p53 — a Jack of all trades but master of none

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Abstract | Cancers are rare because their evolution is actively restrained by a range of tumour suppressors. Of these p53 seems unusually crucial as either it or its attendant upstream or downstream pathways are inactivated in virtually all cancers. p53 is an evolutionarily ancient coordinator of metazoan stress responses. Its role in tumour suppression is likely to be a relatively recent adaptation, which is only necessary when large, long-lived organisms acquired the sufficient size and somatic regenerative capacity to necessitate specific mechanisms to reign in rogue proliferating cells. However, such evolutionary reappropriation of this venerable transcription factor entails compromises that restrict its efficacy as a tumour suppressor.

Cnidarians–bilaterians
Cinidarians comprise an animal phylum of ~9,000 radially symmetrical, mostly marine organisms. Most other animals are bilaterally symmetrical and are classed as bilateria. The cnidarians and bilaterians last shared a common ancestor ~570–700 million years ago.

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doi:10.1038/nrc2728
Published online
24 September 2009

Cancer is a genetic pathology that arises in the adult tissues of long-lived organisms, such as vertebrates, whose tissues retain a substantial regenerative capacity throughout life and, consequently, whose somatic cells accumulate mutations. Occasionally, such mutations corrupt the regulatory mechanisms that suppress untoward somatic cell proliferation, survival and migration, resulting in the progressive outgrowth of a somatic clone. Fortunately, however, abundant evidence indicates that both the genesis and evolution of cancers are actively restrained by various tumour suppressive mechanisms.

Effective tumour suppression requires sensors that accurately discriminate between normal and neoplastic cell growth — allowing the former and forestalling the latter. p53 is the poster child for such selectivity: its potent growth suppressive functions are unleashed only in damaged or transformed cells. Moreover, the tumour suppressive action of p53 is remarkably eclectic and versatile: there is strong selection against p53 pathway function in almost all cancers, irrespective of the cell type or underlying oncogenic mechanism. However, what signals trigger p53 activity during carcinogenesis, how diverse these signals are, and when and for how long during tumour evolution such signals are present are all unknown. Is p53 triggered by some unitary, obligate attribute that is common to cancers of all types or by many distinct signals that, between them, encompass the range of aberrant processes in each different type of cancer? Is tumour suppression a discrete, evolved function of vertebrate p53 or is it merely an adaptation of the other known and venerable roles of p53 in stress and damage responses? Answers to these questions are important for several reasons. Knowing how p53 discriminates between normal and tumour cells might point to attributes of cancer cells that qualitatively distinguish them from normal somatic cells and could be used as tumour-specific targets. Knowing what signals drive selection for loss of p53 function in cancers would help us understand what constrains and dictates the varied trajectories of cancer evolution in different tissues and individuals. Understanding when p53 is triggered during tumorigenesis would indicate whether p53 pathway inactivation is required only transiently at some specific bottleneck in tumour evolution or if it is a persistent requirement of cancers — in which case, restoring p53 function would be therapeutically useful. Identifying which of the many biological functions of p53 are required to forestall tumorigenesis is essential for improving the therapeutic efficacy of p53 restoration in the treatment of cancers.

p53 and cancer — how, why, when and where?

Mammalian p53 family proteins — p53, p63 and p73 — are descendents of an evolutionarily ancient family of transcription factors, the origins of which predate the cnidarian–bilaterian divergence approximately 700 million years ago[1] and may go back as far as the 2 billion year-old divergence of animalia and fungi[2]. Of the three mammalian p53 family proteins, p53 is unique in its pre-eminence as a tumour suppressor. In addition p53 coordinates diverse cellular responses to stress and damage and plays an emerging part in various physiological processes, including fertility[3], cell metabolism[4] and mitochondrial respiration[5], autophagy[6], cell

At a glance

- p53 is an evolutionary ancient transcription factor, the primordial function of which in early metazoans may have been to coordinate transcriptional responses to stress and damage.
- Vertebrate p53 is activated by many types of stress and damage. In its basal 'unactivated' state it also controls various normal physiological functions.
- In vertebrates, p53 acts as an important tumour suppressor and either it or its attendant upstream or downstream pathways are functionally inactivated in virtually all cancers.
- The extent to which the roles of p53 in tumour suppression, stress or damage responses and normal physiology are interdependent or overlap mechanistically is unclear.
- Vertebrate p53 is highly pleiotropic and presides over a diverse range of contingent cell responses. Some of these responses are ostensibly antithetical — for example, p53 coordinates both the 'pause–repair–recovery' and 'senescence and/or cell death' responses to genotoxic injury.
- Such multifunctionality arises from the tortuous evolutionary legacy of p53, and may have led to compromises that degrade the efficacy of p53 as a tumour suppressor.
- The effectiveness of p53-mediated tumour suppression in vertebrates relies on the consistent and reliable activation of the p53 pathway by oncogenic signalling but never by normal mitogenic signals.
- The ARF tumour suppressor seems to be the pre-eminent mediator of p53 activation in cancer cells. ARF has evolved to be specifically induced only by oncogenic signals, which are persistent and obligate attributes of cancer cells throughout their genesis and subsequent evolution.
- However, the trigger for ARF (and hence, the p53 pathway) in tumour cells is not the abnormal persistence of growth signals, which is what makes signals oncogenic, but the aberrantly high signal strength, which is a frequent — but not unfailing — correlate of oncogenesis.
- Therefore, the slapdash evolution of p53-mediated tumour suppression has incorporated a fundamental flaw — it senses only a symptom of oncogenic signalling rather than the oncogenic signal itself.

adhesion[7], stem cell maintenance[8] and development[9]. In normal, unstressed cells, p53 activity is maintained at low levels through a combination of p53 degradation and direct transcriptional squelching, principally mediated by the MDM2 E3-ubiquitin ligase and the related protein MDM4 (also known as MDMX). Such basal, low-level p53 seems sufficient to mediate many, if not all, of the physiological functions of p53 (FIG. 1). By contrast, oncogenic signalling and stress or damage signals elicit a marked increase in p53 activity — mostly by hindering its interactions with MDM2 and MDM4, which triggers p53 accumulation and unleashes its transcriptional activity. Intriguingly, the rapid post-translational regulation of p53 activity by modulating its interactions with MDM2 and MDM4 is a peculiarly vertebrate invention: no direct counterparts of MDM2 or MDM4 exist in invertebrates, and invertebrate p53 homologues lack the key residues with which these proteins interact[10,11].

