602.
31. Palmero I, Pantoja C, Serrano M. p19ARF links the tumour suppressor *p53* to Ras. Nature 1998; 395:125-6.
32. de Stanchina E, McCurrach ME, Zindy F, Shieh SY, Ferbeyre G, Samuelson AV, Prives C, Roussel MF, Sherr CJ, Lowe SW. E1A signaling to p53 involves the *p19*(*ARF*) tumor suppressor. Genes Dev 1998; 12:2434-42.
33. Zindy F, Eischen CM, Randle DH, Kamijo T, Cleveland JL, Sherr CJ, Roussel MF. Myc signaling via the *ARF* tumor suppressor regulates *p53*-dependent apoptosis and immortalization. Genes Dev 1998; 12:2424-33.
34. Sharpless NE. *INK4a/ARF*: A multifunctional tumor suppressor locus. Mutat Res 2005; 576:22-38.
35. Gil J, Peters G. Regulation of the *INK4b-ARF-INK4a* tumour suppressor locus: All for one or one for all. Nat Rev Mol Cell Biol 2006; 7:667-77.
36. Inoue K, Roussel MF, Sherr CJ. Induction of *ARF* tumor suppressor gene expression and cell cycle arrest by transcription factor DMP1. Proc Natl Acad Sci USA 1999; 96:3993-8.
37. Inoue K, Wen R, Rehg JE, Adachi M, Cleveland JL, Roussel MF, Sherr CJ. Disruption of the ARF transcriptional activator DMP1 facilitates cell immortalization, Ras transformation, and tumorigenesis. Genes Dev 2000; 14:1797-809.
38. Kamijo T, Zindy F, Roussel MF, Quelle DE, Downing JR, Ashmun RA, Grosveld G, Sherr CJ. Tumor suppression at the mouse *INK4a* locus mediated by the alternative reading frame product p19ARF. Cell 1997; 91:649-59.
39. Kamijo T, Bodner S, van de Kamp E, Randle DH, Sherr CJ. Tumor spectrum in *ARF*-deficient mice. Cancer Res 1999; 59:2217-22.
40. Moore L, Venkatachalam S, Vogel H, Watt JC, Wu CL, Steinman H, Jones SN, Donehower LA. Cooperativity of *p19ARF*, *Mdm2*, and *p53* in murine tumorigenesis. Oncogene 2003; 22:7831-7.
41. Randerson-Moor JA, Harland M, Williams S, Cuthbert-Heavens D, Sheridan E, Aveyard J, Sibley K, Whitaker L, Knowles M, Bishop JN, Bishop DT. A germline deletion of p14(ARF) but not CDKN2A in a melanoma-neural system tumour syndrome family. Hum Mol Genet 2001; 10:55-62.
42. Rizos H, Puig S, Badenas C, Malvehy J, Darmanian AP, Jimenez L, Mila M, Kefford RF. A melanoma-associated germline mutation in exon 1beta inactivates *p14ARF*. Oncogene 2001; 20:5543-7.
43. Kim WY, Sharpless NE. The regulation of INK4/ARF in cancer and aging. Cell 2006; 127:265-75.
44. Efeyan A, Garcia-Cao I, Herranz D, Velasco-Miguel S, Serrano M. Tumour biology: Policing of oncogene activity by *p53*. Nature 2006; 443:159.
45. Christophorou MA, Ringshausen I, Finch AJ, Swigart LB, Evan GI. The pathological response to DNA damage does not contribute to *p53*-mediated tumour suppression. Nature 2006; 443:214-7.
46. Garcia-Cao I, Garcia-Cao M, Martin-Caballero J, Criado LM, Klatt P, Flores JM, Weill JC, Blasco MA, Serrano M. "Super *p53*" mice exhibit enhanced DNA damage response, are tumor resistant and age normally. Embo J 2002; 21:6225-35.

47. Christophorou MA, Martin-Zanca D, Soucek L, Lawlor ER, Brown-Swigart L, Verschuren EW, Evan GI. Temporal dissection of p53 function in vitro and in vivo. Nat Genet 2005; 37:718-26.
48. Brown JM, Attardi LD. The role of apoptosis in cancer development and treatment response. Nat Rev Cancer 2005; 5:231-7.
49. Levesque AA, Eastman A. p53-based cancer therapies: Is defective p53 the Achilles heel of the tumor? Carcinogenesis 2007; 28:13-20.
