The expanding universe of p53 targets

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Abstract | The p53 tumour suppressor is modified through mutation or changes in expression in most cancers, leading to the altered regulation of hundreds of genes that are directly influenced by this sequence-specific transcription factor. Central to the p53 master regulatory network are the target response element (RE) sequences. The extent of p53 transactivation and transcriptional repression is influenced by many factors, including p53 levels, cofactors and the specific RE sequences, all of which contribute to the role that p53 has in the aetiology of cancer. This Review describes the identification and functionality of REs and highlights the inclusion of non-canonical REs that expand the universe of genes and regulation mechanisms in the p53 tumour suppressor network.

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The *TP53* gene has a prominent role in cancer and much of human biology. The 'guardian of the genome' continues to fascinate investigators because of its many functions. The p53 tumour suppressor can be induced by a range of stresses through transcriptional[1,2], post-transcriptional[3–8] and post-translational[9] control mechanisms. The intrigue caused by this protein has been heightened by recent findings that p53 activities also occur in human development long before the onset of cancer, including during embryo implantation[10]. Many functions have been attributed to p53, including direct roles in repair and recombination, association with proteins involved in genome stability, and chromatin modification[11]. However, its broadest cellular effect is that of a transcription factor (TF)[12]. In its role as a master regulator, the universe of genes subject to p53 control extends across a diverse group of biological activities[13] that include DNA metabolism[11], apoptosis[14], cell cycle regulation[15], senescence[16], energy metabolism[17,18], angiogenesis[19–22], immune response[23], cell differentiation, motility and migration[24–28] and cell–cell communication[29]. Recent studies have demonstrated how p53-dependent activation of microRNA genes can participate in the modulation of various biological activities[30–33]. Approximately 50% of all cancers have a mutation in p53 that alters transcriptional activity (see the International Agency for Cancer Research *TP53* Mutation Database and the *TP53* Website). In most of the remaining cancers, the functions of the p53 pathway are impaired mainly through a reduction in nuclear p53 levels, resulting in multiple changes in stress responses and cellular fate.

As a tumour suppressor, the major p53 functions are to regulate growth arrest and apoptosis (see the review by Vousden and Prives[13]), and the balance of these two cellular events can determine the fate of individual cells. Unlike for other tumour suppressor genes, most *TP53* mutations in tumours are of the missense type and lead to single amino acid changes that predominantly affect residues in the DNA binding domain of the protein, strongly suggesting that targeted sequence-specific DNA binding is crucial for the escape of tumours from p53 suppressor activity. As presented elsewhere in this Focus Issue (see the Review by Brosh and Rotter[34]), p53 mutant status in tumours has been linked to adverse prognosis in different cancer types, a finding that has stimulated the development of various intervention strategies. Studies in animal models in which p53 has been constitutively or conditionally altered[35–37] demonstrate that p53 transcriptional activity is key to tumour suppression. Mouse knock-in approaches were used to show that the tissue-specific predisposition to cancer and tumour onset correlated with the DNA binding and transactivation potentials of p53 mutants in cell systems or *in vitro*[38–40]. Inherited p53 mutations in humans result in the highly penetrant cancer-prone Li-Fraumeni syndrome. The variety of clinical manifestations in patients with Li-Fraumeni syndrome is related to the different abilities of p53 mutant alleles to function as sequence-specific transcription factors[41,42].

Central to transcriptional regulation by the p53 tumour suppressor is target sequence recognition. Over the past 20 years considerable effort has gone into understanding what constitutes a p53 response element (RE), as

At a glance

- p53 is a key tumour suppressor and master regulatory transcription factor that is altered in most human cancers. Several stresses lead to p53 activation, which results in various biological outcomes, including cell cycle arrest and apoptosis. Each of these events seems to contribute to tumour suppression. The p53 network can be affected by variation in p53 levels and the variety of genes targeted.
- The extent of p53 transactivation and transcriptional repression is influenced by many factors, including p53 levels, cofactors and the specific response element (RE) sequences, all of which contribute to the role that p53 has in the aetiology of cancer. Cooperativity in *cis* between p53 and other transcription factors, such as oestrogen receptors, in the activation of canonical and non-canonical REs greatly expands the p53 master regulatory network.
- Essential components in the p53-mediated transactivation of target genes are the p53 RE sequences, which differ individually from the consensus sequence and support p53 transactivation to varying extents. Transactivation assays developed in budding yeast and human cells have been valuable tools for defining and assessing the p53 transcriptional functionality of potential RE targets.
- Non-canonical sequences that differ significantly from consensus can also support transactivation by p53, thereby greatly expanding the p53 transcriptional network. Canononical and non-canonical p53 REs can be transactivated by several p53 mutants with altered functionality, many of which are associated with cancer.
- Using information about the functionality of p53 REs, it seems that in the evolution of humans and primates many DNA metabolism and repair genes have evolved to become responsive to p53 through the inclusion of functional p53 REs.

well as the conditions and factors that affect p53-mediated transcription[43,44] (recent reviews include REFS 12,45). This information is key for identifying the genes that are included in the p53 master regulatory network and understanding their potential roles in tumour suppression and cancer prevention. The depth of genomic influence of p53 is much larger than originally anticipated owing to the 'expanding universe' of genes directly targeted by p53. This Review focuses on the identification and assessment of the p53 transcriptional functionality of potential RE targets; the expansion of the p53 universe through targeting to non-canonical sequences; *cis* interactions with other master regulators, in particular the oestrogen receptor (ER); and changes in the universe of p53 targets owing to cancer-associated p53 mutations that retain function.

Complex transcriptional regulation by p53

The p53 master regulatory network is composed of a vast number of genes that are direct targets for p53-mediated transactivation. Many factors influence the ability of p53 to function as a sequence-specific transcription factor (FIG. 1). The organization, arrangement and localization of binding motifs, as well as the level of p53 expression, also have an important effect on the ability of p53 to transactivate from an RE sequence. There are many examples of p53-regulated genes, including those that encode p21, MDM2, insulin-like growth factor-binding protein 3 (IGFBP3) and tumour protein p53-inducible 3 (TP53I3; also known as PIG3), which contain more than two p53 REs (referred to as clusters) that usually differ in their sequences and p53 binding affinities. In principle, carrying more than one binding site in the promoter would strengthen the responsiveness of the gene to the respective regulator[46,47]. For example, the p21 promoter region has one high-affinity and several low-affinity p53 RE sites. In general, binding affinities seem to dictate the choices between regulating cell cycle arrest (high-affinity site) and pro-apoptotic responses (low-affinity sites)[48]. The position of p53 relative to the transcription start site also seems to be important for the p53-mediated transactivation, as more than 50% of the established REs are located in the 5′ promoter enhancer region of a gene, and ~25% are in the first intron[12]. However, chromatin immunoprecipitation (ChIP) experiments indicate that p53 binding sites also exist at large distances from a transcription start site[49,50]. Elaborate signalling systems allow stresses and growth conditions to be translated into increased p53 stability, as well as nuclear availability; for example, an antisense RNA has recently been described that increases p53 mRNA stability and protein production[5]. Proteins that determine p53 stability and availability to chromosomes are of particular importance, especially MDM2 and MDMX, which can ubiquitylate p53 and lead to its degradation[51,52]. At the most basic level, the amount of available p53 is expected to strongly influence the extent of transactivation.