The extent to which the roles of p53 in tumour suppression, stress or damage responses and normal physiology in vertebrates are interdependent or overlap mechanistically is unclear, and we can only guess at the nature of the primordial function of p53 or when and why during its evolution it acquired its many attributes (BOX 1). A more detailed exposition of the tortuous phylogeny of p53 is dealt with elsewhere. Our specific interest here is in ascertaining the extent to which the unique evolutionary trajectory of p53 may have constrained or embellished its capacity to act as a tumour suppressor in vertebrates.

The ancient and evolutionarily conserved role of p53 as a mediator of DNA damage responses, together with the dramatic genome instability and aneuploidy shown by many p53-deficient cancer cells[12–14], has fostered the celebrated 'guardian of the genome' concept that p53 suppresses cancer principally by preserving genome integrity, permanently crippling somatic cells that sustain DNA damage and so preventing the accumulation of oncogenic mutations. The idea has been further fuelled by evidence that oncogenic signalling can, at least in some circumstances, directly induce genomic damage[15] and by initial evidence for habitual DNA damage in early, pre-malignant neoplastic lesions in humans[16,17]. Activation of p53 by DNA damage is principally mediated through an array of post-translational modifications[18], pre-eminent of which are the direct phosphorylation of p53 and its principal regulators MDM2 and MDM4 through the ataxia–telangiectasia mutated (ATM)–CHK2 or ataxia–telangiectasia and Rad3-related (ATR)–CHK1 kinase pathways. This phosphorylation impairs interactions between p53 and MDM2 and MDM4, inhibiting the capacity of MDM2 and MDM4 to suppress p53 transcription and promote p53 degradation. DNA damage also elicits p53 acetylation and methylation, although the functional roles of these modifications in determining p53 activity are less clear.

Although DNA damage is a potent trigger of p53 activity, its role as the principal axis of p53-mediated tumour suppression has recently been challenged by evidence suggesting that the p53-mediated DNA damage response and p53-mediated tumour suppression are independent and separable p53 functions. Christophorou *et al.* developed a mouse in which the endogenous *Trp53* gene is modified to encode a p53–oestrogen receptor fusion protein ($p53ER^{TAM}$) that is only functional in the presence of a synthetic ligand 4-hydroxytamoxifen (4-OHT)[19]. Because endogenous p53 function in such $Trp53ER^{TAM}$ knock-in animals can be rapidly and reversibly toggled between inactive and active by the systemic administration of 4-OHT ligand, these mice can be used to probe when during tumour evolution p53 function is required for tumour suppression. These authors then investigated the function of p53 in a lymphomagenesis model induced by γ-radiation, a prototypical DNA damage and p53-activating carcinogen. When p53 was in its functional state, irradiation of mice triggered the expected gamut of radiation pathologies, inducing widespread p53-dependent apoptosis in radiosensitive tissues, such as bone marrow, lymphoid organs and the gastrointestinal tract. After recovery from this acute radiation injury p53 function was then deactivated and the tumour incidence was monitored. Remarkably, the mice showed no protection from lymphomagenesis compared with animals in which p53 function had never been restored. The widespread p53-dependent apoptosis induced by DNA damage in the lymphoid organs therefore made no contribution to tumour suppression — the mice shared all of the pain but none of the gain. By contrast, mice in

Squelching
Interference of one transcriptional activator by another is called squelching, and is caused by competition for binding a scarce factor.

which p53 was non-functional at the time of irradiation were spared from all radiation-induced pathologies. Moreover, keeping p53 non-functional at the time of irradiation and restoring p53 transiently at later times elicited no observable side-effects, but proved potently tumour suppressive — all the gain but none of the pain. Further analysis of their switchable p53 model by Christophorou *et al.* showed that the p53-mediated tumour suppressive effect afforded by delayed p53 restoration was entirely dependent on p19^{ARF} (p14^{ARF} in humans), which (at least in mouse cells) is the principal mechanism by which oncogenic signals activate p53 (REF. 19). A recent complementary study using an elegant mouse model that allows the timed excision of the *Trp53* gene[20] came to similar conclusions. Hinkal *et al.* deleted *Trp53* before, concurrently with or after γ-irradiation and monitored the effect on lymphoma latency — essentially the inverse of the experiment by Christophorou *et al.* Once again, p53 status at the time of irradiation had no influence on tumour latency, confirming that the immediate p53 DNA damage response is irrelevant to subsequent lymphomagenesis[21].

p19^{ARF} is encoded by an alternate open-reading frame within the *Ink4a–Arf* locus[22] and directly activates p53 by inhibiting MDM2 (REFS 23–26). Importantly, p19^{ARF} is induced specifically by oncogenic signalling[27] not DNA damage[19,28–30]. This is important as oncogenic signalling is a persistent and mechanistically obligate feature of tumour cells, whereas DNA damage — like other acute p53-activating stresses — is episodic and dispensable. The fact that tumour cells harbour persistent, oncogene-driven p53-activating signals offers encouragement for therapeutic strategies based on restoring p53 in established cancers. This notion has recently been validated *in vivo* by preclinical studies which show that restoring p53 function in established tumours re-engages their inherent oncogene-dependent p19^{ARF}-dependent p53 pathway[31], exerting a profound therapeutic impact[31–33]. The crucial role of ARF in mediating the activation of p53 by oncogenic signals was confirmed by elegant studies by Serrano *et al.* using 'super-p53' mice that harbour an extra transgenic copy of *Trp53* under the control of relatively normal regulatory elements[34]. Such super-p53 mice show superior tumour suppression relative to their diploid counterparts but this increased protection is, once again, completely dependent on p19^{ARF} and is lost in an *Arf*-deficient background[28].

