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DATABASES
Pathway Interaction Database: http://pid.nci.nih.gov/p53
UniProtKB: http://www.uniprot.org
ARF | ATM | BAX | ER | HRAS | MDM2 | MDMX | MYC | p21 | p53 | p63 | p73 | RB | WIP1

FURTHER INFORMATION
Moshe Oren's homepage: http://www.weizmann.ac.il/mcb/MosheOren/
Arnold J. Levine's homepage: http://www.sns.ias.edu/csb/
International Agency for Cancer Research *TP53* Mutation Database: http://www-p53.iarc.fr/
p53 Knowledgebase: http://p53.bii.a-star.edu.sg/index.php
The *TP53* Website: http://p53.free.fr/

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OPINION

p53 ancestry: gazing through an evolutionary lens

Wan-Jin Lu, James F. Amatruda and John M. Abrams

Abstract | Evolutionary patterns indicate that primordial p53 genes predated the appearance of cancer. Therefore, wild-type tumour suppressive functions and mutant oncogenic functions that give celebrity status to this gene family were probably co-opted from unrelated primordial activities. Is it possible to deduce what these early functions might have been? And might this knowledge provide a platform for therapeutic opportunities?

'The p53 gene is mutated in approximately 50% of human cancers'. This common axiom is routinely disseminated throughout the cancer research community but the reasons underlying why p53 is so frequently mutated are less sharply in focus. In its wild-type form, p53 occupies a central position in stress response networks and thereby limits oncogenesis through activities that govern adaptive responses. When cells are challenged by genotoxic agents, radiation, hypoxia or other inappropriate signals, p53 restrains cell growth through activities that arrest the cell cycle and/or promote senescence, DNA repair or apoptosis. Unlike conventional tumour suppressors (which are typically affected by nonsense or frameshift mutations), at least 80% of *TP53* alterations sequenced in tumours are missense mutations[1]. These mutations encode oncogenic activities that are distinct from wild-type and simple dominant-negative variants but, despite extensive efforts, the transforming nature of these mutations remains largely elusive[2]. Therefore, although a consensus in the published literature helps us to understand how p53 functions normally, how p53 variants become endowed with properties that specify oncogenic fates remains controversial. Why are p53 mutations so regularly found in tumours? Are there special properties that impart peculiar activity to the gene and/or its protein product? Or is p53 simply an ordinary protein that happens to occupy a rate-limiting 'hub position' in the larger scale of regulatory networks? In this Opinion article we consider these and related questions from an evolutionary perspective. We first review the evolutionary paths of this ancient gene family, highlighting recent sequence information together with studies in model systems. We then explore the primordial features compared with the derived features of p53 regulatory networks, and ask whether these reveal cancer-relevant insights.

p53 ancestry

Three members of the p53 family are found in humans: p53, p63 and p73. As shown in FIG. 1, all members have an amino-terminal transactivation domain, a central DNA-binding domain and a carboxy-terminal oligomerization domain. The transactivation and oligomerization domains seem to have broadly diverged, whereas the DNA-binding domain is significantly conserved[3]. Notably, p63 and p73 contain a sterile alpha motif (SAM) domain at their extreme C-terminus. This domain probably facilitates protein–protein interactions and, in the context of p73, has been implicated in protein turnover[4]. The SAM domain is absent from p53 and, therefore, on the basis of protein architecture, p63 and p73 share a more recent common ancestor. Given current information, the precise evolutionary relationships among the p53 subfamilies are not clear[3]. Therefore, to simplify our discussion we consider only full-length isoforms and refer collectively to p53 family members that encode a SAM domain as 'p63 and p73-like' proteins and define members lacking a SAM domain as 'p53-like' proteins.

