TRIMming p53's anticancer activity

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Several TRIM proteins control abundance and activity of p53. Along this route, TRIM proteins have a serious impact on carcinogenesis and prognosis for cancer patients. In the past years, a significant increase has been made in our understanding of how the TRIM protein family controls p53 activity.

INTRODUCTION

P53 is one of the most important tumour-suppressor proteins. This tumour-suppressing activity is based on several actions: (i) p53 controls transcription of genes that are involved in cell cycle control, induction of cell death and senescence and regulation of cellular metabolism.[1] (ii) p53 regulates induction of programmed cell death by binding to pro-apoptotic and antiapoptotic proteins in the cytoplasm.[2] (iii) p53 controls DNA repair.[3]

Abundance and activity of p53 are tightly regulated and in differentiated cells they are usually low. In situations with an increased risk to acquire mutations, the p53 protein accumulates and becomes activated through posttranslational modifications.[4] As cells that lack functional p53 are unable to respond suitably to cellular stress, they accumulate mutations that favour the development of tumours.

THE TRIM FAMILY OF PROTEINS

The tripartite motif (TRIM) proteins represent a large protein family comprising >70 members (Figure 1). The name of these proteins comes from a three-domain module present in their N-terminal region also called RBCC motif. This motif consists of a Really Interesting New Gene (RING) domain followed by one or two B-Boxes (B1/B2) and a coiled-coil (CC) region (Figure 1). Proteins with a RING domain frequently display E3 ligase activity. Indeed, several TRIM proteins modify target proteins with ubiquitin, SUMO or ISG15.[5–7] Some members of the TRIM family lack the RING domain and TRIM20 possesses a pyrin domain instead of a RING domain (Figure 1). Several TRIM proteins exist as different isoforms generated by alternative splicing and they may have different functions.[8,9] The role of many TRIM isoforms, however, is still uncharacterized.

Members of the TRIM protein family are involved in many biological processes, and changes in their abundance or activity are associated with several pathological conditions, including viral infections, developmental and neurodegenerative disorders and cancer. Some TRIM proteins are involved in oncogenic chromosomal translocations where the RBCC motif is fused to another gene, and it is an alluring possibility that the RBCC motif could have a role in these translocations. Other members of the TRIM protein family are implicated in cancer development *per se*.[10] Several of these proteins control carcinogenesis by modulating the activity of the p53 tumour-suppressor protein (Table 1).

p53 AND MDM2 (MURINE DOUBLE MINUTE-2), A CONNECTION FOR DESTRUCTION

The tight regulation of p53 is accomplished to a large extent by Mdm2 (Figure 2a). Mdm2 regulates p53 by three different interconnected modes: (i) by inhibiting its transcriptional activity, (ii) by controlling its subcellular localization, and (iii) by modulating its protein stability (Figure 2b).[4] Mdm2 is itself a target gene of p53, and this feedback loop serves as a pivotal mechanism for restraining p53 function in the absence of stress.[4] In response to cellular stress, p53 is activated and this activation is fine-tuned through different interactions of p53 and Mdm2, that interrupt the feedback loop in several ways.[4] Proto-oncogenes such as c-Myc or Ras prevent Mdm2-mediated degradation of p53 via expression of the alternative reading frame protein (p19ARF).[4] Similarly, some ribosomal proteins bind to Mdm2 upon ribosomal stress and sequester Mdm2 away from p53.[4] Other proteins influence the subcellular localization of p53 or its ability to modulate gene transcription.[4] A number of TRIM proteins modulate the abundance and/or the activity of p53 or Mdm2 and by this impinge on p53's tumour-suppressive function.

