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MDM2, MDMX and p53 in oncogenesis and cancer therapy

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Abstract

The MDM2 and MDMX (also known as HDMX and MDM4) proteins are deregulated in many human cancers and exert their oncogenic activity predominantly by inhibiting the p53 tumour suppressor. However, the MDM proteins modulate and respond to many other signalling networks in which they are embedded. Recent mechanistic studies and animal models have demonstrated how functional interactions in these networks are crucial for maintaining normal tissue homeostasis, and for determining responses to oncogenic and therapeutic challenges. This Review highlights the progress made and pitfalls encountered as the field continues to search for MDM-targeted antitumour agents.

Thirty years of research have shown that the tumour suppressor p53 has a crucial role in many physiological processes, and that it is mutated or functionally inactivated in most human cancers. In a substantial proportion of cancers *TP53* (which encodes p53) is wild type but the protein is inactivated; this offers an attractive strategy for cancer therapy based on p53 reactivation. Although clinically approved, p53 activators are still a dream; recent studies in cancer patients have provided proof-of-concept for this approach. Such activators are the product of basic research conducted over the past 20 years that has led to the appreciation of MDM2 and MDMX (also known as HDMX and MDM4) as the two major negative regulators of p53, which now seem to be ‘druggable’ using a variety of strategies.

In this Review, we highlight the major advances in our understanding of the biological function of MDM2 and MDMX, and evaluate the evidence that they are oncogenic. We discuss the physiological roles of MDM2 and MDMX and their associated key signalling pathways, as studies in this area have provided important insights into potential clinical benefits and toxicities that are likely to arise from using MDM2 and MDMX antagonists. Finally, we review the current status of small-molecule and peptidic MDM2 and MDMX inhibitors and emphasize how systems biology approaches have provided rationales for developing novel combination strategies. The emerging picture is one of context: MDM2

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and MDMX should be considered as two of many crucial factors that contribute to tumour development. Thus, their misregulation sets the stage for additional genomic and epigenetic alterations that lead to cancer. Such a perspective should stimulate approaches to identify and to treat patients whose tumours are particularly susceptible to the targeting of defective MDM2–MDMX–p53 circuitry.

The core pathway

Most p53 mutants in human tumours are transactivation-deficient, suggesting that blocking p53-dependent transcription is a crucial event in tumorigenesis[1]. Consistent with this, inhibition of p53 transcriptional activation was the first functional role ascribed to MDM2 (Ref. 2). Amplification of MDM genes or altered expression of MDM proteins is a feature of many tumours[3–10] (Table 1). In many cases, the frequency of MDM protein deregulation is higher in tumours that retain wild-type p53. Taken together, these observations indicate that a major oncogenic role of MDM proteins is to block p53 transcriptional activity.

Although both MDM2 and MDMX can inhibit p53 transactivation function by engaging its amino-terminal transactivation domain via related N-terminal hydrophobic pockets[2,11,12], key differences between MDM2 and MDMX affect their ability to regulate p53, as well as their biochemical functions. For example, although p53-responsive elements have been found in both the *MDM2* and *MDMX* promoters, *MDM2* is more broadly responsive to p53 activation. By contrast, HDMXL, which is an MDMX protein with an 18-amino acid N-terminal extension, is induced by p53 under more selective conditions[13,14]. MDM2 homo-oligomers have E3 ubiquitin ligase activity, which depends on an intact carboxy-terminal RING domain[15]. On binding, MDM2 ubiquitylates p53 and leads to its proteasomal degradation; this keeps p53 levels and activity low in unstressed cells. By contrast, MDMX does not homo-oligomerize and has no intrinsic ubiquitin ligase function, although it can increase or decrease MDM2 ubiquitin ligase activity depending on MDMX abundance[16]. Hetero-oligomerization of MDM2 and MDMX via their RING domains is crucial for the suppression of p53 activity during embryonic development[17,18]. Furthermore, aromatic residues that are present in the RING-proximal C-terminal domains of both MDM2 and MDMX are required for the recruitment of E2 ubiquitin-conjugating enzymes[19–21]. Thus, hetero-oligomerization of MDM2 and MDMX may create a more effective p53 E3 ubiquitin ligase complex, or a more effective inhibitor of p53-dependent transactivation; determining whether these two functions are separable will require additional *in vivo* models.

Given these findings, we focus on the p53–MDM2–MDMX network, as perturbing this pathway has clear implications for tumorigenesis and presents exciting opportunities for cancer therapy. However, it is important to emphasize that both MDM proteins are reported to have p53-independent roles (Box 1). Such functions may explain the apparent selection for deregulation of MDM2 or MDMX in some tumours that express mutant p53.

MDM transcriptional regulation

Gene amplification can lead to increased MDM2 or MDMX protein expression. However, many tumours exhibit high MDM2 and MDMX protein levels without increased copy number. These include melanoma[10], Ewing's sarcoma[22], colon carcinoma[9] and

retinoblastoma[12,23]. Thus, the gene amplification criterion may underestimate the number of tumours in which MDM2 or MDMX overexpression contributes to cancer initiation, maintenance or progression. Studies of tissue-specific regulation of MDM transcription and translation might provide clues to how such high MDM protein levels can be attained. For example, the post-translational stabilization of MDM2 and MDMX in some tumours may occur via the activation of cancer-specific signalling pathways, or the hijacking of normal signalling modules that regulate MDM2 and MDMX levels. As identifying the components of such pathways should lead to the development of novel strategies for reducing MDM2 and MDMX abundance, we review below the mechanisms by which MDM proteins are induced or stabilized in normal tissue homeostasis and cancer (Fig. 1).

Proliferating cells are more sensitive than resting cells to MDM2 and MDMX depletion[24]. It is not surprising, therefore, that links between mitogenic signalling and MDM proteins are emerging. The RAS signalling pathway converges on the activation of ETS transcription factors, and overexpression of RAS leads to ETS-dependent upregulation of both MDM2 and MDMX expression[9,25]. The subsequent inhibition of p53 may increase the likelihood of RAS-expressing cells escaping p53-dependent arrest. As colon carcinomas and melanomas frequently harbour activated RAS and high levels of MDM2 or MDMX, combinations of RAS pathway inhibitors and MDM2 and/or MDMX antagonists might be particularly effective in such tumours (discussed below).

The activity of MYC, another potent mitogen and an oncogene, is also sensed by p53. In *Mdm2*$^{+/-}$- and *Mdmx*$^{+/-}$-heterozygous mice, the threshold for p53 activation in response to MYC is lowered, resulting in lower rates of MYC-induced lymphoma[26,27]. Conversely, one might expect MYC and MDM2 or MDMX to collaborate during tumorigenesis. Intriguingly, MDMX levels seem to be increased in B cells that overexpress MYC before tumorigenesis[28]. However, whether this is a cause or a consequence of MYC-induced transformation of B cells is unclear. Although potential MYC-binding sites have been found in *MDMX* (M.W., unpublished observations), there is little evidence supporting MYC-dependent increases in *MDMX* mRNA. However, there are binding sites for the B cell-specific transcription factor interferon-regulatory factor 8 (IRF8) in both *MDM2* and *MDMX*[29] (M.W., unpublished observations). Furthermore, there is genetic evidence that MDM2 is upregulated by IRF8 during germinal centre B cell proliferation[29]. The induction of B cell proliferation during homeostasis (or following oncogene activation) and the ensuing IRF8-dependent increase in MDM proteins may limit p53 activity as cells undergo genetic recombination events.