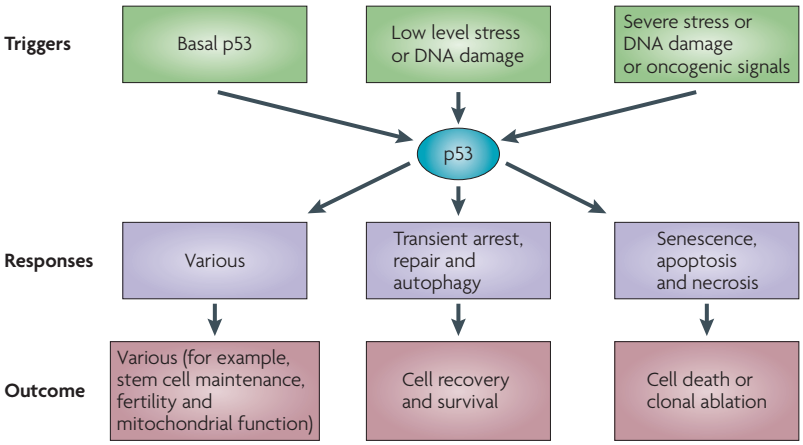

Figure 1 | **Outcomes of p53 activation.** Triggers for p53 activation are depicted in the upper panel. Multiple upstream triggers activate p53, including damage, stress and oncogenic signals. Basal p53 presides over a variety of ill-defined physiological functions: it is not known whether such physiological functions require p53 activation by an upstream signal. Specific responses are represented in the middle panel and the various outcomes are indicated in the lowest panel. Once activated, p53 can instruct a wide range of effector pathways, some of which contribute to the stress and damage response by promoting repair and recovery, and others contribute by ablating the affected cell. How choices between these outputs are selected is unclear, as is the extent to which the different types of output interfere with each other. Several facts that are pertinent to this Review are evident in this depiction of the p53 pathway. First, p53 is the least functionally degenerate part of this pathway, yet it is inactivated infrequently in cancer. Second, multiple inputs, each of which demands its own p53-dependent response, nonetheless all funnel through the same p53. This raises the potential for inappropriate p53 outputs in response to upstream signals (cell death when repair would be more prudent, or repair and survival when cell ablation would be more appropriate). Third, p53 concurrently drives processes that promote tumours and processes that block them. The evolutionarily accreted multifunctionality of p53 has therefore compromised its efficacy as a tumour suppressor.

Not only do such data imply that direct ARF-dependent activation of p53 by oncogenic signals is the pre-eminent mechanism that engages p53 in tumours, but they also call into question the whole part played by DNA damage in p53-mediated tumour suppression, hinting that it may even be dispensable. This is an idea with intriguing implications because DNA damage is not the sole purview of cancer cells. Many, perhaps most, somatic cells incur episodic DNA damage but all of them either repair themselves or sustain somatic mutations that are not oncogenic and hence pose no substantial risk to the organism of which they are a part. Unfortunately, the p53-mediated DNA damage response does not discriminate between damaged cells that are potential tumour cells and those that are not — instead, it efficiently kills or irreversibly arrests them all, an injudicious miscalculation that is directly responsible for the widespread pathologies following whole-body irradiation or chemotherapy. At best, p53-mediated DNA damage seems a blunt and inaccurate tool for suppressing tumours and, at worst, a dispensable relic of the checkered evolutionary legacy of p53 and a liability in patients exposed to radiation or chemotherapy. Indeed, by gradually eroding regenerative capacity through its diligent ablation of damaged cells, the archaic linkage between p53 and DNA damage may even contribute to organismal aging. By contrast, ARF seems to have evolved specifically to respond to oncogenic signals, efficiently engaging p53 but only in those rare cells that, as a consequence of genotoxic injury, acquire oncogenic mutations and therefore have an increased risk of evolving into cancers. Such ideas, although provocative, must nonetheless be considered cautiously because they are based on studies in mice, which differ substantially from humans in several important ways. Of note, unexpected differences may exist in the responsiveness of human p14^{ARF} versus mouse p19^{ARF} to various oncogenic signals — most notably, high levels of oncogenic Ras potently induce p19^{ARF} but not p14^{ARF} (REFS 35,36) in cultured primary cells. It is currently unclear whether this difference is indicative of a fundamental difference in the way that