Cofactors influencing p53 transactivation. Importantly, post-translational and gene-specific chromatin modifications of p53 can strongly influence transactivation at specific promoters[9,53] (FIG. 1). There is a close relationship between the components of the transcription machinery that p53 interacts with and the ability of p53 to activate its target genes[13,54], including some that encode components of the Mediator[55,56] and the SWI–SNF[57] pre-initiation complexes. Several p53 co-activators and co-repressors are histone-modifying enzymes, such as the histone acetyltransferase CREB-binding protein p300 (REFS 46,58–61), the arginine methyltransferase protein-arginine *N*-methyltransferase 1 (PRMT1) and the co-activator-associated arginine methyltransferase (CARM1)[58]. Other co-activator complexes, including SAGA and NuA4–Tip60, also function in p53-dependent gene activation[62–64].

An increasing number of p53 cofactors has been shown to influence promoter-selective p53 transcriptional activity, thereby altering the balance between cell life and death (for a review, see Vousden and Prives[13]). Here, we highlight a few examples of how cofactors can influence p53-dependent transactivation. After severe and irreparable levels of damage, p53 interacts with a series of cofactors that stimulate the transactivation of pro-apoptotic genes and the repression of cell cycle arrest, such as the prolyl isomerase *PIN1* (REF. 65) and the apoptosis-stimulating of p53 protein 1 (*ASPP1*) and *ASPP2* genes[66,67]. The following can also be recruited by p53 to a subset of apoptotic target genes: cellular apoptosis susceptibility protein (CAS; also known as CSE1L)[68], p38-regulated and DNA damage-inducible protein 18 (p18; also known as Hamlet)[69], as well as other transcription factors, including the p52 subunit of nuclear factor-κB (NF-κB)[70], the p53-related p63 and p73 proteins[71], and the p53 isoform p53β[72]. Under conditions of low, transient and repairable damage, p53 mainly interacts with cofactors that strongly

influence cell cycle arrest, such as inhibitor of ASPP (iASPP), which represses the transcription of apoptotic genes[73], haematopoietic zinc-finger (HZF)[74], mucin 1 (MUC1), Y-box factor 1 (YB1)[75,76] and the p53 isoform δ133 (REF. 72).

In addition, there are cofactors that can modulate p53 target selectivity in opposite directions. In the brain-specific homeobox/POU domain protein 3 (Brn3) family of transcription factors, BRN3A promotes growth arrest by p53 and BRN3B promotes apoptosis[77,78]. The list of factors that have a general role in transcriptional regulation of p53 targets in response to DNA damage is growing rapidly, and has recently been reviewed[13]. MicroRNAs that are directly induced by p53 can participate in p53 responses[30–33] and mainly function post-transcriptionally to reduce the stability and translation of target mRNAs. In many cases, the potential for tissue-specific, as well as stress-dependent, regulation of p53 activities by cofactors remains to be clarified[54,79,80] and — except for a few instances (for example, ASPP and iASPP) — the effect that p53 cofactors have in tumorigenesis remains to be established.

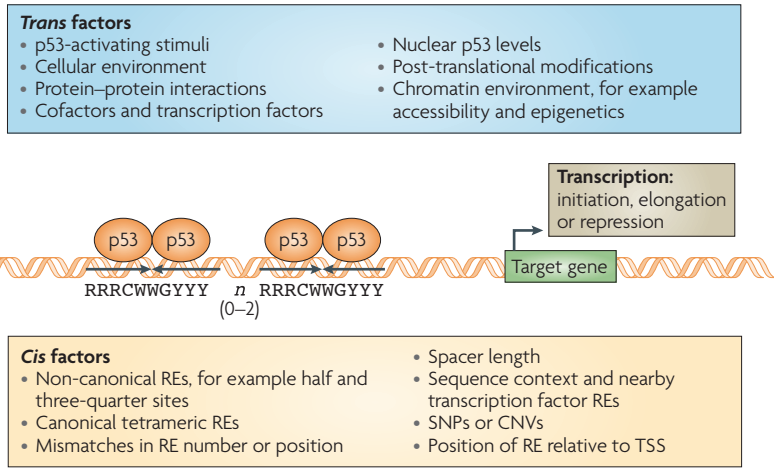


Figure 1 | **Many factors affect p53-dependent transcriptional modulation and the universe of directly targeted genes.** Many factors influence p53-dependent transcription and these can be divided into '*cis*' and '*trans*' factors. The interaction of the p53 sequence-specific protein with target response elements (REs) is essential in the transcriptional modulation of target genes. As discussed in the main text, various intrinsic features of the REs can greatly affect the transactivation potential of p53, including the sequence and organization of the individual functional units in an RE (that is, the monomer binding sites — identified with an arrow) and particularly the spacer separating the two decamer half sites. The distance from and position relative to the transcriptional start site and the presence of nearby or overlapping REs for p53 or other sequence-specific transcription factors functioning in *cis* can also contribute to transcriptional modulation. Variation in RE sequences or number, single nucleotide polymorphisms (SNPs) and unstable DNA elements can also affect p53 transactivation potential[98,99]. The many factors described here could have a different effect on the altered transactivation of many cancer-associated p53 mutants that retain at least some function. In addition to the REs, p53 binding to REs and transactivation potential is affected by various *trans* factors, particularly the levels of available p53. Cellular perturbations can differentially activate signal transduction pathways, resulting in complex patterns of post-translational modifications of p53 as well as p53-interacting proteins, such as MDM2 and MDMX[9,11,196,197]. The cell type and stress responses also influence the availability of cofactors and sequence-specific transcription factors[62,79,80]. These changes result in fine-tuning of p53 nuclear levels and could directly affect p53 DNA binding specificity and the potential for protein–protein interactions[65,198–201]. Chromatin changes, including post-translational modifications of histone tails and remodelling of nucleosomes that can be mediated by p53-recruited histone modifying enzymes also affect transcriptional modulation[54,58]. CNV, copy number variant; TSS, transcription start site.

p53 REs: identification and functionality. Across the hundreds of targeted genes in the p53 network there is large variation in p53-dependent expression[54,81–83]. The sequences of the individual REs are expected to have a substantial functional influence on p53–DNA interactions in terms of binding and the level of transactivation as the amounts of available p53 change. Having established that p53 is a sequence-specific regulatory factor[84–86], considerable efforts were made to determine what constitutes a target RE sequence. Traditional approaches examined *in vitro* binding to defined sequences, along with a corresponding evaluation of *in vivo* transcriptional activity from defined sequences associated with reporters. A generally accepted consensus sequence for p53 binding is composed of two 10-base decamers and a spacer as follows: RRRCWWGYYY…*n*…RRRCWWGYYY (in which R is a purine, Y is a pyrimidine, W is an A or T and the spacer is 0–13, although for functional REs the spacer is <3, as described below).