New genomes uncover deeper roots. p53 family members are widespread among animals and have been reported to be present in many taxa beyond vertebrates, such as ascidians (sea squirts)[5], cnidarians[6,7], platyhelminths and other invertebrates (FIG. 1; see Supplementary information S1 (table) for sequence accession numbers). Because it is not present in the yeast genome, early conventional wisdom held that the p53 gene family emerged in the Animalia,

perhaps in response to characteristic selective pressures on early multicellular organisms. However, the recently published choanoflagellate (*Monosiga brevicollis*) genome shatters this view[8]. Two distinct genes, a p53-like sequence and a p63 and p73-like sequence, are both present in this unicellular protist, which is possibly the closest extant relative of the metazoans. Similarly, a p53-like protein has also been reported in another protozoan, the amoeba *Entamoeba histolytica*[9]. These discoveries revise the p53 evolutionary picture in at least two ways. First, the emergence of this gene family clearly predated the appearance of multicellular animals. Second, both p53-like and p63 and p73-like genes probably existed in the common ancestor of the metazoa and protozoa.

p63 and p73-like genes are noticeably absent from several lineages in which p53-like genes are present (such as flies, nematodes and cnidarians). Furthermore, compared with p63 and p73-like genes, the p53-like genes seem to be more extensively diversified in separate lineages of invertebrates and chordates[10]. Current evidence is ambivalent regarding the phylogenetic origin of the p53 family. For example, the primordial history of these subfamilies could reflect the acquisition of the C-terminal SAM domain by a p53-like gene or, equally likely, the loss of this domain from an ancient form of a p63 and p73 family member. Compelling arguments support either scenario. However, it is worth noting that among the sequenced genomes in which this family is present, p53-like genes are consistently present but the p63 and p73-like genes are not. Similarly, it is p53, not p63 or p73, which is frequently mutated in human cancer cells, although tumour suppression functions of p63 and p73 have been reported in mouse models[11,12]. Are these two facts simply a coincidence? Or do they reflect some degree of meaningful linkage? In the following sections we expand on this theme by examining the evolutionarily conserved features in p53 regulatory networks.

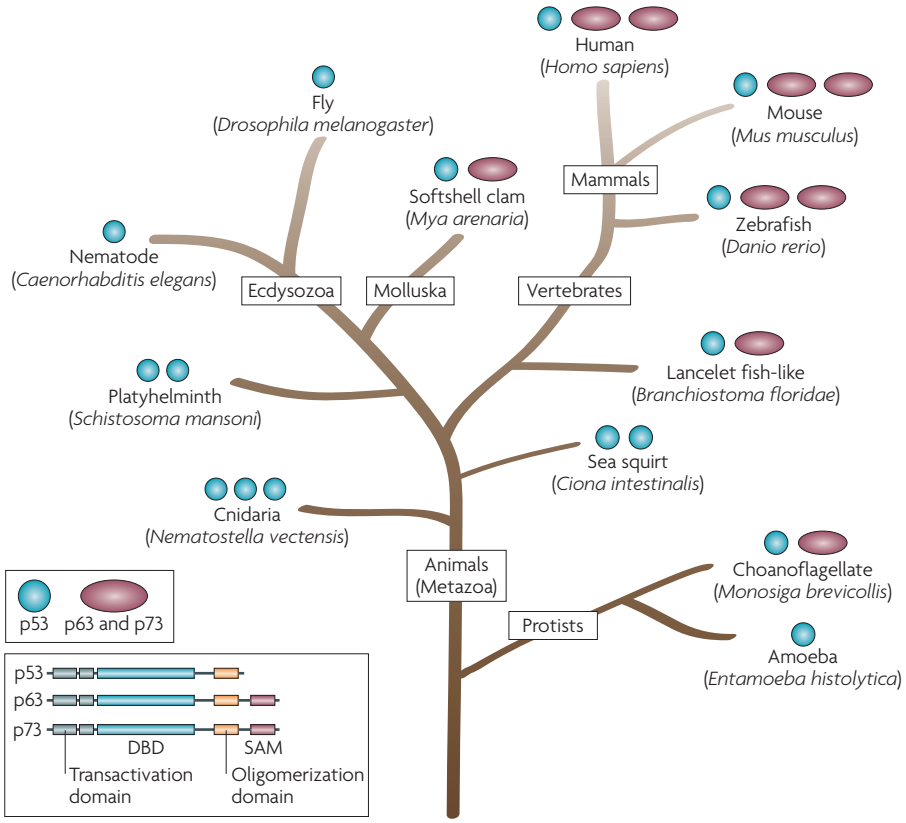

Figure 1 | **Simplified p53 family member tree.** All sequenced genomes with a p53 family member are represented in this simplified family tree. Classification is based solely on the sterile alpha motif (SAM) domain, which is present in the p63 and p73-like subfamily and absent in the p53-like subfamily. p53 gene designations are based on published literature and/or predicted annotations that are available from public databases but, in some organisms, are not yet functionally validated (accession numbers are available in Supplementary information S1 (table)). DBD, DNA-binding domain.