Box 1 | **The origins of p53 tumour suppression**

It is possible that one or more of the conserved functions of p53 in normal physiology (such as stem cell identity, differentiation, innate immunity or homeostasis) evolved first, predating its more celebrated involvement in disaster management. However, it seems more plausible that p53 originally evolved to coordinate transcriptional responses to DNA damage, checkpoint engagement and stress, perhaps initially to safeguard the 'immortal' germ lines of primordial organisms from the hazards of the Cambrian ocean; a role that is still retained by mammalian p63 (REF. 90). By contrast, tumour suppression seems an unlikely evolutionary imperative. Cancer is a disease caused by the clonal accumulation of somatic mutations, which is a protracted process that requires an organism to be long-lived, physically large and maintain substantial regenerative capacity through life. For p53 to have arisen initially as a tumour suppressor, one would have to presuppose that the probability of somatic mutations that could deregulate somatic cell growth control arising before reproductive age in early organisms would have been sufficiently high to degrade organismal fitness were the ancestral p53 not present. Given the small size (few cell divisions), short lives (little time to accumulate mutations) and typically postmitotic body plan of most primordial animals[91], this seems unlikely. A reasonable guess is that the preconfigured proficiency of p53 as an inducer of replicative arrest and cell death made it an attractive tool for later co-option into the role of tumour suppression. This is consistent with the different mechanisms of p53 family regulation by vertebrates and molluscs — two distantly related bilaterian phyla that nonetheless share a susceptibility to cancer owing to their longevity, size and regenerative adult body plan.

human and mouse cells sense oncogenic perturbation of the Ras pathway or, instead, an artefact of *in vitro* cell culture. Telomere biology also differs between humans and inbred mice. Telomere erosion is an important tumour suppressive bottleneck that curtails the evolution of many human cancers by triggering a potent and sustained DNA damage response[37]. However, due to marked differences in the size and maintenance of telomeres in inbred mice, no such p53-activating bottleneck limits the evolution of their tumours[38]. It seems plausible that the additional direct selection against p53 that telomere erosion-induced DNA damage imposes during human cancer evolution would undercut much of the selective advantage afforded by loss of just the ARF pathway, which might partly explain why inactivation of human $p14^{ARF}$ is far less common than inactivation of $p19^{ARF}$ in the mouse. Nonetheless, the notion of improving p53-mediated tumour suppression by decoupling it from p53-mediated DNA damage remains both intriguing and provocative and is further elaborated below.

Of course, merely identifying which signals are responsible for triggering p53 in tumour cells sidesteps the whole pivotal issue of how such activation is restricted to tumours, and not normal proliferating cells. One possibility is that the signals that activate p53 in cancer cells are not 'tumour specific' *per se* but are instead conventional stress and damage signals that preferentially afflict cancer cells because of their straightened circumstances and debauched lifestyles. Although conventional damage signals clearly have a part in episodically triggering p53 during the evolution and treatment of cancer, they cannot be the whole story. Indeed, aside from the rare exceptions of spermatagonia[39] and the neonatal eye[40], ARF is not expressed in normal developing or adult tissues. In addition, although ARF-positive cells accumulate sporadically with age[41], it remains unclear whether these are a consequence of organismal aging *per se* or relics of appositely aborted oncogenic accidents. While much is known about the many factors that regulate ARF expression — either negatively, such as BMI1 (REFS 42,43), CBX7 (REF. 44), TBX2 (REF. 45), E2F3b[46], Pokemon (also known as ZBTB7) (REF. 47), or positively, such as E2F1–E2F3a[48] and the Ras-induced transcription factor DMP1 (REF. 49) — it has been unclear which attribute that is specific to oncogenic and not normal mitogenic signals induces ARF. Recently, however, several studies have directly investigated this issue and come to the general conclusion that the specific trigger for ARF is the aberrantly high strength of oncogenic signals, not their abnormal persistence (FIG. 2). For example, activation of oncogenic MYC at high levels *in vivo* induces $p19^{ARF}$ together with widespread apoptosis[50,51], curtailing tumour expansion. However, by expressing a low level of MYC *in vivo*, proliferation was achieved in the absence of $p19^{ARF}$ induction and apoptosis[52]. Nonetheless, this low level of MYC resulted in tumorigenic expansion, indicating that the threshold that exists for tripping tumour suppressive functions can be subverted. In another study, different levels of oncogenic HRAS were established in mouse mammary tissue *in vivo* by titrating a doxycycline-inducible $Hras^{G12V}$ transgene[53]. High-level $HRAS^{G12V}$ expression, analogous to that in established tumours, elicited an initial burst of proliferation that triggered $p19^{ARF}$–p53-dependent senescence and blocked tumour development. By contrast, low-level $HRAS^{G12V}$ expression induced proliferation without induction of $p19^{ARF}$–p53-dependent senescence, inducing mammary hyperplasia and eventual tumour formation. Such experiments expose a substantial structural shortcoming in the way that p53-mediated tumour suppression has evolved. Rather than responding to the aberrant persistence of oncogenic signals, which is what makes them oncogenic, p53 is triggered by aberrant signal intensity, which is merely a frequent (but not invariable) correlate of such mutations. As a consequence, low-level oncogenic signals fly beneath the radar, driving sedate but progressive tumour expansion that fails to alert tumour surveillance.

Inactivation of the p53 pathway in cancer

The surprisingly diverse range of mutations that inactivate the p53 pathway in cancer has important implications both for understanding the mechanisms of p53-mediated tumour suppression and for the practical management of cancer therapy (FIG. 3). Aside from specialized instances, such as the p53-inactivating proteins of oncogenic viruses, however, it remains unclear why some mechanisms are favoured in particular tumours and not others. The outgrowth of clones with p53-inactivating mutations is driven by selection for tumour growth and survival. Therefore, the way in which the p53 pathway is abrogated in any specific tumour must, to some degree, reflect the upstream p53-activating signals and downstream p53-dependent tumour suppressor pathways that presented the principal impediments at the time of selection. However, a wide range of factors complicates the impact and outcome of such selective pressures. Many of these factors arise from the