Recently, our understanding of the structural interaction between p53 and target DNA sequences has been greatly increased[87–89]. p53 binds as a dimer of dimers, in which each p53 subunit contacts three nucleotides of the RRRCW or WGYYY pentamer, resulting in mutual conformational changes of the protein and the target DNA. There is also limited sequence-independent DNA binding, which can be increased by non B-form DNA structures[90–93]. Other structural studies suggest that the p53 tetramer conformation provides effective interactions with components of the transcription machinery[94] and that binding to DNA can affect the orientation of the transactivation domains in a p53 tetramer[95]. An alternative model of p53 quaternary organization in which p53 binds REs with variable spacer lengths has recently emerged[96]. Although there is much evidence for the conformational flexibility of the p53 DNA binding domain[97], there is little direct structural evidence addressing how p53 might bind to sequences that differ from the canonical RE, including the half and three-quarter sites described below, and how these interactions might affect quaternary conformation and protein–protein interactions.

Among the endogenous human REs that have been validated by p53 binding and corresponding gene expression, nearly 95% have mismatches from the consensus. These differences between target REs suggest degeneracy in the sequence requirement for RE function and raise the possibility that RE sequence differences between individuals[45,98,99] and species[100,101] might be well tolerated in the elaborate p53 network. However, *in vivo* sequence interactions with p53 are usually addressed under conditions of excess p53 and, therefore, may fail to reveal differences in the functionality of REs or the consequences of p53 mutations[83,102–104]. On the basis of *in vitro* dissociation constant (K_d) measurements[45,105,106], the amount of p53 is expected to be a major determinant for binding differences between REs.

A full understanding of the variation in responsiveness to p53 and, therefore, p53-mediated biological consequences requires an understanding of what constitutes a potentially functional RE in cells and the effect of varying levels of p53 on functional response.

The approaches that have been used to identify and validate functional REs are outlined in BOX 1, together with a comparison of the consensus p53 RE sequences obtained with different experimental approaches. These studies have provided broad functionality guidelines[101]

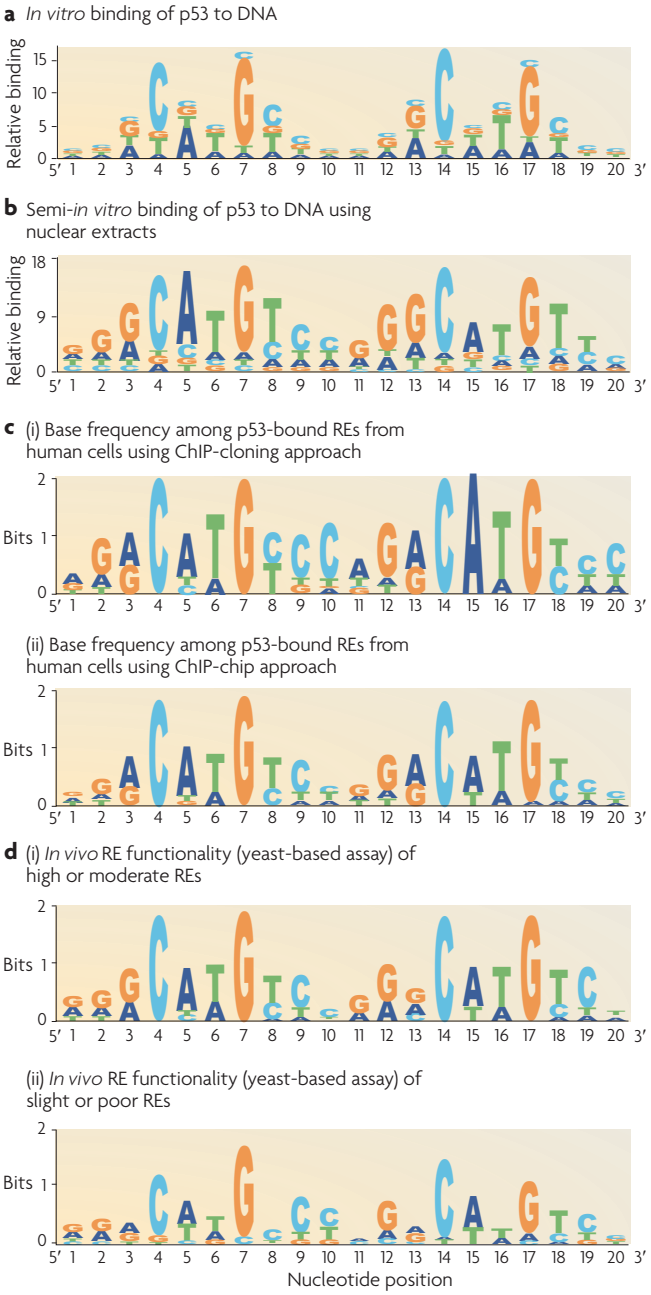
Box 1 | **Techniques to identify and validate functional REs**

Two general approaches have been taken to identify and validate functional response elements (REs): chromatin immunoprecipitation (ChIP) and transactivation in response to induction of p53. ChIP typically involves the treatment of cells with a DNA-damaging agent and identifying and measuring the amount of target region DNA bound by the p53 protein[49,50,81,135,136,186]. The ability of a sequence to support p53 transactivation is often confirmed in mammalian cells using a reporter assay in which the putative target sequence is placed in the context of a minimal promoter upstream of a reporter, and p53 is supplied endogenously or by a transfected plasmid.

More direct methods are needed to assess the *in vivo* functionality of REs. Importantly, most *in vivo* approaches are limited in their ability to identify weakly binding sequences or new sequences or motifs that do not fall into the canonical p53 consensus. Direct assessment of the potential functionality of target REs requires the ability to quantitatively vary levels of intracellular p53, an approach that is analogous to assessing p53 binding *in vitro*. Although this is difficult to attain in mammalian cells, it has been possible by modifying a yeast-based system[187,188] so that the levels of p53 could be modulated over several hundred-fold and transactivation could be determined at individual REs placed upstream of a reporter on a plasmid or in a chromosome[103,107]. Using this approach, the functionality of established individual RE sequences, most of which deviate from consensus, can vary over 200-fold from high (for example, *CDKN1A* (also known as p21) and *RRM2B* (also known as p53R2) that require low levels of p53) to weak (requiring high p53 levels; for example, *BAX* and *NOXA* (also known as *PMAIP1*)) to poor responders (for example, *TP53I3* (also known as *PIG3*)). The quantitative findings obtained with the yeast system have corresponded well with more qualitative results in human cells[107,189]. A recently developed semi-*in vitro* system based on the ability of p53 in nuclear extracts of human cells to bind to REs attached to beads has shown a good correlation between p53 binding and the *in vivo* functionality of REs[107,113].

Sequence logos provide a convenient way of summarizing the effect of individual bases in a consensus sequence on a particular biological outcome. The figure shows p53 logos derived using different methods. Although the potential for p53 to bind to sequences is important for identifying possible target sites in the genome, the results obtained with purified p53 show limited agreement with *in vivo* binding or the functionality of specific REs, or even binding in nuclear extracts. The CATG sequence in the CWWG core is especially prevalent in the logos that correspond to *in vivo* binding and high or moderate functionality.