Much more remains to be discovered regarding the regulation of *MDM2* and *MDMX* transcription. Although activated p53 was thought to exclusively upregulate *MDM2*, new data have revealed p53-dependent increases in MDMX expression under certain conditions[14]. These mechanisms may contribute to the inhibition of p53 in response to both physiological stresses and p53-targeted therapeutics. Mutation of the p53 response elements in the *Mdm2* and *Mdmx* promoters *in vivo* will be required to establish their role in the p53 response. Additionally, the factors that control tissue-specific transcriptional regulation of *MDM2* and *MDMX* have not been systematically investigated. For example, it will be interesting to determine the tissue-specific and stress-specific factors that control the

expression of *MDM2* from the P2 promoter. Originally identified as p53-responsive, it is now clear that other transcription factors can modulate expression from this promoter[30,31].

MDM post-transcriptional regulation

Additional layers of post-transcriptional control of MDM proteins are continually being revealed. Endogenous mechanisms include regulation by microRNAs (miRNAs) and by post-translational modifications (PTMs). Intriguingly, viruses may subvert these mechanisms as part of their life cycle. We discuss below each of these regulatory circuits as they relate to cancer.

PTMs

Multiple kinases are known to modify both MDM proteins and (perhaps unsurprisingly) the downstream effects cluster into those that inhibit and those that activate p53. The AKT kinases regulate cell growth and survival, and they are activated in human cancers; among their targets are MDM2 and MDMX. Phosphorylation of MDM2 at Ser166 and Ser186 (Ref. 32), and of MDMX at Ser367 (Ref. 33), leads to their stabilization, and is associated with p53 inhibition. Casein kinase 1α (CK1α)-mediated phosphorylation of MDMX increases its affinity for p53 and may therefore reduce p53 activity[34]. Following genotoxic stress, DNA-dependent protein kinase (DNA-PK) phosphorylates Ser17 of MDM2 (Ref. 35), and ABL (also known as ABL1) phosphorylates Tyr99 of MDMX[36]. In each case, this leads to the dissociation of p53 from its negative regulators.

Following DNA damage, the ataxia-telangiectasia mutated (ATM) and CHK kinases also phosphorylate multiple serine residues in or close to the RING domains of MDM2 and MDMX[37]. This can lead either to the dissociation of MDM oligomers or to the destabilization of MDM2 or MDMX[38–41]. The downstream consequences of the ensuing p53 activation are determined in a tissue-specific manner[42]. Conversely, dephosphorylation of these residues may inhibit p53. Indeed, protein phosphatase 1D (PPM1D) can dephosphorylate both MDM2 and MDMX, thereby contributing to its oncogenic function[43,44]. The CK1δ kinase phosphorylates MDM2 in the acidic domain, and this also leads to p53 activation[45]. Mutation of some of these phosphorylation sites is associated with tumorigenesis in knock-in mouse models[46,47] (discussed below).

miRNA links to p53 pathway control by MDM2 and MDMX

Recent studies have added numerous miRNAs to the gene constellation that p53 regulates[48,49], which in turn contribute to its activation. Importantly, several studies have indicated that some p53-induced miRNAs contribute to the downregulation of MDM2 and MDMX. Although perhaps expected in normal cells, it was surprising to find that in multiple myelomas in which p53 is wild type, p53 can induce the expression of *mir-192*, *mir-194* and *mir-215*, which subsequently downregulate MDM2 expression[50]. This would be expected to activate p53. However, hypermethylation of the promoter region of all three miRNAs impairs MDM2 downregulation on p53 activation, which should blunt p53 function in these tumours[50].

In addition to genotoxin-induced degradation of MDMX, *MDMX* mRNA levels is also reduced on DNA damage. This results, in part, from the action of *mir-34a*[51]. The *mir-34a* target site colocalizes with a single nucleotide polymorphism (SNP) in exon 11 of the *MDMX* mRNA[52]. Sequence alignment indicates that the C allele of the SNP would disrupt *mir-34a* binding[52]. In addition, a SNP (SNP34091) in the *MDMX* mRNA 3′ untranslated region (UTR) was recently shown to be an illegitimate target site of miR-191 that was nevertheless targeted[53]. However, the effects of these miRNAs in modulating MDMX levels, and therefore p53 regulation *in vivo*, require additional study.

Although the data suggest that p53, microRNAs, MDM2 and MDMX form regulatory circuits to facilitate the rapid activation of p53 on stress, a recent *mir-34*-knockout mouse model suggests caution in such an interpretation. These studies have revealed that the *mir-34* family does not have a substantial effect on p53 tumour suppressor functions *in vivo*[54]. Thus, the biological importance of the p53-miRNA regulatory network for tumour suppression and stem cell biology remains to be clarified, and additional *in vivo* models should illuminate this[48].

The study of virus-induced cellular miRNAs is also beginning to reveal some surprises. For example, p53-independent induction of *mir-34a* by Epstein-Barr virus (EBV) seems to be necessary for the transformation of B lymphocytes[55]. This again underscores the context-dependent effects of *mir-34a* induction. EBV infection also leads to the expression of miRNAs that have seed complementarity to both *MDM2* and *MDMX*[55]. However, the downregulation of MDM2 and MDMX would be expected to induce growth inhibition; thus it is likely that additional cellular transcripts with target sites that are similar to the MDM mRNAs are the primary targets. Whether other viruses might use miRNAs to modulate *MDM2* or *MDMX* mRNA levels to inhibit the p53 feedback loop is unclear.

Regardless, virus-induced upregulation of MDM2 by both transcriptional and post-transcriptional mechanisms has been reported. EBV is associated with several tumour types, and it expresses latency antigens that stabilize MDM2 (Ref. 56). MDMX levels are also fairly high in EBV-infected B cells, although the underlying mechanism is unclear. Kaposi's sarcoma-associated herpes virus (KSHV) expresses vIRF4, a factor that binds MDM2 and that seems to switch its ubiquitin ligase activity to p53 degradation[57]. Both EBV and lymphoid cells exhibit B cell tropism, and it is clear that small changes in the levels of MDM proteins in these cells render them susceptible to transformation[58]. Tumour-associated SNPs in the *MDM2* promoter are also correlated with the increased risk of hepatitis C-induced cancer[59]. Together, these data suggest that targeting MDM proteins in virus-associated malignancies may have therapeutic benefit. In support of this, the MDM2 antagonist nutlin 3a induces cell death in KSHV-positive tumour cells[60].

MDM2 and *MDMX* as oncogenes: the evidence

Cell-based studies

MDM2 and MDMX have oncogenic properties *in vitro*, but this is only manifest in the context of other oncogenic lesions. For example, attempts to use primary cells to demonstrate that MDM2 alone has transforming activity have been unsuccessful. This is

probably because MDM2 overexpression at supraphysiological levels is toxic to many normal cells, suggesting that it may have other cellular targets[61]. MDM2 is also inherently unstable, making it difficult to express the protein at levels that are sufficient to inactivate p53 and to induce tumorigenesis. By contrast, the overexpression of MDM2 in immortalized mouse NIH3T3 cells renders them tumorigenic[62]. Thus, the effects of MDM2 are manifest in a background of pre-existing genetic changes. Conversely, MDMX has a long half-life and can generally be expressed at extremely high levels. MDMX can immortalize primary mouse embryonic fibroblasts and can accelerate the growth of human fibroblasts[63,64]. However, despite its ability to inhibit p53, MDMX overexpression alone is insufficient for the robust transformation of either human or mouse cells.

In vivo studies

Tables 2,3 list the tissue-specific effects of *Mdm2*- or *Mdmx*-knockout mice, as well as several *in vivo* models that have shed light on their oncogenic roles. Early transgenic mouse models showed that MDM2 overexpression could induce carcinoma[65] and lymphomas or sarcomas[66]. MDM2 overexpression does not seem to accelerate the onset of tumorigenesis in *Trp53* (which encodes p53 in mice)-null animals. Together, these data provide evidence that a major oncogenic effect of MDM2 is mediated through p53 inhibition. It is possible that positional effects and a lack of wild-type gene architecture contribute to the phenotypes of transgenic animals. However, a more recent mouse model used a knock-in of a human tumour-associated *MDM2* SNP at the endogenous *Mdm2* locus. Data from this model confirm that a subtle increase in MDM2 levels engenders tumorigenesis at a rate that is similar to that observed in *Trp53*-hemizygous animals[67]. Conversely, haploinsufficiency of MDM2 delays the onset of MYC-induced lymphoma *in vivo*[27].