50. Collado M, Serrano M. The senescent side of tumor suppression. Cell Cycle 2005; 4:1722-4.
51. Collado M, Serrano M. The power and the promise of oncogene-induced senescence markers. Nat Rev Cancer 2006; 6:472-6.
52. Dimri GP, Itahana K, Acosta M, Campisi J. Regulation of a senescence checkpoint response by the E2F1 transcription factor and *p14*(*ARF*) tumor suppressor. Mol Cell Biol 2000; 20:273-85.
53. Wei W, Hemmer RM, Sedivy JM. Role of p14(ARF) in replicative and induced senescence of human fibroblasts. Mol Cell Biol 2001; 21:6748-57.
54. Voorhoeve PM, Agami R. The tumor-suppressive functions of the human INK4A locus. Cancer Cell 2003; 4:311-9.
55. Olsen CL, Gardie B, Yaswen P, Stampfer MR. Raf-1-induced growth arrest in human mammary epithelial cells is p16-independent and is overcome in immortal cells during conversion. Oncogene 2002; 21:6328-39.
56. Takaoka M, Harada H, Deramaudt TB, Oyama K, Andl CD, Johnstone CN, Rhoades B, Enders GH, Opitz OG, Nakagawa H. Ha-Ras(G12V) induces senescence in primary and immortalized human esophageal keratinocytes with p53 dysfunction. Oncogene 2004; 23:6760-8.
57. Lee AC, Fenster BE, Ito H, Takeda K, Bae NS, Hirai T, Yu ZX, Ferrans VJ, Howard BH, Finkel T. Ras proteins induce senescence by altering the intracellular levels of reactive oxygen species. J Biol Chem 1999; 274:7936-40.
58. Vafa O, Wade M, Kern S, Beeche M, Pandita TK, Hampton GM, Wahl GM. c-Myc can induce DNA damage, increase reactive oxygen species, and mitigate p53 function: A mechanism for oncogene-induced genetic instability. Mol Cell 2002; 9:1031-44.
59. Moiseeva O, Mallette FA, Mukhopadhyay UK, Moores A, Ferbeyre G. DNA damage signaling and p53-dependent senescence after prolonged beta-interferon stimulation. Mol Biol Cell 2006; 17:1583-92.
60. Mallette FA, Gaumont-Leclerc MF, Ferbeyre G. The DNA damage signaling pathway is a critical mediator of oncogene-induced senescence. Genes Dev 2007; 21:43-8.
61. Khan SH, Moritsugu J, Wahl GM. Differential requirement for p19ARF in the p53-dependent arrest induced by DNA damage, microtubule disruption, and ribonucleotide depletion. Proc Natl Acad Sci USA 2000; 97:3266-71.
62. Khan S, Guevara C, Fujii G, Parry D. p14ARF is a component of the p53 response following ionizing irradiation of normal human fibroblasts. Oncogene 2004; 23:6040-6.
63. Eymin B, Claverie P, Salon C, Leduc C, Col E, Brambilla E, Khochbin S, Gazzeri S. p14ARF activates a Tip60-dependent and p53-independent ATM/ATR/CHK pathway in response to genotoxic stress. Mol Cell Biol 2006; 26:4339-50.
64. Vassilev LT, Vu BT, Graves B, Carvajal D, Podlaski F, Filipovic Z, Kong N, Kammlott U, Lukacs C, Klein C, Fotouhi N, Liu EA. In vivo activation of the p53 pathway by small-molecule antagonists of MDM2. Science 2004; 303:844-8.
65. Vassilev LT. Small-molecule antagonists of p53-MDM2 binding: Research tools and potential therapeutics. Cell Cycle 2004; 3:419-21.
66. Chene P. Inhibition of the p53-hdm2 interaction with low molecular weight compounds. Cell Cycle 2004; 3:460-1.
67. Issaeva N, Bozko P, Enge M, Protopopova M, Verhoef LG, Masucci M, Pramanik A, Selivanova G. Small molecule RITA binds to p53, blocks p53-HDM-2 interaction and activates *p53* function in tumors. Nat Med 2004; 10:1321-8.
68. Ventura A, Kirsch DG, McLaughlin ME, Tuveson DA, Grimm J, Lintault L, Newman J, Reczek EE, Weissleder R, Jacks T. Restoration of *p53* function leads to tumour regression in vivo. Nature 2007; 445:661-5.
69. Xue W, Zender L, Miething C, Dickins RA, Hernando E, Krizhanovsky V, Cordon-Cardo C, Lowe SW. Senescence and tumour clearance is triggered by *p53* restoration in murine liver carcinomas. Nature 2007; 445:656-60.