Part **a** of the figure shows the logo for *in vitro* binding, originally presented in REF. 45. The effects on DNA binding, the dissociation constant (K_d), of single nucleotide changes were compared with the highest affinity sequence identified. The height of a base is proportional to the effect of that change on the binding affinity. Part **b** of the figure shows the sequence logo developed from semi-*in vitro* binding results obtained from doxorubicin-activated p53 in the nuclei of lymphoblast human cells[113]. Part **c** of the figure shows logo representations of p53 REs identified based on *in vivo* occupancy studies. Part i shows the results that were obtained from a ChIP-cloning approach originally presented in REF. 50 and part ii shows the results that are from a ChIP-chip study originally presented in REF. 135. In both logos, the height of a letter at each given position of the p53 RE is proportional to the frequency of its corresponding nucleotide at that position among the identified p53 REs in p53-bound DNA sequences. Part **d** of the figure is a logo representation of the sequence features of p53 REs grouped based on their relative transactivation potential, as determined from yeast-based assays[98,101–103]. The criteria for functional scoring are described in the text and in Supplementary information S1 (figure). Note the difference at positions 3 and 13 for high functionality and *in vitro* binding compared with those positions for the other *in vivo*-derived logos. The WebLogo3 free online tool (see the WebLogo website) was used to generate logos that depict the frequency of bases at each position of the p53 REs[190].



that are described in Supplementary information S1 (figure) (the assessments of predicted transactivation capacity have been updated to include results from a recent analysis of non-canonical REs, such as half and three-quarter sites[107], see below). The *in vivo* quantitative analyses of functionality have yielded insights into the effect of changes in the consensus CWWG core and the spacer on transactivation. For example, altering C or G in either decamer can dramatically reduce the responsiveness to p53. The arrangement CATG allows much stronger transactivation than the three other possibilities (CAAG, CTAG or CTTG). The strong binding associated with CATG[45] might reflect bending capabilities[108,109] or greater flexibility of the DNA. Unlike *in vitro* binding, a spacer of a few bases between decamer regions dramatically affects functionality in cells, as well as the binding of REs in a semi-*in vitro* assay using human cell extracts[107]. This is consistent with the fact that most validated human REs have a spacer that is less than three bases[12,50] and suggests that variation in spacer length may be an important mechanism for maintaining a particular level of functionality[107].

Functionality seems to be strongly influenced by the bases surrounding the CWWG core, particularly the bases GGG and TCC for the high and moderate functionality groups of p53 REs, which have been shown to increase binding at full-site REs[107,110] (D.M., A.I. and M.A.R., unpublished observations) (see Supplementary information S1 (figure)), and by the overall number of mismatches. Notably, the level of p53-mediated transactivation could have a role in RE sequence selection during evolution, resulting in differences between the *in vitro* DNA binding potential and the *in vivo* frequency of specific RE sequences. For example, there may be a selective advantage in retaining weak p53 REs[100,101] compared with high-affinity p53 binding sites. The weaker REs could allow fine-tuning of responses through the regulation of p53 protein levels[54,80] or by specific post-translational modifications that could affect DNA binding affinity[111,112]. Technical limitations of ChIP-based approaches may skew the identified REs towards high-affinity p53 binding sites[50].

The functionality guidelines described in Supplementary information S1 (figure), which have been developed partly on the basis of quantitative analysis of REs that are responsive to p53, have proved to be useful in searches for single nucleotide polymorphisms (SNPs) that could affect stress responses from potential REs[98] and therefore possibly influence the individual risk of developing cancer. Subsequent examination of these alleles in yeast- and human cell-based systems and using a semi-*in vitro* DNA bead assay[113] confirmed that there were differences in the capability of p53 to transactivate from these allelic REs.

The relative importance of individual bases in p53 REs is described in the sequence logos in BOX 1, which summarize the *in vitro* and *in vivo* binding of p53, as well as the ability of an RE to support p53 transactivation. Although the potential for p53 to bind to different DNA sequences is important for identifying possible target sites in the genome, the *in vitro* results show limited agreement with the *in vivo* binding results or the functionality of specific REs in cells. The CATG sequence in the CWWG core is especially prevalent in the logos for *in vivo* binding and high and moderate functionality.

In summary, the biological effect of the p53 regulatory network and the consequences for tumour suppression are likely to be strongly influenced by the potential functionality of the p53 target RE sequences, as well as by the level of available p53 (REF. 102), cofactors and stress-dependent post-translational modifications that can affect p53 interactions with the cofactors or the assembly of the transcriptional machinery at target promoters.

Transcriptional repression by p53. The p53 tumour suppressor can function as a repressor and an inducer of gene expression[114–118]. Repression is detected for ~15% of the recently described validated target REs[12] that are associated with various genes involved in cell proliferation, cell cycle control, apoptosis and cytoskeleton organization, including genes that encode important cancer-promoting factors, such as survivin (also known as baculoviral IAP repeat-containing protein 5 and BIRC5)[119], Myc[120], stathmin (also known as STMN1)[116] and vascular endothelial growth factor A (VEGFA)[20]. Interestingly, p53-dependent repression of Myc has also recently been linked to the p53-dependent induction of miR-145 (REF. 121).

Reports differ on the *cis*-element requirements for p53-dependent repression, mainly because of the difficulty in identifying canonical p53 REs in the promoters of repressed genes[114]. However, there are some examples that suggest that the organization and/or the sequence of a p53 RE can have a role in transcriptional repression. In the multidrug resistance 1 (*MDR1*) promoter[122], the head-to-tail arrangement of the two pentamer binding sites comprising a p53 decamer leads to p53-mediated repression, as does a three-nucleotide spacer between two decamers in the survivin gene[119]. Many downregulated genes contain proposed target sequences that differ from the p53 binding consensus, suggesting both direct and indirect mechanisms of p53 repression[119,123]. A p53 target-repressing sequence change of CWWG to CCAG was identified in the promoter of *LASP1*, which is expressed in hepatocellular carcinoma[124]. Recent functional studies by Wang *et al.*[125] have led to the description of a new p53 consensus site for repression in which only the C and G nucleotides in the CWWG core domain are conserved, and a specific dinucleotide combination in the newly defined CXXG core motif can determine the repression by p53 of a target promoter.

As p53 has dual activation and repression properties, how these two activities are regulated is one of the challenges in the field of p53 research. At least part of the regulation must be context dependent, including the location of the p53 RE at the target gene and its relationship with proximal and/or overlapping binding sites of other transcriptional cofactors[114]. Other proposed mechanisms for p53-mediated repression include interference with the basal transcriptional machinery, recruitment of chromatin modifying factors to reduce promoter accessibility and the recruitment of transcriptional

co-repressors[114]. p53 can compete with other TFs, such as SP1, E2F1 and BRN3A, for partially overlapping or adjacent binding sites, leading to a reduction transactivation[119,126–134]. However, the rules dictating which cofactor will be predominantly recruited by p53 at a target promoter for the activation or repression of gene expression are still elusive.

Non-canonical sequences expand p53 universe

Although many sequences have been validated as target p53 REs (TABLE 1), genome analysis and ChIP binding studies indicate that many more genes might be targeted by p53. Genome-wide approaches have been used to find sites of p53 binding and potential p53 target genes[50,81,135], and projections from smaller-scale but high-resolution studies[136] suggest that thousands of sites in the genome are bound by p53. Although binding does not necessarily lead to transactivation, these results suggest that the complexity of the p53 network is far from being fully described. Nearly 95% of natural REs have at least one mismatch from the consensus sequence and around 10% of the validated REs have novel sequences that are not clearly related to the consensus sequence (see Supplementary information S2 (table)).