Given the many functional similarities between MDM2 and MDMX, and the frequent overexpression of MDMX in some human cancers, it was logical to predict that MDMX overexpression *in vivo* would be tumorigenic. However, mouse models of MDMX overexpression have yielded conflicting results. In one study, transgenic mice that ubiquitously expressed MDMX developed tumours with a latency that was comparable to that of *Trp53*-hemizygous mice[68]. However, a subsequent study found no evidence for enhanced tumorigenicity following high-level *MDMX* transgene expression in many tissues[28]. Differences in transgene introduction strategies and mouse genetic backgrounds may contribute to these disparate results, which underscores the idea that the context in which MDMX is overexpressed is probably a major determinant of its oncogenic activity.

On the basis of the finding that MDM proteins are the targets of multiple kinases, two mouse models have been generated to test the effect of MDMX and MDM2 PTMs on p53 regulation and the subsequent biological sequelae in different tissues. In the first mouse model, Wang *et al*.[46] mutated three serines in the C terminus of MDMX to alanine ($MDMX^{3SA}$). Phosphorylation of these residues by ATM and CHK2 triggers MDMX degradation. Therefore, $MDMX^{3SA}$ is resistant to DNA damage-induced degradation, which ultimately attenuates p53 function. Consistent with this, $MDMX^{3SA}$ mice are extremely radioresistant. Surprisingly, although there was no increase in spontaneous tumour formation in $MDMX^{3SA}$ animals, these mice were more prone to *Myc*-induced

tumorigenesis[69] (discussed below). These data reveal that MDMX modification is important for preventing oncogene-induced tumorigenesis, but does not affect the tumorigenicity that is associated with background mutagenic events. By contrast, the mutation of Ser394 to alanine (Ser394Ala) in mouse MDM2 *in vivo* removes an ATM phosphorylation site, engenders radioresistance and increases the rate of spontaneous tumorigenesis to that observed in *Trp53*-hemizygous animals[47]. Furthermore, the predominance of T cell lymphomas in the MDM2-Ser394Ala mice recapitulates the range of tumour types found in *Trp53*-deficient mice. Together, these data indicate that the phosphorylation of both MDM2 and MDMX C termini is required for the p53 response to high levels of ionizing radiation. However, p53 sensing of endogenous (and potentially oncogenic) DNA damage *in vivo* seems to specifically require modification of the MDM2 C terminus.

Cooperating lesions

Both the cell-based and the *in vivo* data discussed above indicate that the full oncogenic potential of MDM2 and MDMX is only revealed in the presence of additional genetic lesions. *In vitro* culture and mouse models have provided some insight into the nature of these lesions. The transduction of normal human and mouse fibroblasts with oncogenic RAS variants leads to senescence, which is generally p53-dependent. However, co-expression of MDMX leads to cellular transformation *in vitro*[63,64,70]. Importantly, this requires the p53-binding domain of MDMX, suggesting that p53-inhibitory activity of MDMX is crucial for transformation[63]. A recent *in vivo* model of melanoma has also provided evidence for the cooperation between MDMX and RAS during tumorigenesis. Following melanocyte-specific expression of MDMX and RAS, the time to onset of melanomas was reduced and the tumours that developed were more aggressive[10]. Furthermore, the finding that melanomagenesis in *Trp53*-hemizygous mice is accelerated by MDMX overexpression confirms that p53 is the main target of MDMX in this cancer. The recapitulation of some of the genetic and clinical features of melanoma in this study should inspire additional *in vivo* models to dissect the tissue-specific role of MDMX in other tumour types.

Similar to RAS, the oncogenic activity of MYC can be limited by p53 activation. This was clearly established in mouse models in which the deletion of *Trp53* decreased the time to onset of MYC-driven B cell lymphoma[71]. Although p53 mutations are commonly found in human and mouse lymphomas, a substantial proportion retains the wild-type *Trp53* allele. This suggests that there are additional ways to subvert p53 activation in B cell neoplasms. An obvious candidate is MDMX, and two mouse models have addressed its potential role in MYC-induced lymphomagenesis. Marine and colleagues[28] found that MYC-induced lymphomas were not accelerated in mice with ubiquitous overexpression of wild-type *Mdmx*. By contrast, Wahl and colleagues[46] reported a dramatic acceleration of MYC-induced lymphomas in the presence of the $MDMX^{3SA}$ mutant. As MDMX levels were lower in this study, the amount of MDMX present cannot explain the variation in results. As $MDMX^{3SA}$ mice have an attenuated response to DNA damage, and because MYC is known to induce genotoxic stress, disruption of the MYC–DNA damage–MDMX circuit might contribute to increased tumorigenesis in $MDMX^{3SA}$ mice. Intriguingly, one of the ATM target sites (MDMX-Ser367) was mutated in a squamous cell carcinoma sample, and this occurred in the context of *TP53* heterozygosity[72]. However, further clinical and

experimental studies are required to establish the importance of MDMX phosphorylation in human cancer. Although there are correlations between high levels of NMYC and MDM2 in neuroblastoma cell lines[73] there is no direct *in vivo* evidence that MYC and elevated MDM2 levels cooperate to accelerate tumorigenesis.

In addition to their cooperation with oncogenes, the expression of MDM proteins can accelerate tumorigenesis that is caused by the loss of tumour suppressors. For example, retinoblastoma (which is caused by the loss of proteins of the RB tumour suppressor family) is accelerated by MDMX overexpression[12]. This is important from a clinical perspective because retinoblastomas retain wild-type *TP53* and may therefore benefit from treatment with MDM2 and/or MDMX antagonists. Loss of the PTEN tumour suppressor leads to the activation of the AKT kinases and increases the level of MDM2 (Ref. 74). This is associated with an attenuated p53 response. Although AKT has multiple downstream targets in addition to MDM2, tumour cells with activated AKT pathways are sensitive to MDM2 antagonists[75,76].

Together, these data indicate that the oncogenic stress that is generated by the loss of tumour suppressors may lead to the selection of tumour cells with elevated MDM2 or MDMX levels. In this regard, understanding the homeostatic roles of MDM proteins will provide insights into the mechanisms by which they are deregulated during cancer (Box 2). Furthermore, the failure of MDM2 and MDMX overexpression in isolation to robustly induce tumours may indicate that ‘too much is not enough’. Rather, their full oncogenic potential may only be unleashed in the context of other neoplastic changes.

p53 reactivation in cancer therapy

Around 22 million cancer patients have defects in p53 signalling[77]. Although ∼50% of these patients harbour mutant p53, which has lost its tumour suppressor function, the other 50% retain a wild-type *TP53* allele. The function of p53 is attenuated by MDM2, MDMX and other signalling modules. Mouse models clearly demonstrate that the inactivation of p53 promotes tumorigenesis, whereas restoration of p53 causes the regression of established tumours[78–80]. Although deletion of *Mdm2* can cause p53 activation in both tumour tissue and normal tissue[81], and p53 upregulation in the haematopoietic system engenders myeloablation[82,83], the MDM2 antagonist nutlin 3a (discussed below) is well-tolerated in mouse preclinical models[84]. Mouse tissues also seem to tolerate the p53 activation that accompanies *Mdmx* deletion[85]. These data indicate that wild-type p53 is a valid therapeutic target, and that reactivation of p53 via selective targeting of either MDM2 or MDMX is a viable strategy for cancer therapy.