checkered evolutionary legacy of p53, during which it has consolidated control over several distinct biological functions that consequently all become subject to varying degrees of co-selection. As we shall see, such *de rigeur* evolutionary retooling and functional diversification has also frequently led to compromising solutions that restrict the efficacy of p53 as a tumour suppressor. Any impact of negative selection is also constrained by the differing degrees of functional degeneracy in pathways upstream and downstream of p53, rendering some more robust than others. For example, as DNA damage signalling is mediated through the functionally overlapping ATM–CHK2 and ATR–CHK1 kinases that modify p53, MDM2 and MDM4, total abrogation of DNA damage signalling is virtually impossible. Likewise, several ARF-independent mechanisms couple oncogenic signalling to p53 activation, including metabolic stress and the indirect induction of DNA damage owing to promiscuous cell cycle progression. Substantial degeneracy is also evident in downstream effector functions, such as p53-mediated apoptosis, which is mediated by multiple BH3-only apoptotic effectors and involves both transcriptional and non-transcriptional mechanisms, as well as the large and functionally diverse repertoire of p53 target genes[54]. Indeed, the whole p53 tumour suppressor machine shows broad functional redundancy, thwarting tumorigenesis through at least two independent mechanisms: growth arrest and cell death.

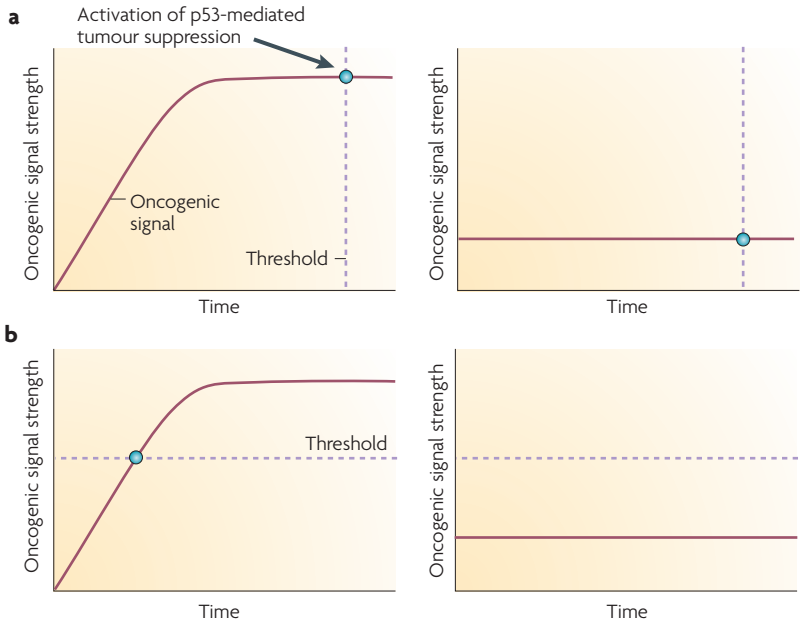

Figure 2 | **Aberrantly high intensity and not the abnormal persistence of oncogenic signalling triggers p53-mediated tumour suppression. a** | A model for p53 activation based on a persistent oncogenic signal. The threshold for activation is triggered by signals persisting beyond a specified time point, so the duration of the signal determines activation. Note that the intensity of the oncogenic insult does not influence when activation occurs (left panel compared with right panel). **b** | A model for activation based on the strength of the oncogenic signal. The threshold for activation is fixed at a specific level, making signal intensity the crucial determinant of activation (left panel). Low-level, persistent oncogenic signals evade this means of activation and undetectably drive tumorigenesis (right panel).

Given the redundancies upstream and downstream of p53, it is perhaps surprising that p53 is not functionally inactivated in more tumours — less than half of all tumour cell lines and even fewer primary tumours harbour mutant p53 (REF. 55) (see Further Information for a link to the IARC p53 mutation database). After all, direct inactivation of p53 seems to present the most economical and effective means of inactivating the whole tumour suppressor pathway at a stroke. However, tumours are seldom optimized for growth, as evident by their high apoptotic indices, necrotic cores, hypoxia and abundant cytopathologies, so complete abrogation of p53 tumour suppressor activity is not a necessity. Indeed, progressive tumour growth can occur even if p53-mediated apoptosis proceeds unchecked, provided that cell proliferation is more rapid[56]. Another potentially important determinant of the p53 pathway in cancer is positive selection for retention of p53 pathway components. p53 is not only involved in ablating cells that sustain severe damage or oncogenic signalling but also in coordinating the pause-and-repair and autophagic responses that facilitate the recovery of cells from transient damage or metabolic stress. Paradoxically, this may mean that tumour cells are initially more dependent on p53 functions for the adroit management of cell cycle checkpoints, repair and nutrient responses than their normal counterparts, at least until additional oncogenic mutations shatter any last vestiges of homeostatic competence. Such pro-survival properties of p53 would blunt direct selection against p53 itself and redirect selective pressure against other, albeit less efficient, inactivating mutations elsewhere in the p53 pathway. Alternatively, they might drive selection for partial loss-of-function p53 mutations that retain p53 attributes that are advantageous to tumour cells: provocatively, such mutant p53 forms would have gain-of-function properties when activated by upstream oncogenic and stress signals. Clearly, the inopportune shunting of rogue cells into repair and survival modes by such mutant forms of p53 has substantial potential to promote tumorigenesis[57–60]. Such considerations might explain the paradox of why p53-inactivating mutations confer a selective advantage only relatively late in the ontogeny of many tumour types. Analogous positive selection may also promote the retention of p53 pathway genes: for example, retention of $p19^{ARF}$ has recently been shown to promote the survival of metabolically stressed cells by promoting autophagy[61]. Moreover, the p53 target gene *CDKN1A*, which encodes p21, has a crucial role in cyclin–cyclin-dependent kinase assembly during the normal cell cycle[62]; ATM–CHK2 and ATR–CHK1 are needed for the biochemical resolution of damaged DNA to allow cell cycle progression; and both MDM2 and, probably, MDM4 have a wide range of p53-independent essential cellular functions[63] that might make their direct inactivation less palatable for certain cell types.