The recent finding that a half site (which has 10 bases rather than 20) can function as a p53 target RE has led to the expansion of the universe of genes that might be directly controlled by p53 (REF. 22). An SNP identified in a 25 base pair region of the promoter of vascular endothelial growth factor receptor 1 (*VEGFR1*; also known as *FLT1*) gene results in a sequence that can function as a p53 target RE, bringing together the VEGF-mediated angiogenesis pathway and the p53 stress response pathway. The observed C to T change in the first decamer (GGACAcTGCT) resulting in GGACATGCTCccctgGGACcTGagC creates a perfect consensus half site containing the strong CATG core in approximately 5% of the population. Unexpectedly, the putative RE was functional and could bind p53 even though there was a five-base spacer and three mismatches in key positions in the second half site. In fact, the half site was sufficient for functionality. This finding helped to explain the limited responsiveness of half sites separated by over ten bases[107] and suggested that non-canonical sequences might have a general role in the p53 network.

Subsequent studies in yeast and human cells established the functionality of half and three-quarter p53 RE sites[107]. The functionality of these sites in terms of *in vivo* binding and transactivation can be similar to weakly or modestly responding full-site REs, such as those associated with the *BAX* and aryl hydrocarbon receptor-interacting protein (*AIP*) genes. Typically, the half-site responsiveness is less than 10% of the level of p53-mediated transactivation at a complete *CDKN1A* RE. As established for full-site REs using the yeast-based system, the CWWG core is important and CATG is the strongest functional sequence[107]. Similar findings of half-site functionality were obtained with human osteosarcoma cells (SaOS2) on the basis of p53 binding and transactivation[107] (D.M., A.I., M.A.R., unpublished observations). The response to DNA-damaging agents (such as ultraviolet and infrared irradiation, as well as doxorubicin and 5-fluorouracil

Table 1 | **Sequence conservation of p53 targets in humans and rodents***

Biological function‡	Total REs	Canonical p53 REs in humans§	Non-canonical p53 REs in humans‖	Sequence conservation¶	Functional conservation¶
Apoptosis	37	27 (1)	10 (2)	8 (2)	12
Cell cycle, senescence, development and differentiation	28	20 (1)	8 (3)	14 (7)	12
DNA repair	15	10 (1)	5	3 (1)	0
Cytoskeleton, cell adhesion, angiogenesis and migration	18	11 (2)	7 (4)	7 (3)	5
Feedback and regulation	10	8	2	6	5
Cytokine production and inflammation	11	7 (1)	4 (3)	4 (2)	5
Transcription and translation	14	9	5 (1)	10 (3)	9
Various	13	9 (2)	4 (3)	6 (2)	5
Total number of p53 REs analysed	146	101 (8)	45 (16)	58 (20)	53
		69%	31% (11%)	38% (14%)	36%

*The human p53 REs have been validated in various studies: 138 are described in REF. 12; 4 are from the *FLT1* and *RAP80* promoters, as described in the text, and 4 are described in REF. 96. ‡Several REs are associated with genes that may be involved in more than one biological process according to the Gene Ontology database. Presented is the most common and validated biological process for each gene. §Number of REs with two decamers that may have mismatches. Number of REs with no mismatches in parentheses. ‖Number of REs that lack a complete canonical RE but contain either half or three-quarter sites or new sequences that vary widely from consensus (the number of the new sequences are in parentheses; see text and Supplementary information S2,S3 (tables). All the half and three-quarter sites contain a perfect consensus decamer. ¶Conservation of sequence and functionality from humans down to rodents, as well as other species, has been analysed according to methods described in REF. 96. Among the REs described in REF. 12, 31 had been previously analysed in REF. 96. The number of non-canonical sequences is indicated in parentheses. All the non-canonical sequences are half or three-quarter sites. Complete documentation for all 146 REs analysed has been deposited with *Nature Reviews Cancer* and the information is available on request from the authors.
RE, response element.

exposure) can differ between half sites[137] (M.A.R., A.I., D.M., unpublished observations), suggesting the involvement of additional control factors, as is the case for complete REs.

Despite the functionality of REs with only ten bases, tetramerization is still required for efficient binding and transactivation in yeast and human cells[107] (D.M. and M.A.R., unpublished observations). This is consistent with the lower dissociation constants for wild-type p53 binding to a half site compared with a dimeric mutant protein[138]. Possibly, sequence-specific binding of p53 to the half site along with weaker, nonspecific DNA binding provides opportunities for tetramer-derived transactivation. Nevertheless, a dimeric form of p53 created by the L344A mutation can bind to the p53 half site[138], although it binds with sixfold less affinity than wild-type p53 (REF. 105). On the basis of binding by carboxy-terminal p53 fragments that contain tetramerization or dimerization mutations, the dimer form of p53 is required for nonspecific DNA binding[139]. The L344A mutant, as well as another mutant — N345S — that fails to form tetramers, also retained modest transactivation activity towards half sites, based on luciferase reporter assays in human cells[107,140] (D.M. and M.A.R., unpublished observations). The three-quarter sites are also functional and exhibit greater transactivation than half sites. Recently, tetramerization was also found to increase p53 transactivation from other non-canonical REs[141] that contain a specific two-nucleotide spacer between quarter sites. The contribution of non-specific DNA binding of p53 to the interaction with non-canonical REs remains to be clarified[54,107,141]. It is unclear whether p53 tetramers are formed before interaction with the target DNA or are assembled after binding of the separated dimers to half sites. The half sites are transactivated only when p53 levels are high, unlike full-site REs, some of which can mediate transactivation even at very low p53 levels[103,107].

A summary of canonical compared with non-canonical sequences is shown in TABLE 1, and the sequence conservation of 146 validated REs that can support p53 transactivation is shown in BOX 2 (138 are from REF. 12). The corresponding genes were categorized using the Gene Ontology database (see the DAVID Bioinformatics Resources website)[142]. Among the REs, 45 (31%) seem to be non-canonical. This group includes REs with one consensus decamer only (which contain mismatches in the CWWG core in the other

Box 2 | **Evolution of the p53 network through REs**

Evolutionary analysis has provided useful insights into potential p53 targets as the inclusion or exclusion of genes in a transcriptional network could be accomplished by changes in regulatory sequences as well as the transcriptional regulator. Once rules for response element (RE) functionality are established it is relatively straightforward to address how changes in RE sequences contribute to the evolutionary inclusion or exclusion of genes in the p53 network. Recently, the evolution of the p53 network has been addressed using a combination of functionality rules (see Supplementary information S1 (figure)), *in silico* analyses and direct evaluation of RE functionality. Validated p53 REs from human p53-regulated genes were examined across many species with an emphasis on primates and rodents[100,101], and conservation of both RE sequence and functionality were displayed in a 'heat map' format. Comparisons with rodents are especially relevant because p53 is highly conserved (>85%) and human p53 substitutes well for the mouse protein in whole animals[191].