Several pharmacological strategies have been proposed for the activation of wild-type p53 (Table 4). However, interfering with MDM2 and MDMX is the most direct approach. First, reducing MDM2 and MDMX abundance in cancer cells should enhance p53 activity. Second, inhibitors of MDM2 E3 ubiquitin ligase function should increase p53 level and activity. Third, protein–protein interaction (PPI) antagonists that selectively disrupt p53–MDM2 or p53–MDMX N-terminal interaction should activate p53. In addition, attenuation of MDM2 ubiquitin ligase activity should increase p53 levels and activity. This may be

achieved using PPI antagonists of the heterodimerization of MDM2 and MDMX RING domains, or of the interaction between MDM2 and E2 ubiquitin-conjugation enzymes (designated MDM2/E2). There are also more indirect approaches, which is discussed in Box 3. Additionally, as the pharmacological reactivation of mutant p53 is an attractive, though challenging, strategy, we refer readers to reviews that discuss this option in detail[77,86].

Modulating protein expression

A straightforward strategy to reduce MDM2 and MDMX protein levels in cancer cells is to specifically target them using small interfering RNA (siRNA), short hairpin RNA (shRNA) or miRNA approaches. Unfortunately, siRNA therapy is still hampered by delivery and cellular uptake issues[87], making this approach currently unrealistic. A benzofuroxan derivative (NSC207895) that selectively inhibits MDMX expression has been identified[88]. This molecule downregulates MDMX levels and causes p53-dependent transactivation of proapoptotic genes in several cancer cell lines. NSC207895 seems to repress the *MDMX* promoter, although the underlying molecular mechanism of promoter-specific targeting has not yet been revealed[88]. However, NSC207895 also clustered with known DNA-damaging agents, such as methyl methanesulphonate (MMS) and camptothecin, in a cross-species chemogenomic profiling screen[89]. As DNA-damaging agents induce MDMX degradation[90] it is possible that the effects of NSC207895 on MDMX protein levels and p53 activation involve more than the repression of *MDMX* transcription.

The molecular chaperone heat shock protein 90 (HSP90) is highly expressed in many cancers[91]. Treatment with the HSP90 inhibitor 17-allylamino-17-demethoxygeldanamycin (17-AAG; also known as tanespimycin) destabilizes MDMX in several cancer cell lines. Co-treatment of such cancer cells with 17-AAG to downregulate MDMX, and nutlin 3a to antagonize the MDM2–p53 interaction, yielded synergistic cytotoxic effects, probably owing to enhanced p53 activation[92]. Currently, no compounds are reported to selectively reduce *MDM2* transcription and protein abundance.

Targeting protein–protein interactions

Genome-wide protein–protein network studies have provided a detailed map of disease-associated PPIs[93,94]. However, flat, featureless and extended PPI surfaces are considered undruggable with small-molecule compounds[95]. Small-molecule PPI antagonists that disrupt the p53–MDM2 interaction have been identified, including nutlin 3a[96] and MI-219 (Ref. 97). These compounds reactivate wild-type p53 by competing with it for binding to the hydrophobic cleft in the MDM2 N terminus. RG7112 (also known as RO5045337), a derivative of nutlin 3a with better potency and pharmacological properties, is in Phase I clinical trials (see the ClinicalTrials.gov website; see Further information), and the results of the first proof-of-mechanism study for RG7112 in patients have just been published[98]. A substantial proportion of liposarcomas express wild-type p53 and have amplified *MDM2*; thus, patients with liposarcomas of this class provide a suitable population in which to assess the ability of RG7112 to activate p53 and elicit downstream responses, as well as to determine the side effects from the use of this compound. The results of the analysis must be considered preliminary as only a small number of patients (20) were enrolled. However, the data indicate that RG7112 reaches its target in a solid tumour, and increases the level of

macrophage inhibitory cytokine 1 (MIC1; also known as GDF15), which is a secreted protein product of a p53 target gene. Interestingly, although RG7112 treatment generally correlated with increased p53 and p21 levels, and decreased proliferation, the data did not reach significance. As the tumours were not microdissected, the modest results might reflect the use of heterogeneous tumour tissue in which only a small proportion of cells responded to RG7112 at the time of analysis. Notably, p53 pathway reactivation resulted in at least one adverse event in each patient, the most frequent being related to haematological toxicities. The authors suggested that RG7112 may be considered in neoadjuvant therapy when combined with existing clinically approved cytotoxic agents. However, the radiosensitivity of the haematopoietic system of mice to p53 activation[83] suggests that such an approach would probably exacerbate the toxicity that is associated with RG7112. Rather, we suggest that combining non-genotoxic molecularly targeted therapeutic agents with p53 agonists might be a safer alternative.

Although nutlin 3a and MI-219 robustly activate p53 in cancer cells with overexpressed MDM2, they do not consistently elicit p53-dependent effects in cancer cells that overexpress MDMX[99–102]. This was surprising because the sequence of the MDMX N-terminal p53-binding domain is very similar to that of MDM2. However, subtle structural differences in the MDMX N-terminal p53-binding pocket dramatically reduce the binding affinity of both nutlin 3a and MI-219 for MDMX; this is supported by *in vitro* binding studies showing that nutlin 3a is 500-fold less potent against MDMX compared with MDM2 (Ref. 103). Despite this, a customized ocular formulation of nutlin 3a enhanced the efficacy of topotecan in a p53-dependent manner when it was subconjunctivally administered to MDMX-overexpressing retino-blastomas *in situ*[104]. However, it is not clear to what extent this synergy depends on the disruption of MDMX–p53 complexes versus MDM2–p53 complexes at the high dose of nutlin 3a used, and whether downregulation of MDMX by topotecan may also contribute. The small molecule SJ-172550 was identified as an MDMX–p53-selective antagonist[105] that exhibited additive cytotoxicity with nutlin 3a in MDMX-amplified retinoblastoma cells. However, subsequent analyses showed that SJ-172550 forms covalent adducts with cysteine residues in the p53-binding domain of both MDM2 and MDMX. The thiol reactivity precludes this chemical scaffold from further development and optimization as a selective MDMX inhibitor[106]. Although a selective MDMX small-molecule inhibitor does not currently exist, it is conceivable that MDMX-selective inhibitory polypeptides could be computationally designed. The extended length of such molecules may overcome some of the limitations that are associated with the use of small peptides. The expression of MDMX-targeted polypeptides would be a valuable addition to the study of MDMX function in normal and cancer cells.

Mouse genetic models strongly suggest that MDM2 and MDMX are the two major p53 antagonists *in vivo*[107,108] and that they exert non-overlapping inhibitory activities towards p53 (Refs 24,109). Therefore, dual antagonists for p53–MDM2 and p53–MDMX may effectively reactivate p53 in cancer. RO-5963 was identified as a dual inhibitor of p53–MDM2 and p53–MDMX[103]. Structural analyses suggest that the compound induces p53 activity via an unusual mechanism: it triggers the formation of MDM2–MDMX homodimers and heterodimers via interactions with their N-termini[103]. Intriguingly, although RO-5963

showed better *in vitro* binding affinity for MDM2 than nutlin 3a (IC_{50} values of 17.3 nM and 18.7 nM, respectively) it has poorer pharmacological properties. For example, at equimolar doses, nutlin 3a is a more potent p53 activator in cultured cancer cells[103]. Furthermore, in contrast to nutlin 3a, RO-5963 kills cells with both high MDM2 levels and high MDMX levels. This suggests that the disruption of p53–MDMX, rather than p53–MDM2, complexes may contribute to its cellular efficacy[103]. Further *in vivo* studies are required to understand whether this dual inhibitor will be useful in the clinic given its apparent weaker antagonism of p53–MDM2.