It is clear that variations in the sequence and sum of selective pressures that act against the p53 pathway must influence greatly the diversity of p53 pathway-inactivating mechanisms in cancer. However, selection, whether positive or negative, can only operate on the genetic and epigenetic diversity available to it and there are

substantial variations in the rates and types of endogenous mutation between different tissue types and cell lineages and selection. To some extent, this may reflect the differing vulnerabilities of genes depending on their levels of expression or chromatin status. However, different tissues are also exposed to diverse endogenous and exogenous carcinogens. A pertinent example of this is the form of the inactivating mutation in *TP53* that is evident in tumours that arise in lung, skin and liver as a result of exposure to ultraviolet irradiation, tobacco carcinogens and aflatoxin, respectively[64].

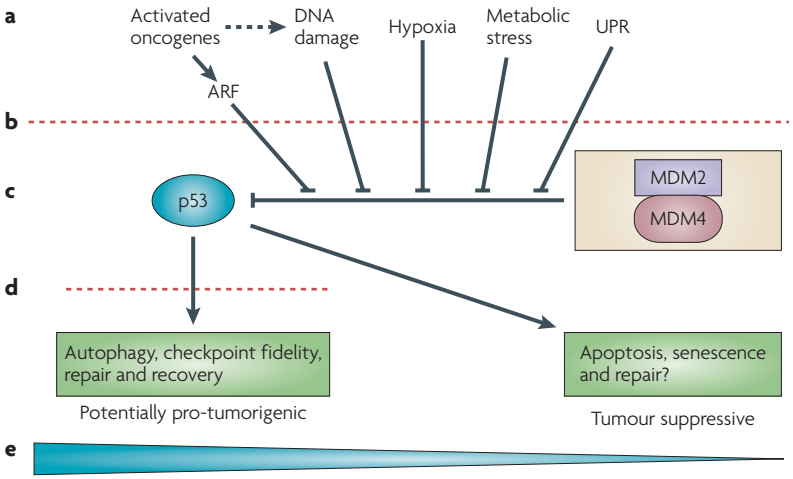

Figure 3 | **Potential mechanisms for evasion and circumvention of p53-mediated tumour suppression.** Effective engagement of p53-mediated tumour suppression can be circumvented (during tumorigenesis) or abrogated (in established tumours) at various points. **a** | p53 activation requires signals of sufficient strength and tenacity. Oncogenic signalling below a crucial activating threshold can drive tumorigenesis but fails to induce ARF or activate p53. Similarly, overly expeditious resolution of DNA or other damage attenuates p53 activation even if damage is inaccurately repaired, favouring survival and recovery of the injured cell over its ablation. **b** | Mutations that inactivate key components of p53 upstream activating signals abrogate p53 activation. However, many distinct signals can potentially activate p53, so the prevalence of mutations in each pathway will depend on several factors: the extent of functional degeneracy in each pathway; the extent to which that pathway contributes to p53 activation in the tumour; and the intrinsic frequency with which particular mutations affect particular genes in each pathway. **c** | p53 is functionally inactivated in many cancers by the loss of p53 expression through gene loss or epigenetic mechanisms, or by structural mutations, some of which may retain partial agonistic functions that promote oncogenesis. **d** | The principal p53 outputs that permanently ablate potential tumour cells are induction of cell death and replicative senescence. These programmes can be defeated by various mechanisms — for example, p53-induced apoptosis can be abrogated by overexpression of BCL-2 or BCL-X_L proteins, loss of p53 apoptotic effectors, such as PUMA and NOXA, or precocious survival signalling in specialized somatic niches. p53-induced senescence can be compromised by inactivation of *INK4A* or *CDKN1A*. As both programmes can efficiently block tumour growth, the extent to which inactivation of either programme alone can abrogate p53-dependent tumour suppression is unclear. The contribution made by p53-mediated DNA damage repair in tumour suppression is less clear. In most instances the DNA damage repair machinery correctly fixes the lesion; however, on occasions, it merely resolves the lesion biochemically, incorporating mutations and truncating the DNA damage signals that would otherwise activate p53 and forestall survival and clonal expansion of the mutated cell. **e** | p53 has evolved to protect cells as well as to eliminate them. Shifting p53 output away from apoptosis and senescence towards transient arrest, repair or autophagy can promote survival of mutated, damaged clones. Because tumours are caused by a net imbalance between cell gain and cell loss, even subtle shifts to the left may substantially augment the probability of tumour cell survival. UPR, unfolded protein response.

Redesigning p53 — intelligently

Like some pervasive computer operating systems, p53 is an archetypical example of the unintelligent design and compromise that is inherent in evolution — a multifunctional, multipurpose transcriptional coordinator that has only lately been retasked to the job of tumour suppression in large, long-lived organisms. Shackled by its evolutionary legacy, the need for backward compatibility and multifunctionality limits the efficacy of p53 as a tumour suppressor. At the end of the day p53, together with all our other suppressor mechanisms, fails half of humanity. But need this be so?

Can we learn from the flaws inherent in the haphazard and unintelligent design of p53 and, perhaps one future day in a different ethical world, improve on it? After all, 'p53 2.0' would be the ultimate firmware update for a species bent on achieving a long and cancer-free life. On the basis of what we know, it seems plausible (FIG. 4). Effective tumour suppression is required only through a reproductive age, after which we are cast adrift like so much Darwinian flotsam, so room for improvement seems likely. Indeed, substantial potential for increasing and extending tumour suppression without a trade off against fitness or longevity must exist, as evident from the lack of variation in the lifetime cancer risk between mammalian species — mice (10^{11} cells) have the same (~35%) lifetime risk of cancer over their 2–3 year lifespans as humans (10^{14} cells) over 70 years or blue whales (10^{17} cells) over 110 years. So, how might such an improvement be achieved? Here three speculative recipes are outlined.