Upstream regulators

In mammals, the p53 regulatory network includes a complex array of upstream regulators and downstream effectors[13]. The emerging picture reflects a hub position for p53, whereby it integrates a wide range of signals to promote adaptive responses to stress. Upstream control of the p53 regulatory network falls into three regulatory models: stabilization, anti-repression and promoter-specific activation[14]. The degree to which these models extend beyond mammals is not yet known. However, the content of non-mammalian genomes, combined with studies in *Drosophila melanogaster* and *Caenorhabditis elegans*, offers some surprises and tentative conclusions. For example, the first two mechanisms of p53 control, stabilization and anti-repression, involve Mdm proteins as pivotal central regulators (FIG. 2). This idea is underscored by studies in mice and fish showing that lethal *Mdm2*- and *Mdm4*-deficient phenotypes are genetically rescued by eliminating p53 (REFS 15–17). However, genes encoding MDM2-like proteins are absent from non-vertebrate lineage, and, furthermore, studies in *D. melanogaster* suggest that genotoxic activation of p53 can occur without altering the protein level of p53 (REF. 18; J.M.A., unpublished observations). Taken together, these observations indicate that ancient circuits linking DNA damage to p53 activation existed before the emergence of MDM2-mediated regulation. Therefore, non-vertebrate p53 regulatory networks offer us an opportunity to understand how p53 activation may occur independently of the destabilizing functions that are conferred by MDM2.

What upstream regulatory mechanisms are conserved? One strong candidate is checkpoint kinase 2 (CHK2). Like its mammalian counterparts, the *D. melanogaster* CHK2 orthologue directly phosphorylates p53 (REFS 19,20) and is required for the induction of apoptosis in response to ionizing radiation[20]. It is not known whether CHK2 directly regulates CEP-1, the *C. elegans* p53 orthologue, but CHK2, together with orthologues of ataxia–telangiectasia mutated (ATM), ataxia–telangiectasia and Rad3-related protein (ATR) and ATL-1, the *C. elegans* ATR homologue, are necessary for cell death that is induced by ultraviolet radiation[21,22]. These cross-species similarities qualify CHK2 as a highly conserved p53 regulator, and indicate that ancestral pathways probably included direct activation of p53 through phosphorylation and perhaps other post-translational modifications (FIG. 2).

Downstream effectors

Output modalities of the p53 regulatory network that contribute to growth repression have been extensively reviewed[13]. Here, we consider evidence from invertebrate models that may distinguish ancient and derived functions. Like their human counterparts, non-mammalian p53 proteins are intimately engaged by and essential for proper genotoxic stress responses that provoke apoptosis[23–25]. As illustrated in FIG. 2, broadly conserved transcriptional targets are commonly recruited among vertebrates and invertebrates to control apoptosis, and include members of the BH3-only Bcl-2 subfamily and inhibitor of apoptosis antagonists (FIG. 2). Likewise, common sets of targets that promote DNA repair, such as XRCC5 (also known as Ku80), ribonucleotide reductase (RNR) and MutS homologue (MSH) proteins have been independently observed in mammals and flies[18,22,26]. By contrast, p21-mediated cell cycle arrest seems to be less broadly conserved and might be restricted to vertebrates. For example, the gene encoding p21 in zebrafish is probably a p53 target[17,27], but the p21 orthologue in flies is not a p53 target gene[18,26].

Interestingly, p53 can still regulate the cell cycle in worms and flies, albeit through different sets of target genes. In energy-deprived cells, *D. melanogaster* p53 can mediate G1/S cell cycle arrest independently of p21 through a mechanism that involves cyclin E[28]. Similarly, CEP-1 mediates the ultraviolet radiation-induced arrest of germ cell proliferation through a direct target — PHG-1, a homologue of the human protein growth arrest-specific 1 (GAS1)[29]. Regulation of autophagy and metabolism are newly appreciated outputs from the p53 regulatory network that may turn out to be broadly conserved and quite ancient. Consistent with this view is the fact that p53 regulates autophagy in mice[30,31] and worms[32].