Peptide antagonists of p53–MDM2 and p53–MDMX interactions have been developed using structure-based rational design and phage display methods[110–112]. Although peptides and mini-proteins can antagonize PPIs more efficiently owing to their larger interaction surfaces, most of them are unstable *in vivo* and are poorly internalized, which compromises their ability to antagonize intracellular PPIs. To overcome these drawbacks, stapled peptides with improved cellular uptake and stability have been developed[113]. The stabilized α-helix of p53 variant 8 (SAH-p53-8) peptide has nanomolar binding affinity to the N-terminal p53-binding pocket of both MDM2 and MDMX *in vitro*. The IC_{50} of SAH-p53-8 for MDM2 is tenfold better than that of nutlin 3a, and the peptide also disrupts p53–MDMX interactions[110]. Additionally, *in vitro* assays indicate that SAH-p53-8 has a higher affinity for MDMX compared with MDM2 (Ref. 110). However, SAH-p53-8 is far less efficient at disrupting the p53–MDM2 interaction in cells and it requires nutlin 3a for optimal activity in cells that overexpress both MDM2 and MDMX. One interpretation is that SAH-p53-8 is a poorer antagonist of the MDM2–p53 interaction in cells with high MDM2 levels[10,110], possibly because its effective intracellular concentration is much lower than that of nutlin 3a. Indeed, cellular uptake of stapled peptides such as SAH-p53-8 requires pinocytosis[113], which is less effective than the uptake of small molecules through passive diffusion. Furthermore, SAH-p53-8 uptake is attenuated in the presence of serum (Y.-C.L. and G.M.W., unpublished observations). Inefficient uptake probably accounted for the need to use SAH-p53-8 at high concentrations (15–30 μM) to induce cytoxicity in a melanoma model[10]. Also, pharmacological properties of SAH-p53-8 might affect its affinity for MDM2 *in vitro* versus in the cellular microenvironment, as occurs with ABT-737, a peptidomimetic that antagonizes the interaction of BCL-XL with pro-apoptic BH3 family members[114]. Despite these issues, it is noteworthy that SAH-p53-8 is effective at high concentrations in a mouse model of melanoma in which MDMX is overexpressed[10]. In this model, SAH-p53-8 treatment increased cytotoxicity and sensitized melanomas to the chemotherapeutic agent cisplatin and the BRAF-V600E inhibitor vemurafenib (also known as PLX4032). This suggests a potential clinical utility of combined stapled peptides combining p53 agonists and BRAF inhibitors.

Targeting ubiquitin ligase activity

Small molecules that inhibit MDM2 ubiquitin ligase activity have been identified in high-throughput screens. These include HLI98 (Ref. 115), the derivative MPD compounds[116], MEL23 and MEL24 (Ref. 117). Treating cells with these compounds stabilized p53 and MDM2 and subsequently led to p53 activation. MPD37 (the most potent among such compounds) was shown to bind the MDM2 RING domain, although whether this prevents

MDM2–MDMX heterodimer formation is unclear[116]. These MDM2 E3 ubiquitin ligase inhibitors exhibit some p53-independent cytotoxicity, especially at higher concentrations, which may be due to the inhibition of other cellular RING domain E3 ubiquitin ligases.

An important gap in our knowledge of MDM2–p53 regulation is the identity of the E2 ubiquitin-conjugating enzyme or enzymes involved in the ubiquitylation of p53 *in vivo*. Although several E2s can transfer ubiquitin to p53 *in vitro* [118,119], the intracellular E2s that are required for p53 ubiquitylation have not been identified. This is probably due to the low affinity and the transient nature of E2–E3 interactions[120,121]. Conceivably, MDM2 might use different E2s to regulate itself, MDMX and/or p53. Alternatively, different developmental stages or different tissues might require discrete MDM2–E2 pairs to modulate p53 activities. Also, discrete MDM2–E2 pairs might be used in normal or malignant tissues. Thus, the identification of the bona fide intracellular E2s will undoubtedly bring us a more complete picture of p53–MDM2 regulation. Once specific E2s are identified, the relatively weak nature of MDM2–E2 interactions may render them particularly susceptible to selective PPI antagonists[122]. This in turn would prevent p53 ubiquitylation and trigger p53 activation.

Combination approaches

Conventional single-agent cancer therapy increases the likelihood of resistance. To minimize the emergence of resistant clones and to achieve maximal therapeutic response, combinations of different classes of therapeutic agents[123] are commonly used. Indeed, the MDM2 antagonist nutlin 3a has been combined with non-targeted genotoxic agents to augment efficacy (reviewed in Ref. 124). We discuss below recent rational combinations of targeted agents and MDM2–MDMX antagonists.

The PI3K–AKT pathway usually has an anti-apoptotic role to promote cancer cell survival. This may be partly due to AKT-dependent stabilization of MDM2 (Refs 125,126). Thus, targeting both the AKT and p53 axes simultaneously might be particularly effective in cancer cells. Consistent with this, inhibition of the PI3K–AKT pathway sensitizes acute lymphoblastic leukaemia (ALL) cell lines to nutlin 3a-induced p53 activation[127]. More than 80% of acute myeloid leukaemias (AMLs) express constitutively activated MAPK, and 50% overexpress MDM2 (Ref. 128). Nutlin 3a synergizes with AZD6244 (also known as selumetinib), an inhibitor of downstream MAPK signalling, to induce apoptosis in AML[128], providing proof-of-concept for this rational combination treatment.

Early combination regimens were mainly determined empirically. However, in the post-genomic era, several research institutes have approached combinatorial targeted therapy in a systematic manner[129,130]. In one such study[130], as expected, p53 mutation status was the strongest indicator of nutlin 3a resistance; interestingly, BRAF mutation was associated with nutlin 3a sensitivity in the same study[130]. This suggests that targeting BRAF, as well as p53 pathways, should generate additive or synergistic effects. Indeed, co-treatment of melanomas with the p53-activating stapled peptide SAH-p53-8 and the BRAF inhibitor PLX4032 significantly enhanced cytotoxicity when compared with single-agent treatment[10].

Insensitivity to MDM2 antagonists can arise from a variety of mechanisms, including p53 mutation, the presence of dominant survival pathways or the inhibition of death-inducing pathways. In an effort to identify genes that protect cells from p53-induced apoptosis, a genome-wide siRNA screen revealed that knocking down ATM and MET (also known as HGFR) kinases exhibited synthetic lethality with nutlin 3a[131]. At first this was surprising, because ATM kinase activates p53 in response to DNA damage. However, context is likely to be the key, and the protective role of ATM may be separate from its function as a DNA damage-activated kinase. Together, these results indicate that ATM and MET affect multiple downstream regulators in order to modulate the cellular response to MDM2 antagonists. Although a detailed molecular mechanism was not elucidated, it seems that the MET and MDM2 signalling pathways are used during development for cell survival[132]. This underscores the idea that signalling networks in normal cells are frequently usurped during tumorigenesis to promote cancer cell growth.

Concluding remarks

The intricacies of the MDM2–MDMX–p53 regulatory circuit have attracted the attention of academics, translational scientists and drug companies. Collaborations between these groups have inspired the implementation of numerous drug discovery projects and the design of clinical trials[133]. Ultimately, this will benefit cancer patients, although currently only one MDM2 antagonist, RG7112 (see the ClinicalTrials.gov website; see Further information), is in clinical trials and many challenges still lie ahead. For example, with the obvious exception of p53, we still do not understand how MDM2 and MDMX expression is regulated during development or in adult tissues during homeostasis. Insight here may reveal signalling pathways that can be targeted for therapeutic benefit. Are MDM2 and MDMX involved in the modulation of pathologies other than cancer? Given the role of p53 in the control of core metabolic pathways, this is entirely possible. Finally, how can we effectively select patients for clinical trials of MDM2- and MDMX- targeted therapies? A reliance simply on MDM gene amplification is inadequate. Combined screening for additional markers such as the loss of miRNA clusters that regulate MDM2 or MDMX, the presence of deubiquitylases that stabilize these oncogenes or druggable kinases that cooperate with MDM proteins should all be considered.