There is functional conservation for several REs, such as the p53 target REs of the cell cycle and proliferation genes *SNK*, *CDKN1A* (which encodes p21), apoptotic protease-activating factor 1 (*APAF1*) and p53 upregulated modulator of apoptosis (*PUMA*; also known as *BBC3*). However, for most p53 target REs there is considerable variation in sequence and functionality; some REs are detected only in primates[101,192–194]. TABLE 1 summarizes the canonical and non-canonical sequences as well as sequence conservation for 146 validated REs (136 from REF. 12) that can support transactivation. Only around one-third of the REs found in humans are functionally conserved in rodents (complete documentation for all 146 REs analysed across 16 species has been deposited with *Nature Reviews Cancer* and the information is available on request from the authors). Compared with genes related to checkpoint controls, the p53 target sequences of human apoptosis genes generally seem to be less conserved both in terms of sequence and predicted functionality in rodents. This variation contrasts sharply with the RE target sequences of master regulatory proteins such as nuclear factor-κB (NF-κB) and nuclear factor erythroid 2-related factor 2 (NRF2), which are well preserved across species[100].

Surprisingly, among the entire group of 15 p53-targeted human REs from 12 genes affecting DNA metabolic activities, none is functional in rodents. This result confirms previous results in which six of these DNA metabolic genes were investigated in depth and were also found to be lacking in compensating functional RE sequences[101]. This suggests that these 12 genes are not part of the direct p53 regulatory network in rodents. In humans, the DNA metabolic genes (TABLES 1,2) are under the influence of p53 through p53-targeted canonical and non-canonical REs. This finding was recently extended to three REs in the promoter of receptor-associated protein 80 (*RAP80*; also known as *UMC1*), a gene that can influence BRCA1-mediated double-strand break repair[137]. The p53 REs of human *RAP80* are not detected in rodents, and potentially compensating sequences related to p53 REs are predicted to lack transactivation function.

These observations suggest that the paths that led from a common ancestor to the concerted evolution of modern rodents and primates may have resulted in the selective inclusion of genes that affect DNA metabolism into the p53 network. Although several hypotheses can be offered for the differences in human and rodent p53 REs, the additional coordinated regulation in response to stresses in primates may ensure added genome protection in organisms that have longer lifetimes and, therefore, are at greater risk for DNA damage-induced disease, particularly cancer. Also, exposure to a broader range of environmental agents, such as ultraviolet light damage to skin, may increase the need for greater inducibility of DNA repair[195].

decamer), three-quarter sites, REs with a spacer >13 bases (that is, 13 bases is the maximum spacer in the original consensus sequence) and REs with mismatches in both CWWG cores. Examples of such non-canonical REs are shown in Supplementary information S3 (table). All of the half and three-quarter site REs contain a perfect consensus half site (RRRCWWGYYY). In the group of non-canonical REs, there are 16 novel REs that differ widely from full- or even half-site consensus. Most of these 16 REs have been characterized by *in vitro* binding or by reporter assays; however, only a few were validated by *in vivo* binding and endogenous gene expression. Therefore, given the potential functionality of half sites and the expected large number of half and three-quarter sites across the genome, non-canonical REs can greatly expand the p53 universe of transcription targets. In a preliminary genomics screen, we have identified over 1,400 consensus p53 half sites containing a CATG core motif in the genome within 2 kb of a transcriptional start site (D.M., A.I. and M.A.R., unpublished observations).

Interestingly, further analysis of the validated REs (BOX 2; TABLE 1) has revealed that several of the non-consensus REs are maintained during evolution, based on a comparison of rodents and primates. This is consistent with our previous observations of selection for sites that can weakly respond to p53 (REFS 100,101). In addition, evolutionary analysis of p53 REs has been helpful in locating REs, as well as investigating the evolution of p53-responsive systems (BOX 2). For example, it seems that the p53 responsiveness of the DNA repair and metabolism set of genes in humans has evolved separately from that in mice, suggesting differences in the responsiveness to cancer-inducing agents between mice and humans (TABLE 2).

The functional dependency of half-site REs on the type of inducing stress[137], the levels of p53 and the possible cooperation between p53 and other transcription factors indicate that the expression of the associated genes would be particularly sensitive to perturbations of the cellular microenvironment. This feature, as exemplified by the crosstalk between p53 and ER in the regulation of *VEGFR1*, suggests that regulatory modules containing non-canonical p53 REs could be associated with important genes for p53-mediated tumour suppression. Determining the relationship between expression levels and responsiveness at canonical and non-canonical target REs is important for addressing the consequences of p53 alterations in cancer aetiology, particularly those mutations that retain transactivation capabilities, as discussed below.

cis interactions between networks

Gene promoters typically contain target sequences for multiple TFs, raising the possibility of *cis* interactions between master regulatory networks. Several examples of interactions between p53 and other TFs or cofactors have been reported on the basis of experiments in mammalian cells that have focused on various p53-regulated promoters (FIG. 2c). For example, as noted above, p53 can compete at sequences that overlap with the target REs of other TFs, leading to repression. There are several other TFs that have a negative or positive effect on p53-mediated transactivation, including NF-κB[142–145], nuclear factor-Y[146–148], SP1 (REFS 127,129,130), interferon regulatory factor 1 (IRF1)[149,150], ER[151–153] and SMAD[154,155]. The p53 homologues p63 and p73 also have complex interactions with p53, although reports differ on the outcome of

Table 2 | **Human genes related to DNA metabolism and/or repair processes with validated p53 REs**

Gene	Full name of gene	RE Type*	Entrez Gene link
DDB2	Damage-specific DNA binding protein 2	Consensus	DDB2
FANCC	Fanconi anemia, complementation group C	Half RE	FANCC
GADD45A	Growth arrest and DNA damage-inducible, alpha	Half RE	GADD45A
GPX1	Glutathione peroxidase 1	Half RE	GPX1
MLH1	mutL homolog 1	Full RE	MLH1
MSH2 RE1	mutS homolog 2	Full RE	MSH2
MSH2 RE2	mutS homolog 2	Half RE	MSH2
PCNA	Proliferating cell nuclear antigen	Three-quarter RE	PCNA
PMS2	Postmeiotic segregation increased 2	Full RE	PMS2
UIMC1 (also known as *RAP80*) RE3	Ubiquitin interaction motif-containing 1	Full RE	UIMC1
UIMC1 RE4	Ubiquitin interaction motif-containing 1	Full RE	UIMC1
UIMC1 RE5	Ubiquitin interaction motif-containing 1	Half RE	UIMC1
RRM2B	Ribonucleotide reductase M2 B	Half RE	RRM2B
SCARA3	Scavenger receptor class A, member 3	Half RE	SCARA3
XPC	Xeroderma pigmentosum, complementation group C	Full RE	XPC

*All of the half and three-quarter sites contain a decamer with no mismatches from consensus. RE, response element.

the crosstalk, which depends on specific target genes or cell type[71,135,156,157]. The relative expression of p63 or p73, as well as p53 splice and promoter variants, could be an underlying source of differences in these interactions[72,158]. Also, TFs can drive proteins that affect the stability of p53. Interestingly, a polymorphism has been described that indicates that *MDM2* can be controlled by the ER[159,160].

Despite the large amount of information regarding the multiple factors that influence p53 responses, there are no clear molecular rules that dictate the recruitment of different combinations of transcription factors and cofactors at a given promoter in a specific cell type and in response to a given stress condition. Studies to clarify these aspects are needed, particularly to address the tissue-specific risk of developing cancer in relation to alterations in the p53 pathway.