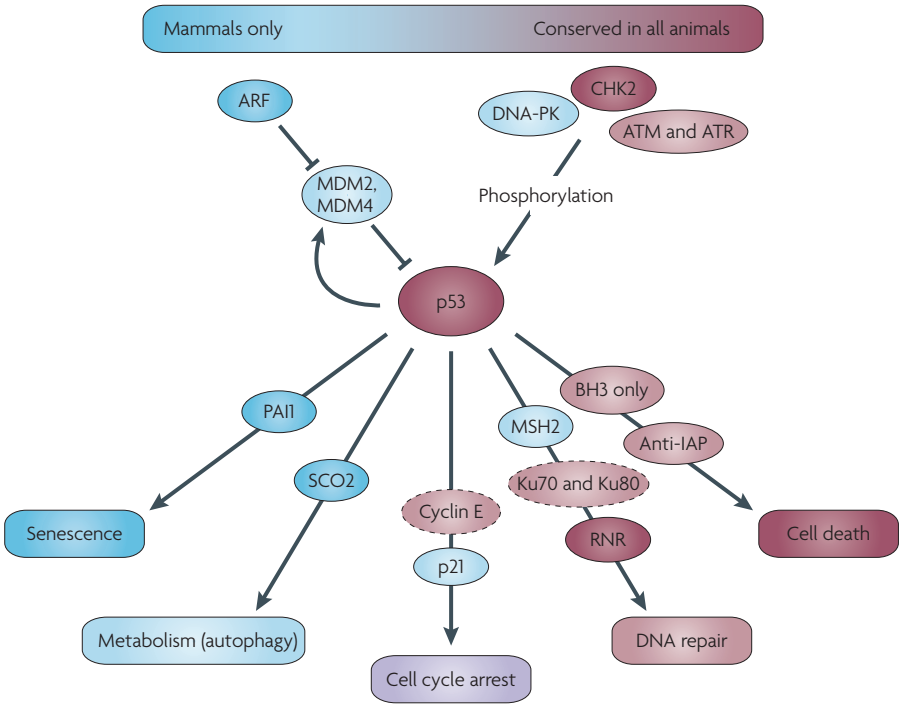

Figure 2 | **A simplified evolutionary schematic of the p53 regulatory network.** Selected upstream regulators, downstream effectors and output terms are colour-coded to indicate conservation across species on the basis of current evidence. The colour gradient illustrates the range of conservation inferred from available genomic data and functional studies in vertebrates (mouse and zebrafish) and invertebrates (*Drosophila melanogaster* and *Caenorhabditis elegans*). Cellular processes and associated genes that are specific to mammals are depicted in blue (for example, ARF). Broadly conserved processes and/or gene functions that span mammals and invertebrate model systems are depicted in red. Regulators and effectors that have been empirically tested for direct links to p53 are depicted with solid outlines and those which are deduced or presumed are shown with dotted outlines. Some downstream targets (such as SCO2 and plasminogen activator inhibitor 1 (PAI1) have not been tested beyond mammalian systems. ATM, ataxia–telangiectasia mutated; ATR, ataxia–telangiectasia and Rad3-related protein; CHK2, checkpoint kinase 2; DNA-PK, DNA protein kinase; IAP, inhibitor of apoptosis; MSH2, MutS homologue; RNR, ribonucleotide reductase.

Other outputs from the p53 network, such as senescence, may be limited to mammalian systems, and it seems fair to assume that output processes that are specific to invertebrates (but which are yet to be identified) may also exist. So, overall, some output modalities seem to be universally represented across phyla (such as promoting apoptosis or DNA repair), although others tend to be specific to certain taxa. Another interesting lesson is that although some p53-regulated outputs are highly conserved, the effectors that couple p53 to a given cellular process are not necessarily shared and can vary across phyla (FIG. 2). A compelling example of this principle is perhaps best illustrated by a conserved axis of regulation involving RNR. In both mammals and flies this enzyme is an important p53 effector but, intriguingly, different subunits are the relevant target[26,33].