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Glossary

Antagonists	Chemical substances that interfere with or inhibit the physiological activity of other biological entities such as proteins or enzymes
miRNAs	Derived from an RNA polymerase II transcribed precursor, miRNAs are a class of non-protein coding mRNA that reduces the expression of cellular proteins through various mechanisms

Hemizygous	A genetic status in which one allelic copy of a gene is deleted or otherwise inactivated
Haploinsufficiency	A genetic status in which a single wild-type copy of an allelic pair is present, but the level of expression of the product is insufficient to give wild-type function
Myeloablation	The depletion of bone marrow cells
Neoadjuvant therapy	Administration of therapeutic agents to reduce tumour volume before giving a primary treatment such as surgery
IC_{50}	The half-maximal inhibitory concentration, which is the concentration of a compound causing 50% inhibition of biological or biochemical function

Box 1

p53-independent roles of MDM proteins

Roles for the MDM proteins beyond p53 regulation are elusive, partly owing to the lack of a strong overt phenotype in *Trp53*–MDM double-knockout mice and cells in culture. However, MDM2 is reported to ubiquitylate numerous targets in addition to p53, and both MDM2 and MDMX are overexpressed in some p53-mutant tumours. This suggests that both proteins may have p53-independent roles in tumorigenesis, as discussed in recent reviews[61,134,135]. Intriguingly, MDM2 can regulate gene expression and DNA repair by interacting with chromatin or chromatin-associated factors. For example, MDM2 associates with and ubiquitylates oestrogen receptor and androgen receptor[136–140]. This leads to changes in the expression of hormone-responsive genes and enhances cell proliferation in some contexts[137,141]. MDM2 may also have a role in the modification of the cellular epigenetic status. For example, MDM2-dependent degradation of RB can increase the levels and activity of the DNA methyltransferase DNMT3A[142]. This is associated with the silencing of tumour suppressor genes, and suggests that targeting this p53-independent function of MDM2 is a potential therapeutic strategy. MDM2 also interacts with the histone methyltransferase SUV39H1. Depending on the context, this leads to SUV39H1 degradation or to the repression of p53 target genes[143,144]. Given that loss of SUV39H1 is associated with epigenetic reprogramming, genomic instability and tumorigenesis[145,146], further studies into the links between MDM2 and chromatin modifiers are warranted. MDM2 may also engender genomic instability, a hallmark of many cancers, by interacting with and inhibiting proteins that are involved in the DNA damage response[134]. In contrast to observations with SUV39H1, however, this does not require MDM2 ubiquitin ligase function[147]. Despite its homology with MDM2, no clear role for MDMX in the regulation of epigenetic modulators has been demonstrated. However, MDMX is reported to interact with and inhibit transcription factors of the E2F and SMAD families[148,149]. Therefore, MDMX may have a broader influence on gene expression beyond the modulation of p53-dependent transcription. Additionally, MDMX may participate in a ‘failsafe’ mechanism to preserve genome integrity[150], although the molecular mechanism (or mechanisms) by which this occurs are unclear.

Box 2

Homeostatic roles of MDM proteins

Ancestral roles of the p53 family were probably geared towards reproductive fitness. Together with other family members, p53 ensures that cells with DNA damage are eliminated during development and enhances the rate of embryonic implantation. Loss of MDM proteins activates p53 in most tissues studied, although the onset of p53-dependent apoptosis seems to be confined to radiosensitive tissues such as the thymus and spleen[24,81]. Thus, the MDM proteins probably evolved to block spurious p53 activation. Given that lymphoid cells generate DNA strand breaks during somatic recombination, MDM proteins in these cells may buffer against 'damage'-induced p53 activation. Interestingly, T cell activation is accompanied by the increased transcription of both *MDM2* and *MDMX*, suggesting that p53–MDM2–MDMX autoregulatory feedback loops are important in this tissue[151].

Intestinal cells are sensitive to p53, probably because they are highly proliferative. Moreover, recent data have suggested that intestinal DNA damage leads to p53 activation via modulation of MDM2 (Ref. 47). Consistent with this, intestinal loss of MDM2 induces apoptosis; together with haematopoietic cell depletion, this is thought to cause the death of *Mdm2*-deficient animals[81]. Therefore, as in the haematopoietic system, the role of MDM proteins is likely to restrict p53 activation unless genomic integrity is threatened.

The subventricular zone (SVZ) of the adult brain is the putative site of proliferation for adult neurogenesis and the location of specific neuronal stem cells[152]. Levels of MDM proteins are relatively high in the brain, and the deletion of either MDM gene in the SVZ leads to p53-dependent apoptosis[24,81,85]. This underscores the importance of MDM proteins in the protection of proliferative cells during homeostasis. Strikingly, *MDM2* and *MDMX* are amplified or overexpressed in glioblastomas at high frequency[153]. As glioblastomas are derived from putative stem cells in the SVZ[152], p53 inhibition by MDM proteins may contribute to the onset or the maintenance of these tumours.

p53-induced mRNAs and microRNAs are associated with liver pathology[154,155], and excessive p53 activation in adipocytes can contribute to insulin resistance[154]. Thus, MDM proteins may serve a protective role in these tissues by blocking p53 activation. MDM2 may also modulate adipocyte differentiation in a p53-independent manner[156]. The additional roles of MDM2 in adipocytes may come at a price, however, as amplification of *MDM2* in these cells is associated with liposarcoma[157].

Box 3

Alternative MDM2- and MDMX-dependent p53-activating approaches

The small-molecule compound reactivation of p53 and induction of tumour cell apoptosis (RITA; also known as NSC 652287) was found to directly bind p53 and to block the p53–MDM2 interaction[158]. RITA was shown to cause p53-dependent accumulation and activation via a high-throughput cell proliferation assay using *TP53*$^{+/+}$ and *TP53*$^{-/-}$ HCT116 cell lines. Its proposed mode of action was via binding to the p53 amino-terminal domain, leading to a p53 conformational change and the dissociation of MDM2 (Ref. 158). However, in addition to its ability to bind to p53, RITA causes protein–DNA crosslinks[159] and is also metabolized to a reactive species[160]. Given these additional undesired properties, it is perhaps not surprising that RITA is cytotoxic in cells with both mutant and truncated p53 (Ref. 161).

JNJ-26854165, a tryptamine derivative, was reported to activate p53 by preventing the MDM2–p53 complex from binding to the proteasome, thereby blocking p53 degradation. However, preclinical studies have revealed activity in p53 wild-type and p53-mutant cancer cells and a general genotoxic effect, indicating that this compound is not p53-specific[162]. Although JNJ-26854165 entered Phase I clinical trials (see the ClinicalTrials.gov website; see Further information), the programme was halted owing to cardiotoxicity and an MDM2-independent mechanism of action (W. Hait, personal communication).

The MDMX^{3SA} and MDM2^{S394A} mouse models[46,47] show that p53 is not activated by DNA damage signals if MDM2 and MDM4 are not phosphorylated correctly. Small-molecule therapeutics that mimic the effect of MDM2^{S394A} or MDMX^{3SA} may be useful both in basic research and in the clinic. For example, in patients undergoing radiotherapy, acute p53-dependent apoptosis in normal radiosensitive tissues is a major source of side effects. Transient inhibition of p53 reduces cell death in normal tissues without compromising p53 tumour suppressor function[163]. Therefore small molecules that block DNA damage-induced MDM protein phosphorylation should transiently inhibit p53 during radiotherapy, thereby reducing unwanted side effects.