***cis* interactions with the ER master regulator.** Recently, new complexity in the p53 network was revealed: p53 and ER can function *in cis* at a promoter to synergistically increase the responsiveness of potential p53 targets[161]. This synergy is created through a p53 non-canonical half-site target RE and an ER half-site RE located ~250 nucleotides upstream (FIG. 2a). This extends the observation (discussed above) of transcription from the *VEGFR1-T* allele, which contains a p53 half-site RE. In transfection assays that included p53, ER and a 1 kb region of the *VEGFR1-T* promoter linked to a reporter, the presence of ERα or ERβ resulted in an ER ligand-dependent, synergistic increase in p53-mediated expression. This finding also merged three major pathways that affect carcinogenesis: p53, VEGF-mediated angiogenesis and ER-mediated hormone responses. Although ER may directly interact with p53 (REFS 151,152), the observed synergy was specifically due to the action of ER in *cis*, as mutation of the ER target sequence prevented ER stimulation. The binding of ER seemed to be dependent on p53 — possibly through a co-activator, such as the thyroid hormone receptor-associated protein (TRAP)–mediator complex.

Because a five-nucleotide ER half-site sequence is thought to frequently occur in the genome, we investigated the generality of the ER–p53 synergy (D.M., A.I. and M.A.R., unpublished observations). The p53 half-site RE in the 1 kb promoter region of *VEGFR1-T* in the reporter plasmid was replaced by various half sites or by well-established full-site REs for p53-induced transactivation. These full-site REs could be weak or moderate (such as those from *AIP* and growth arrest and DNA damage-inducible 45 (*GADD45*)) or strong (for example, that from *CDKN1A*). Remarkably, not only was there ER and p53 synergy in the responsiveness to p53, but the greatest effects were also obtained with the half sites and the weaker REs (up to fivefold; see the example in FIG. 2b).

As the p53 responsiveness of REs can be greatly increased by an ER site in *cis*, does this apply to mutant p53s that retain transcriptional function? We recently

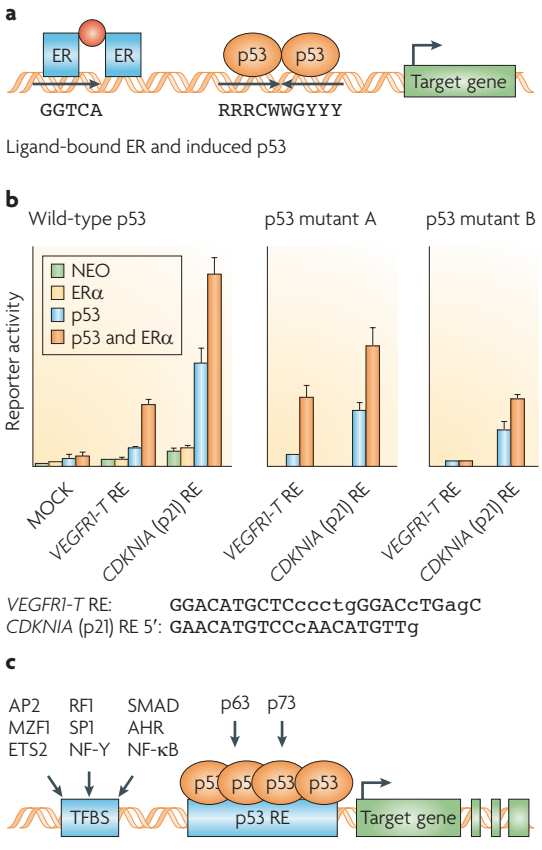


Figure 2 | **Cooperation in *cis* between p53 and other master regulators to drive transactivation at canonical and non-canonical p53 REs. a** | Transcriptional responses owing to in *cis* interactions between p53 and oestrogen receptor (ER), as determined for a promoter region of vascular endothelial growth factor receptor 1 (*VEGFR1*) that contains a non-canonical half-site p53-response element (RE) (*VEGFR1-T* RE) and a half-site ER RE (ERE)[161] located ~200 nucleotides upstream. Activation of p53 (for example, after DNA damage) results in the interaction of p53 with its half-site RE in the *VEGFR1* promoter, leading to limited transactivation. If the ER pathway is also activated by an ER ligand, then ER can interact with its ERE, leading to a synergistic increase in transactivation. **b** | The level of transactivation and synergy with ER is highly dependent on the sequence and strength of the p53 RE. As expected, the replacement of the original half-site RE in the *VEGFR1* promoter with a canonical p53 RE derived from the *CDKN1A* (p21) target gene results in higher p53-driven transactivation; however, the transactivation is increased twofold by the presence of activated ER. p53 mutants with altered transactivation (but not loss of function) can also cooperate with ER in the transactivation of the reporter. The individual transactivation characteristics of the p53 mutant can affect the response to ER (presented here is a comparison between G279R and R337C, corresponding to p53 RE mutants A and B, respectively; (D.M., A.I. and M.A.R., unpublished observations)). **c** | Examples of how other sequence-specific transcription factors could function in *cis* with p53. Partially overlapping REs can result in negative interactions, such as in the case of p53 and SP1 REs. For some transcription factors combinatorial interactions have been inferred based on the significant over-representation of cognate REs among p53 targets (AP2, myeloid zinc finger 1 (MZF1) and ETS2)[135], and for others (interferon regulatory factor 1 (IRF1), SP1, nuclear factor-Y (NF-Y), SMAD, aryl hydrocarbon receptor (AHR) and nuclear factor-κB (NF-κB)) there are reports of functional interactions with p53 that, unlike for *VEGFR1*, were not shown to be cooperative and seemed to be mediated by canonical promoter elements[147,154,202]. In the case of p63 and p73, interactions may occur through shared REs; the relative affinity may dictate the expression of the associated gene. For example, relative differences in the effect of a C or G instead of a W in the CWWG were reported in comparisons between p53 and p63 proteins interacting with DNA[157,203]. NEO, neomycin control; TFBS, transcription factor binding site.

examined several cancer-associated p53 mutants for their ability (with and without ER expression) to transactivate the *VEGFR1-T* motif reporter plasmids described above that contain various half- and full-site p53 REs (D.M., A.I. and M.A.R., unpublished observations). The transcriptionally inactive G279E mutant was also non-functional in this system. However, for two mutants (FIG. 2b), which have reduced transactivation at several REs, including the *VEGFR1-T* RE and the full-site p21 RE, the presence of ERα greatly increased p53 responsiveness. The increase in responsiveness was due to ER functioning in *cis*, as inactivation of the ER target sites removed the stimulation. Therefore, for some p53 mutants, cellular ER status and in *cis* cooperation may be important for reactivating or potentiating residual transactivation capabilities. This might contribute to the poorer cancer prognosis for ER-negative tumours. It will be interesting to assess whether there is an in *cis* interaction between p53 isoforms[72] and ER. The ability of ER to synergize with mutant p53 and increase transactivation may provide a means for assessing whether a mutant protein has retained structural integrity, which could prove useful for developing chemical modifiers.

These results demonstrate the potential for ER to interact in *cis* with weak p53 targets that might normally be undetected. This finding raises many questions, including the generality of the synergy between ER and p53 across the genome and the requirements for the synergy, such as the distance between the ER and p53 targets, as well as variations in target sequences. For example, there are over 600 *VEGFR1-T* promoter-like motifs in the human genome (D.M., A.I. and M.A.R., unpublished observations) based on an *in silico* search of 2 kb promoter regions upstream of transcriptional start sites using the following motif: (half ERE) … (<250 nucleotides) … (half p53 RE) … (<250 nucleotides) … (half ERE).