Tumour suppression — a sideshow

So, what selective pressures actually shaped the evolution of p53 function? Although firm conclusions are not yet possible, it is safe to say that protection against tumour formation was probably not the ancestral function of this gene or its regulatory network. Support for this idea comes from two main lines of evidence. First, the existence of family members in simple, short-lived organisms and protists (FIG. 1) suggests that ancestral p53 genes predated the need to suppress the deregulated growth of cells in specialized tissues. Second, until recently, human life expectancy did not exceed ~29 years, and it is therefore unlikely that late-onset diseases applied pivotal selective pressures at the population level[34]. Furthermore, mice and fish lacking p53 often survive longer than their feral counterparts[34], which usually do not die of cancers and, therefore, from an evolutionary point of view, cancer was probably not a considerable threat to reproductive success. Together these observations suggest that the tumour suppressive activity of p53 was probably co-opted from other more primordial functions. The relatively late appearance of *ARF* gene orthologues in the vertebrate lineage — they are absent from both zebrafish and puffer fish genomes[35] (J.F.A., unpublished observations) — seems consistent with this deduction as the corresponding proteins arguably represent fundamental links between oncogenic stress and p53 (REF. 36).

Accordingly, if the p53 family was not fixed in animal populations for cancer-related functions, then what ancestral activity was it selected for? The regulation of apoptotic death is a plausible candidate, as

current evidence suggests that the control of apoptotic death predated the regulation of the cell cycle by p21 (FIG. 2). However, this does not explain the presence of p53 in unicellular genomes, and other output modalities — such as DNA repair and metabolic regulation — are also attractive contenders as ancient outputs from this regulatory network. Fully gratifying solutions to this mystery could emerge from studies that reveal unappreciated requirements for p53 or its relatives p63 and p73. In this context, developmental and/or physiological roles in stem cell biology[37–39] and germline tissues[12,40] seem promising. Equally mysterious, but equally relevant, are questions related to the types of stress that primordial p53 genes might have been responding to, and it is worth noting that most experimental paradigms for activating p53 are not encountered in the real (or primordial) world. Therefore, stimuli currently known to activate the protein provide us with only partial clues about the selective pressures exerted on the p53 family[34]. Replication repair stress is a plausible source of adaptive pressure that may have been responsible for selecting or shaping p53 regulatory networks. However, empirical evidence to support this idea is scant, and the actual extent of replication repair stress that occurs *in vivo* is not clear.

Conclusions and insights

If genes that encode p53-like proteins were not originally selected for cancer prevention, it is reasonable to ask whether knowledge of primordial p53 functions and conserved topologies in the p53 network can really illuminate new insights regarding cancer-related functions. Although it may be some time before firm solutions to this question emerge, we suspect that the answers will be affirmative and might be forthcoming from models that have not yet been contemplated. Support for this optimism comes from several fronts. First, important gaps in our understanding of p53 remain, and it is likely that p53 exerts functions that are yet to be discovered. Consistent with this idea, oncogenic phenotypes that are associated with somatic p53 mutations in tumours have not yet been recapitulated by any combination of mutations in known effectors. Second, as discussed above, DNA damage pathways can engage p53 without the involvement of MDM2 or stabilization of p53 (REF. 18; J.M.A., unpublished observations). Therefore, unappreciated upstream pathways to p53 activation might exist that could potentially be exploited as cancer therapies. Third, p63 and p73-like genes are restricted to certain taxa only but, where tested, are required for viability. By contrast, the p53-like subfamily is rather ubiquitous among animal taxa and, paradoxically, these genes are not required for viability (BOX 1). This suggests that members of the p53 family that lack SAM domains possess uniquely important properties. A final reason for optimism is that unanticipated (and sometimes profound) insights have consistently emerged through studies that elucidate the properties of cancer-relevant molecules (such as Wnt and hedgehog) in model organisms that do not develop spontaneous tumours[41–46]. Seen in this light, future studies that decipher the functional roles for previously unknown p53 family members in primitive animals and protists may lead to transformative discoveries.