Increase TP53 copy number. A major reason for the eventual failure of p53-mediated tumour suppression is the erosion of the pathway by inactivating mutations[65], so why not incorporate extra copies of the most vulnerable (that is, non-redundant) p53 pathway genes into the germ line? The feasibility of adding to our miserly diploid legacy was recently tested in 'super' mice[34,66], in which mice were provided with additional, single transgenic copies of *Trp53* or *Ink4b–Arf–Ink4a*. Such super-p53 animals (*Trp53*$^{+/+/+}$) had a significantly augmented DNA damage response, decreased susceptibility to virus infection and increased resistance to chemical carcinogenesis, all without any detectable demerits in organismal health or lifespan[34,67]. Provision of an additional, normally regulated *Arf* allele also seemed to confer increased tumour resistance without any discernible downside[66], although interpreting the attributes of these super-ARF mice is complicated because an additional copy of the entire *Cdkn2a–Cdkn2b* locus was inserted, which also encodes the cyclin-dependent kinase inhibitors INK4A and INK4B. Remarkably, when the super-p53 and super-ARF animals were intercrossed they showed the additional benefit of increased longevity, which was not observed in either parental strain[68]. Doubling up on *TP53* and *ARF* copy numbers would seem to be a prudent and relatively non-controversial first approach to upgrading *Homo sapiens*.

Augment the intrinsic activity of p53. In cells that retain a functional p53 pathway, the effectiveness of p53-mediated tumour suppression depends on three factors: foolproof detection of oncogenesis, accurate discrimination between normal and neoplastic signalling, and effective and irrevocable ablation of the offending cell. Unfortunately, as discussed, the efficiency of each of these has been compromised by evolutionary heritage. The unique and requisite hallmark of oncogenic signals is their aberrant persistence. Unfortunately, p53 did not evolve to detect the abnormally persistent signals but, instead, responds to atypically high signal flux, a frequent but by no means invariable consequence of the oncogenic mutations that deregulate growth signals. Nonetheless, as the makeshift solution of evolution has been to use thresholds of signalling as the arbiter of normal versus neoplastic growth, might lowering the threshold for p53 activation be a strategy for improving tumour suppression? Some supportive evidence has come from an unusual mouse that expresses a truncated, constitutively active form of p53 (p53 m)[69]. Animals with such increased p53 activity are significantly protected from spontaneous tumorigenesis; however, they age prematurely and have a reduced lifespan. Mice expressing the natural p44 variant isoform of p53 (ΔNp53 in humans[70,71]) that lacks a transactivation domain show a similar augmentation of the specific activity of p53, together with a progeric phenotype[72,73]. The notion is that reducing the threshold for p53 activation makes it a more assiduous tumour suppressor, but also increases the likelihood of p53 activation in normal regenerating tissues. This has fostered the popular idea that p53 is locked into a 'zero-sum game' — diligent tumour suppression comes at the cost of decreased longevity[74] — a classic instance of antagonistic pleiotropy[75]. However, there remains controversy over the interpretation of these ageing phenotypes[76,77] and, as noted above, the widely differing efficacies of tumour suppression in different mammalian species indicates that the efficiency of tumour suppression and lifespan are not inexorably tethered. Additional evidence for the separability