The abundance of these motifs suggests that the universe of p53 target sequences is much larger than indicated by investigations that focused on full-site REs or even half sites. These observations set the stage for investigating other transcriptional factors that might have a role in p53-targeted gene expression. Using bioinformatics-based composite module pattern searches, REs for kruppel-like factor/paired box 4 (KLF/PAX4), SP1 and NF-κB are over-represented among p53 target promoters (REF. 135; A. Jegga, personal communication). This suggests that these proteins can participate in p53-dependent transactivation and that their dysregulation in cancer cells could modulate p53 functions in some tissue types or stages of tumorigenesis. For example, PAX4 was proposed to function as a tumour suppressor in melanoma[162] but as a survival gene in insulinoma cells[163]. Other TFs were shown to modulate p53-dependent transactivation in *cis*; however, unlike for VEGFR1, the functional interactions were dependent on canonical REs and were not synergistic (FIG. 2c).

Functional p53 mutants change the universe of p53-regulated genes. Cancer-associated p53 mutants can be divided into three functional categories: complete loss of function, gain of function and altered spectrum. Most of these mutants lack direct sequence-specific transcriptional activity. The latter two categories are not surprising as cancer mutations are typically missense and affect amino acids in the DNA binding domain of p53 (REF. 42) (see the International Agency for Cancer Research *TP53* Mutation Database). Also, in some cases mutant p53 proteins are abundantly expressed in the cell, partly owing to the loss of the MDM2 negative-feedback loop[164]. For gain-of-function p53 mutants[165,166], altered p53 can form complexes with NF-κB[167] and nuclear factor-Y[168] and are recruited to new promoters (for example, vitamin D3 receptor (VDR))[167]. Mutant p53 proteins were recently shown to bind to SMAD2–p63 complexes and affect the migratory potential of epithelial cells, therefore directly affecting metastatic potential[169].

Approximately 30% of the p53 mutants that are associated with cancer retain transactivation activity towards at least a few REs (see the International Agency for Cancer Research *TP53* Mutation Database). Although the reported functionality of mutants may differ between studies (partly because of the methods for evaluating RE functionality), evidence of sequence-specific transactivation suggests that at least some transactivating function has been retained. Among these p53 functional mutants there are various changes in transcription patterns from individual REs that can depend on p53 expression levels. For example, several transcriptionally active mutants identified in breast[170–172] and adrenal gland cancers[173] have subtle defects in the transcription at REs that are revealed at the low expression levels that can be achieved in a yeast-based system[102,107,174].

Many of the functional mutants affect the spectrum and levels of transactivation from various REs. There are even mutants (such as S121F, T123A and N288K[175–177]) for which the responsiveness at some REs — that are weakly transactivated by normal p53 — is greatly increased. Changes in the ability of p53 to function at various REs can diversify the downstream biological responses and might be an important component in some cancers. This was demonstrated for apoptosis and radiation survival, in which expression of the T125R mutant led to γ-radiation sensitivity and resistance to ultraviolet irradiation; expression of the wild-type protein resulted in the opposite phenotype[177].

The altered networks resulting from mutations in p53 might be expected to affect prognosis. This has been shown for Li-Fraumeni syndrome, in which the onset of cancers is delayed in individuals harbouring altered-function mutations[41,172]. However, in sporadic breast cancer the functional classification of p53 mutations did not provide additional prognostic value compared with the assessment of the presence or absence of p53 mutations[178]. On the contrary, the transactivation potential of p53 mutant alleles correlated with a worse outcome in specific stages of sporadic colorectal cancer[179].

These findings with functional, cancer-associated mutants have broad implications for the evolution of master regulatory networks. As a single mutation can

alter the range of genes transactivated and the levels of responsiveness, such changes could allow the rapid functional evolution of many components in a system. In this sense, p53 may represent a 'master gene of diversity' (REF. 102), at the organismal level and at the tissue level, in terms of the emergence of cancers. A specific example of evolutionary changes in the p53 sequence was described for the mole rat and was proposed to be part of the adaptation to hypoxic environments[180,181].

Conclusion

The cellular responses to internal and external stresses that might ultimately lead to cancer can be strongly influenced by p53. The universe of genes that are subject to direct control by p53 is much larger than originally anticipated based on previously established consensus sequences. Similarly, the number of known signalling pathways that respond to diverse cellular perturbations and lead to p53 activation is increasing. The integration of all these p53-inducing signals and p53-influenced activities is likely to affect the role of p53 as a tumour suppressor in different tissues. There is considerable flexibility in what constitutes an RE and this may be augmented by *cis*-association with other regulators, as suggested by synergistic interactions of p53 with ER. Many factors influence p53 transcriptional responses at individual genes, including cofactors that may be stress-specific, the strength of RE binding and the levels of p53 in response to stress. On a more general level, the influence of these factors in regulatory networks should be considered as analogue rather than binary, in the sense that instead of a simple on–off response (as for most components in regulatory networks) there can be considerable variation and flexibility in the transactivation response.

Although there has been substantial progress in identifying REs and understanding their interactions with p53, much remains to be done. For example, additional functional non-canonical REs need to be identified and investigated to develop rules for how REs are engaged by p53. Functional interactions between p53 and other master regulators are likely to affect the role of p53 as a tumour suppressor, as are SNPs in p53 REs or other components of the p53 regulatory system. Variations in p53-driven transcription between tissues and stress responses should also be characterized. The increasing dimensions of the p53 transcriptional network increases the number of potential therapeutic targets, which may provide greater opportunities for intervention strategies focused on the modulation of wild-type or mutant p53 functions in cancer. The influence of individual and combined chemotherapeutic agents that target the p53 system (that is, RITA[182] and Nutlins[183]) and mutant proteins (such as p53 reactivation and induction of massive apoptosis (PRIMA1)[184] and the carbazole derivative PhiKan083 (REF. 185)) remains to be addressed. Determining the relationship between expression levels and responsiveness at canonical and non-canonical target sequences is important for evaluating the consequences of cancer-associated p53 mutations, particularly those that retain transactivation capabilities. Because of the complexity and control of the universe of genes in the p53 master regulatory network, the role of p53 and target sequences in cancer and overall human biology remains a challenge even after 30 years of intense investigation.

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DATABASES

Entrez Gene: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=gene
ASPP1 | *ASPP2* | *GADD45* | *LASP1* | *MDR1* | *PIN1* | *TP53* | *VEGFR1*
UniProtKB: http://www.uniprot.org
CARM1 | CSE1L | ER | Hamlet | iASPP | IGFBP3 | IRF1 | MDM2 | MDMX | p21 | p300 | PRIMA1 | PRMT1 | stathmin | survivin | TP53I3 | VDR | VEGFA

FURTHER INFORMATION

Michael A. Resnick's homepage: http://www.niehs.nih.gov/research/atniehs/labs/lmg/cs/index.cfm
DAVID Bioinformatics Resources website: http://david.abcc.ncifcrf.gov/home.jsp
International Agency for Cancer Research *TP53* Mutation Database: http://www-p53.iarc.fr
***TP53* Website:** http://p53.free.fr/Database/p53_database.html
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