What significant lessons might therefore be learnt from a deeper knowledge of p53 evolution? We have suggested that elucidating primitive functions might uncover hidden activities but, conversely, it is also plausible to consider using primitive systems as filters to highlight cancer-relevant elements of the network. Another unsolved area that seems ripe for discovery relates to the oncogenic nature of mutant *TP53* alleles themselves. In human cancers, *TP53* missense mutations can reside *in trans* to a deletion. This, together with other data, excludes a strict dominant-negative classification for at least some alleles and argues that the fully transformed state involves gain-of-function activity conferred by missense mutants[47,48]. However, 30 years after the protein was described and 20 years after meaningful mutations were found, the oncogenic activities conferred by most p53 variants remain mysterious[2]. Using the parlance of classical genetics, the transforming nature encoded by these alleles can be thought of as neomorphic activity. As illustrated in FIG. 3, this category can be further subdivided. For example, some neomorphic alleles are 'accessorized variants' that might add functionality in addition to wild-type activity. Others are 'devolved variants', with deranged activities that might preserve some wild-type functions only. And still others might produce new activities with no resemblance to wild-type functions at all. Given current evidence, most *TP53* missense alleles are probably not accessorized variants, as the hyper-expression that often occurs in human tumours is not consistently accompanied by the upregulation of known p53 target genes[49]. Therefore, it seems plausible that *TP53* mutant alleles could be either devolved or unrelated variants. Consequently, if oncogenic evolution

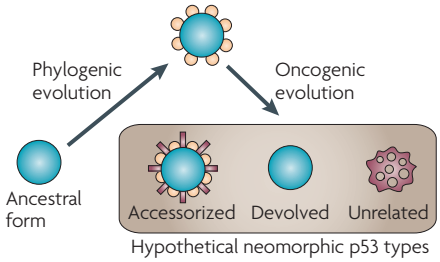

Figure 3 | **Hypothetical changes during phylogenic and oncogenic evolution of p53.** Data indicate that a strict dominant-negative classification for at least some mutated p53 alleles is not correct, and suggest that the fully transformed state involves gain-of-function activity conferred by missense mutants[47,48]. Possible neomorphic p53 gene products are shown in the box. Compared with wild-type alleles, these alleles could encode accessorized, devolved or entirely unrelated functions. Note that some functions acquired during phylogenic evolution of p53 seem to be lost during oncogenic evolution (such as p21 regulation). Therefore, missense p53 mutations could produce either devolved or unrelated variants.

Box 1 | **p53 in development**

Animals can live without p53. Mice, nematodes, and fruit flies lacking p53 are all viable and, to some degree, fertile[23–25,50]. However, interestingly, p53 is preserved in most of the eukaryote lineages (FIG. 1), and non-essential roles for p53 in development have been identified. For example, during embryogenesis p53 contributes to neural tube closure in mice, mesoderm specification in frogs and programmed cell death in flies[51,52]. It is also noteworthy that developmental contributions of this gene family may also be distributed among complex isoforms of the p63 and p73 paralogues, which theoretically allows compensation in certain knockout strains[53]. Furthermore, in vertebrate lineages the genes encoding p63 and p73 seem to have acquired essential developmental roles, as mice deficient for either of these genes are embryonic lethal[48,54–56]. This seems perplexing and perhaps counter-intuitive, as the non-essential p53-like subfamily is far more widely represented among disparate taxa (FIG. 1). Recent studies indicate that members of this gene family exert functions in germline tissues and during pregnancy[12,23,40,57,58]. Therefore, one possible view is that activities in embryonic and/or germline tissues shaped the evolutionary patterns of this gene family.

produces variants of the devolved class, then knowledge of primordial p53 functions could be an important guide towards answering fundamental questions in cancer research.

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UniProtKB: http://www.uniprot.org
ATM | ATR | CEP-1 | CHK2 | GAS1 | p53 | p63 | p73 | XRCC5

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John M. Abrams' homepages: http://www4.utsouthwestern.edu/abramslab/research.htm; http://www8.utsouthwestern.edu/UTSW/FacDir/CDA/FindAFaculty/Results/FacDir_FacSearchRes/0,2357,10011,00.html; http://www.utsouthwestern.edu/graduateschool/geneticsanddevelopment.html
International Agency for Cancer Research *TP53* Mutation Database: http://www-p53.iarc.fr
p53 Knowledgebase: http://p53.bii.a-star.edu.sg/index.php
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