At a glance

- MDM2 and MDMX are RING domain proteins that exert their oncogenic effects primarily by inhibiting the p53 tumour suppressor protein.
- Each protein is overexpressed in diverse tumour types by mechanisms including gene amplification and post-translational stabilization; this is generally more frequent in tumours with a wild-type *TP53* allele.
- Despite their similar structures, only MDM2 has intrinsic E3 ubiquitin ligase activity. Although MDM2 alone can inhibit p53, its RING-dependent heterodimerization with MDMX has an important role in p53 inhibition.
- Both MDM2 and MDMX interact with multiple other partners. Aberrant interactions with these partners may also affect gene expression and genome stability.
- Structure-based drug design has yielded several MDM antagonists that block MDM–p53 interactions, leading to p53 activation. At least one agent has progressed to clinical trials.
- Systems biology studies are providing the rationale for using MDM protein antagonists in combination with both approved and experimental pathway-targeted anticancer drugs.

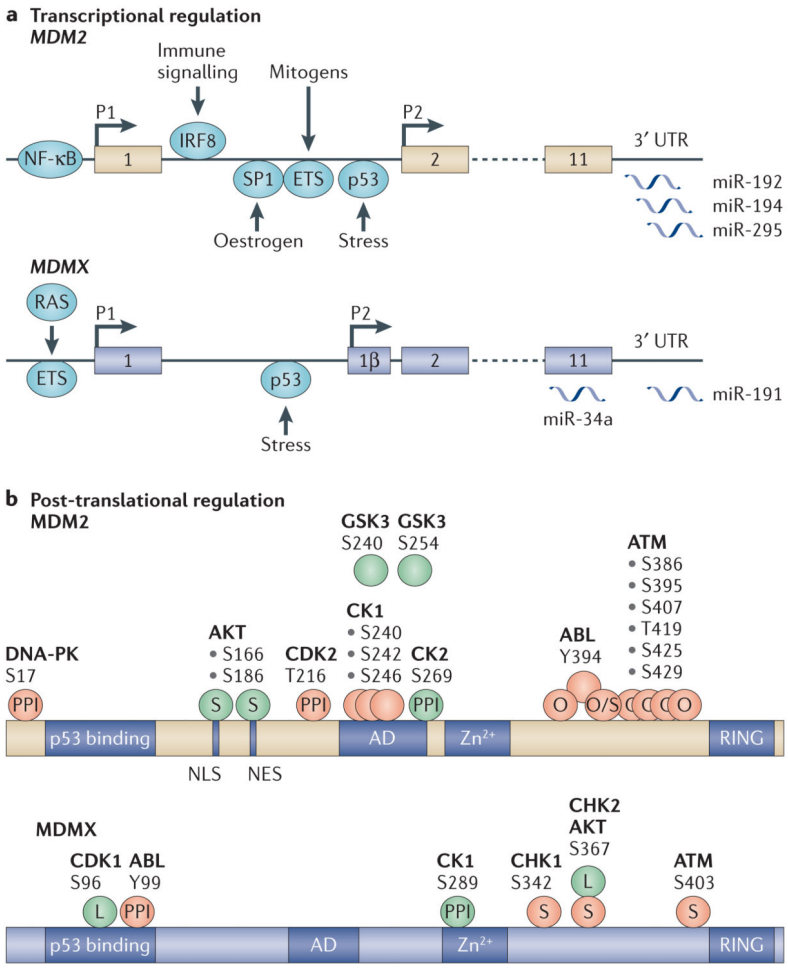

Figure 1. Transcriptional and post-translational regulation of MDM2 an d MDMX

a | Transcriptional regulation of MDM2 and MDMX is shown. The first coding exon for both *MDM2* and *MDMX* is exon 2. An ATG in an additional exon (1β) in *MDMX* can give rise to a longer MDMX-L protein. p53-induced transcription is mediated by the P2 promoter for both *MDM2* and *MDMX*, whereas basal transcription initiates from the P1 promoter. Additional transcription factors (shown in blue) can positively modulate the expression of *MDM2* and *MDMX* from both the P1 and the P2 promoters. Several microRNAs (miRNAs) have been proposed to block translation of either *MDM2* or *MDMX* mRNA. In contrast to the situation with *MDM2*, one of the miRNAs that targets *MDMX* mRNA (miR-34a) binds to the coding sequence, rather than to the 3′ untranslated region (UTR). **b** | In addition to transcriptional regulation, both MDM2 and MDMX are subject to diverse post-translational modifications that affect their ability to bind to p53, to bind to each other and to interact with other cellular proteins that can dramatically affect their stability and that of p53. For simplicity, we focus on phosphorylation to show the diversity of the sites that are modified and the consequences of their modification. Shown in green are phosphorylation sites that are reported to increase MDM2- or MDMX-dependent inhibition of p53. Phosphorylation sites shown in orange are reported to inhibit MDM2- and MDMX-dependent p53 inhibition. In many cases, the precise mechanisms by which phosphorylation modulates the relationship

between p53 and MDM2 or MDMX is unclear. Phosphorylation can disrupt the interaction with p53 (by inhibiting protein–protein interaction (PPI)), can modulate the stability of MDM2 or MDMX (S), change oligomerization status (O), modulate stability and oligomerization (O/S) or can alter subcellular localization (L). Residue numbering is for the human proteins, and it should be noted that not all kinase consensus sites are shared between humans and mice. AD, acidic domain; ATM, ataxia-telangiectasia mutated; CDK, cyclin-dependent kinase; CK, casein kinase; DNA-PK, DNA-dependent protein kinase; GSK3, glycogen synthase kinase 3; IRF8, interferon regulatory factor 8; NES, nuclear export signal; NF-κB, nuclear factor-κB; NLS, nuclear localization signal; Zn^{2+}, zinc finger domain.

Table 1

Frequency of MDM gene or protein alterations in selected human cancers

Tumour type	MDM2*			MDMX*			Detection‡	Refs
	Overall	p53 wild-type	p53 mutant	Overall*	p53 wild-type	p53 mutant		
Glioblastoma§	14%	20%	0%	7%	8%	0%	Genome	5
Well-differentiated liposarcoma§	70%	70%	0%	0%	0%	0%	Genome	8
Cutaneous melanoma //	37%	46%	0%	68%	85%	100%	Protein	10
Breast	31%	80%	14%	38%	80%	24%	Protein	6
Oesophagus	18%	21%	14%	NA	NA	NA	Genome	3
Osteosarcoma	16%	14%	14%	35%	NA	NA	Genome	7,8
Colorectal	9%	65%	35%	49%	NA	NA	Genome (MDM2); protein (MDMX)	4,9

NA, not available.

*The overall frequency at which alterations were present for both MDM2 and MDMX are listed. All numbers are converted to percentages for clarity. Where possible, the fraction of tumour samples with wild-type or mutant p53 that displayed *MDM2* or *MDMX* amplification is listed.

‡Detection method was either immunofluorescence or western blot (protein) or gene amplification (genome).

§Glioblastoma and well-differentiated liposarcoma show clear mutual exclusivity for MDM deregulation and p53 mutation, whereas this is less clear in other tumour types (for example, colorectal cancer).

// Note that the sample size with validated mutant p53 in the cutaneous melanoma study was small (n = 3) and so more studies are required.

¶Data correlating amplification status with p53 status were not available in these studies.