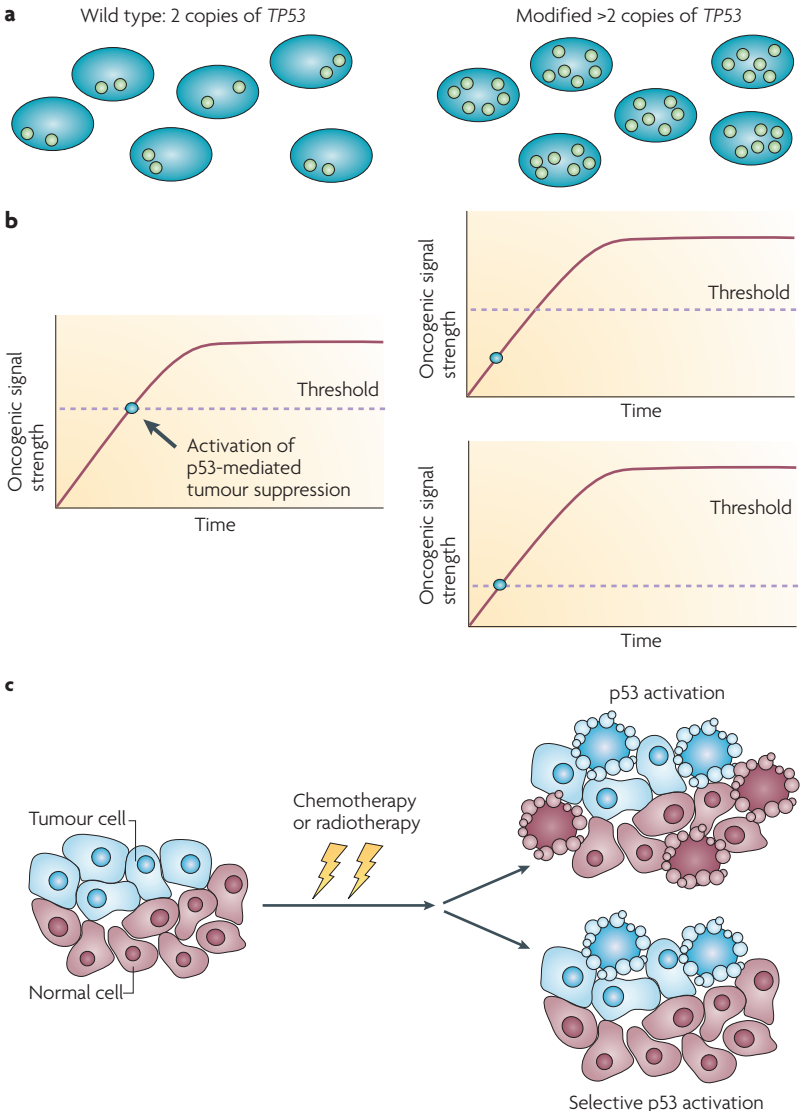

Figure 4 | **Rational redesign of p53-mediated tumour suppression.** Based on our knowledge of p53, we speculate how to redesign and improve p53-mediated tumour suppression, overcoming the shortcomings that are borne of evolutionary compromise. We propose three upgrades. **a** | Increase *TP53* copy number. A major reason for the failure of p53-mediated tumour suppression is its erosion by inactivating mutations or loss, so why not increase the number of copies of *TP53* and/or other vulnerable members of the pathway? Possible downsides include toxicities associated with increased gene dosage, the concurrent increase in the covert pro-tumorigenic activities of p53 and an increased probability of acquiring dominant-negative p53 mutation. In addition, this strategy does nothing to improve the accuracy of detection of oncogenic signals or the capacity of the p53 pathway to discriminate between normal and neoplastic cells. **b** | Increase the intrinsic activity of p53. The effectiveness of p53-mediated tumour suppression relies on foolproof tumour cell detection. As evolution has used thresholds of signalling as the arbiter of normal versus neoplastic signalling, lowering the threshold for p53 activation or increasing its basal activity might increase sensitivity to oncogenic signals. Possible downsides include higher basal levels of senescence and apoptosis owing to constitutively increased p53 activity, increased sensitivity to genotoxic injury in radiosensitive tissues and decreased regenerative potential and longevity. **c** | Improve the selectivity and discrimination of p53-mediated tumour suppression. p53 efficacy as a tumour suppressor hinges on both accurate discrimination between normal and neoplastic signalling and the effective and irrevocable ablation of tumour cells. Normal soma might be protected from the depredation of p53 by confining its expression only to cells that are at real risk of tumorigenesis. Were this possible, it might be even better to replace p53 altogether with a less nuanced and more robust cytotoxic gene. Possible downsides may arise from loss of physiological p53 functions in normal somatic cells.

of p53-mediated tumour suppression and organismal aging comes from studies of mice with reduced MDM2 or MDM4 activity. Animals with a hypomorphic allele of *Mdm2* (REF. 78) or that are haploinsufficient for either *Mdm2* or *Mdm4* (REFS 79,80) show a constitutive augmentation of p53 activity and increased responsiveness to p53-activating signals and are significantly protected from cancers. Despite increased basal rates in radiosensitive tissues and brain, such mice seem healthy, age at a normal rate and have a normal lifespan[78–80]. Taken together, such studies suggest that increasing the inherent activity of (or at least ability to activate) p53 can improve its effectiveness as a tumour suppressor. This conclusion has potentially practical applications given the feasibility of inhibiting the interaction between p53 and MDM2 (and perhaps MDM4) pharmacologically[81,82]. However, such an approach has caveats. Of most immediate concern is that too much unfettered p53 activity is highly toxic. Germline inactivation of either *Mdm2* (REFS 83,84) or *Mdm4* (REF. 85) triggers early p53-dependent embryonal death, and lineage-specific conditional deletion of *Mdm2* or *Mdm4* is similarly toxic to later stages of development[86–88]. Merely restoring p53 systemically in an adult *Mdm2*-deficient mouse in the absence of any p53-activating signal is sufficient to trigger abrupt p53 target gene activation in every tissue, apoptotic collapse of radiosensitive tissues and inescapable death[89].

Improve the selectivity and discrimination of p53-mediated tumour suppression. Unfortunately, neither increasing *TP53* copy number nor raising the baseline of constitutive p53 activity improves the capacity of the p53-mediated tumour suppressor pathway to discriminate between normal and neoplastic signals or its response to low-level oncogenic signals. To do this one would need to blunt the toxic activities of p53 in the normal soma and simultaneously accentuate them in tumour cells. One step towards this might be to use the exquisite discriminatory power of the *ARF* promoter to drive p53 expression selectively in response to oncogenic signals. One could go one better by replacing ARF-driven *TP53* with a more generically toxic gene product, so bypassing the irritating tendency of p53 to engage protective and repair programmes that undercut its tumour suppressive actions.

In the end of course, it may be that the Byzantine contingencies of the evolutionary heritage of p53 stymie even the best schemes of mice and men. *Sic transit Gloria mundi*.

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Acknowledgements

The authors thank the Ellison Medical Foundation for support. Melissa R. Junttila is the Enrique Cepero, Ph.D. Fellow of the Damon Runyon Cancer Research Foundation.

DATABASES

Entrez Gene: http://www.ncbi.nlm.nlm.nih.gov/entrez/query.fcgi?db=gene
CDKN1A
NCI Nature Protein Interaction Database (PID):
http://pid.nci.nih.gov/index.shtml
p53
UniProtKB: http://www.uniprot.org
ATM | ATR | BMI1 | CBX7 | CHK1 | CHK2 | DMP1 | HRAS | MDM2 | MDM4 | MYC | p14^{ARF} | p19^{ARF} | p53 | p63 | p73 | TBX2

FURTHER INFORMATION

Gerard Evan's homepage:
http://cancer.ucsf.edu/evan/index.php
International Agency for Research on Cancer (IARC) TP53 database: http://www-p53.iarc.fr

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