Table 2
Phenotypes of mice with modifications at the *Mdm2* and *Mdmx* loci

Tissue	Genotype	p53 regulation strategy*	Phenotype‡	Refs
CNS	*Mdm2*$^{-/-}$	Nestin-Cre;*Trp53*$^{LSL/-}$	Embryonic lethal; apoptosis	24
	Mdmx$^{-/-}$	Nestin-Cre;*Trp53*$^{LSL/-}$	Apoptosis and arrest	
	Mdm2$^{FM/FM}$	Nestin-Cre	Embryonic lethal; apoptosis	109
	Mdmx$^{FX/FX}$	Nestin-Cre	Embryonic lethal; apoptosis and arrest	
	Mdm2$^{-/-}$	*Trp53*$^{ER/-}$	No effect	81
	Mdmx$^{-/-}$	*Trp53*$^{ER/-}$	Apoptosis only in SVZ	
Intestine	*Mdm2*$^{FM/FM}$	Villin-Cre	Apoptosis	164
	Mdmx$^{FX/FX}$	Villin-Cre	Apoptosis in proliferating cells	165
	Mdm2$^{-/-}$	*Trp53*$^{ER/-}$	Apoptosis	81
	Mdmx$^{-/-}$	*Trp53*$^{ER/-}$	Apoptosis	85
Smooth muscle	*Mdm2*$^{FM/FM}$	*Sm22*-CreER^{T2}	Cell death	166
	Mdmx$^{FX/FX}$	*Sm22*-CreER^{T2}	No effect	
Erythrocyte	*Mdm2*$^{lox/lox}$	*Epor*GFP-Cre/+	Embryonic lethal; apoptosis	167
	Mdmx$^{lox/lox}$	*Epor*GFP-Cre/+	Arrest only in fetal erythropoiesis	
Heart	*Mdm2*$^{FM/-}$	*Myhc*-Cre	Embryonic lethal; apoptosis	168
	Mdmx$^{FX/-}$	*Myhc*-Cre	Apoptosis	169
	Mdm2$^{-/-}$	*Trp53*$^{ER/-}$	No effect	81
	Mdmx$^{-/-}$	*Trp53*$^{ER/-}$	No effect	85
Thymus and spleen	*Mdm2*$^{-/-}$	*Trp53*$^{ER/-}$	Apoptosis	81
	Mdmx$^{-/-}$	*Trp53*$^{ER/-}$	Apoptosis	85
Testis	*Mdm2*$^{-/-}$	*Trp53*$^{ER/-}$	Arrest	81
	Mdmx$^{-/-}$	*Trp53*$^{ER/-}$	Not reported	85
Lung, kidney and liver	*Mdm2*$^{-/-}$	*Trp53*$^{ER/-}$	No effect	81
	Mdmx$^{-/-}$	*Trp53*$^{ER/-}$	No effect	85

CNS, central nervous system; *Epor*, erythropoietin receptor; FM, floxed *Mdm2* allele; FX, floxed *Mdmx* allele; GFP, green fluorescent protein; *Myhc*, myosin heavy chain; *Sm22*, smooth muscle protein 22 (also known as transgelin); SVZ, subventricular zone.

*Knockout of MDM2 or MDMX is embryonic lethal, and therefore either MDM knockout or p53 expression must be rendered conditional. This is usually achieved using Cre-mediated recombination. Note that the *Trp53*^{LSL} and *Trp53*^{ER} systems are to some extent 'leaky'. Thus, adult animals can only be obtained by reducing *Trp53* to hemizygosity.

‡Phenotype is for the adult tissue, except where the knockout was embryonic lethal.

Table 3
Phenotypes of mice with alterations of MDM2 and MDMX

Mouse model	Effect	Phenotype	Refs
Knock-in mice			
MDM2-S394A	Removes ATM phosphorylation site	Radioresistant and accelerated spontaneous tumours	47
MDM2-C305F	Disrupts interaction with ribosomal proteins	Accelerated MYC-induced lymphoma	170
MDM2-C462A	Disrupts RING domain and MDMX binding; ubiquitin ligase activity lost	Embryonic lethal	171
MDM2 (SNP309G/G)	Increases MDM2 expression	Accelerated spontaneous tumorigenesis	67
MDMX RING deletion	Removes all RING-associated functions	Homozygote is embryonic lethal	18
MDMX-C462A	Disrupts RING domain and MDM2 binding	Homozygote is embryonic lethal	17
MDMX-3SA	Removes AKT, ATM and CHK2 phosphorylation sites	No accelerated spontaneous tumorigenesis; accelerated MYC-induced lymphoma	46
Overexpression models			
MDM2 transgenic	BLG promoter-driven mammary gland expression of wild-type *MDM2* cDNA	Inhibits mammary gland development and increased mammary gland tumours	65
MDM2 transgenic	Entire wild-type *MDM2* gene and promoter construct	Increased spontaneous tumours	66
MDM2 S166D/S186D	MMTV-driven *MDM2* cDNA mimicking constitutive AKT phosphorylation	No increase in spontaneous tumours; accelerated ERBB2-induced tumours	172
MDM4 transgenic	Non-targeted transgenic; Cre-activated wild-type cDNA	Accelerated spontaneous tumorigenesis	68
HA-tagged MDM4	Knock-in of Cre-activated wild-type cDNA at *ROSA26* locus	Homozygous overexpression is embryonic lethal; no increase in spontaneous or MYC-induced tumours	28

ATM, ataxia-telangiectasia mutated; BLG, β-lactoglobulin; MMTV, mouse mammary tumour virus.

Table 4

Approaches to target MDM2 and MDMX

Targeting approach	Compound	Class	Target	Proposed working mechanism	Remarks
Modulating protein expression	NSC207895 (Ref. 88)	Small molecule	MDMX	Inhibits MDMX transcription	Highly related to DNA damage agents[89]
	17-AAG[92]	Small molecule	HSP90	HSP90 inhibitor	Destabilizes MDMX through an undefined mechanism[92]
Targeting protein–protein interaction	Nutlin 3a[96]	Small molecule	MDM2 N-terminal p53-binding pocket	Disrupts p53–MDM2 interaction	The derivative, RG7112, is in Phase I clinical trial
	MI-219 (Ref. 97)	Small molecule	MDM2 N-terminal p53-binding pocket	Disrupts p53–MDM2 interaction	Preclinical development
	SJ-172550 (Ref. 105)	Small molecule	MDMX N-terminal p53-binding pocket	Disrupts p53–MDMX interaction	Forms adducts with cysteine in MDM2 and MDMX[106]
	RO-5963 (Ref. 103)	Small molecule	Both MDM2 and MDMX N-terminal p53-binding pocket	Disrupts p53–MDM2 and p53–MDMX interactions	Causes MDM2 and MDMX homodimers and heterodimers[103]
	WK 298 (Ref. 173)	Small molecule	MDMX	Disrupts p53–MDMX interaction	No cellular activity[173]
	AM-8553 (Ref. 174)	Small molecule	MDM2 N-terminal p53-binding pocket	Disrupts p53–MDM2 interaction	
	SAH-p53-8 (Ref. 110)	Peptidic compound	Both MDM2 and MDMX N-terminal p53-binding pocket	Disrupts p53–MDM2 and p53–MDMX interactions	Cellular uptake requires pinocytosis[113]
	PMI peptide[112]	Peptidic compound	Both MDM2 and MDMX N-terminal p53-binding pocket	Disrupts p53–MDM2 and p53–MDMX interactions	No cellular activity[175]
	pDI peptide[111]	Peptide	Both MDM2 and MDMX N-terminal p53-binding pocket	Disrupts p53–MDM2 and p53–MDMX interactions	Cellular activity only in context of adenoviral delivery[111]
	RITA[158]	Small molecule	p53 N-terminal domain	Disrupts p53–MDM2 interaction	Showed p53-independent activity[161]
Targeting E3 ubiquitin ligase activity	HLI98 (Ref. 115)	Small molecule	MDM2	Inhibits MDM2 ubiquitin ligase activity	Showed p53-independent activity[115]
	MPD[116]	Small molecule	MDM2 RING domain	Inhibits MDM2 ubiquitin ligase activity	Based on HLI compound. Potent derivative showed p53-independent activity[116]
	MEL23 and MEL24 (Ref. 117)	Small molecule	MDM2	Inhibits MDM2 ubiquitin ligase activity	
Activating p53 via other mechanisms	JNJ-26854165	Small molecule	MDM2	Inhibits p53–MDM2–proteasome interaction	Showed p53-independent activity[162]

HSP90, heat shock protein 90; RITA, reactivation of p53 and induction of tumour cell apoptosis; SAH-p53-8, stabilized α-helix of p53 variant 8.