p53 CONTROL WITHIN THE TRIM19-DEFINED PROMYELOCYTIC LEUKAEMIA PROTEIN BODIES

TRIM19, better known as promyelocytic leukaemia protein, is the most investigated TRIM protein that controls p53 activity. It normally functions as a tumour-suppressor protein and inhibits colony formation of transformed cells in soft agar and tumour growth in nude mice.[11,12] Several mechanisms have been attributed to this tumour-suppressive function, one of which is the ability of TRIM19 to stimulate p53-dependent transcription (Figure 3).[13,14] As TRIM19-deficient mice are resistant to the lethal effects of γ-irradiation and as DNA damage-induced apoptosis is prevented in TRIM19-negative cells, it is most likely that TRIM19 is an obligatory component of p53 activation in response to DNA damage.[15,16] TRIM19 mediates recruitment of p53 and modifying enzymes such as HIPK2 (homeodomain-interacting protein kinase 2) and CBP (CREB-binding protein) into discrete nuclear structures called PODs (promyelocytic oncogenic domains), which fosters p53 stabilization and posttranslational modifications.[17] HIPK2 phosphorylates p53 at serine 46, a modification that is further enhanced by Axin.[18,19] As TRIM19 is unable to activate p53 in Axin-deficient cells, Axin is most likely also an essential part of the

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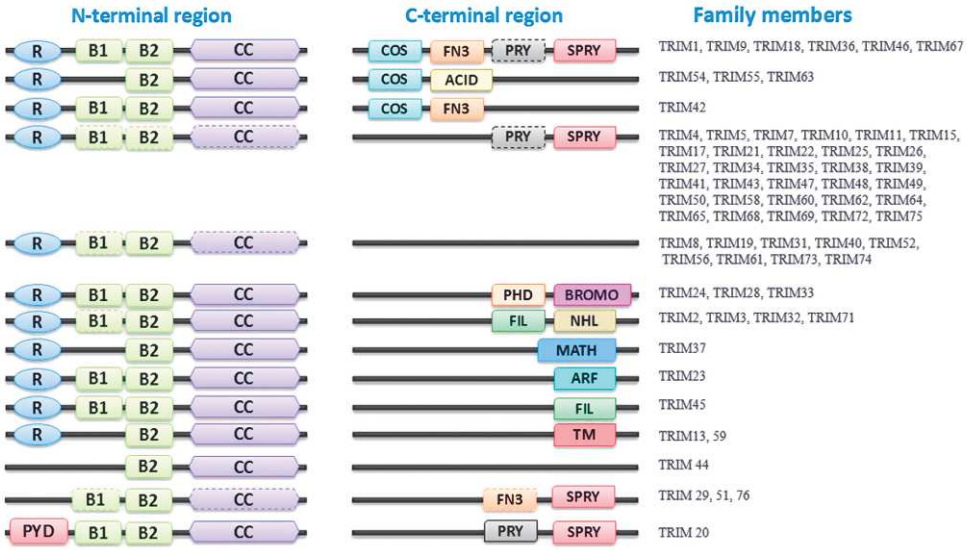

Figure 1. The TRIM protein family. TRIM proteins are characterized by the presence of a tripartite motif in the N-terminal part that usually consists of a RING domain (R), one or two B-boxes (B1, B2) and a coiled-coil region (CC). The C-terminal region may or may not contain a COS (C-terminal subgroup one signature), FN3 (fibronectin type III), PRY (SPRY-associated), SPRY (domain in SPla and the RYanodine receptor), ACID (acidic), TM (transmembrane), PHD (plant homeodomain), BROMO (bromodomain), FLMN (filamin), NHL (NHL-1, HT2A, Lin-41-related), MATH (Meprin and TRAF Homology) or ARF (ADP-ribosylation factor) domain. PYD: pyridin domain. Domains that are encircled with dots are not present in all members (adapted from Short and Cox[77] and modified according to Ozato *et al.*[76]).

Table 1. TRIM proteins involved in the regulation of p53 activity

TRIM protein	*Mechanism*	*Reference*
Increase in p53 activity		
TRIM8	Induces degradation of Mdm2 Increases p53 phosphorylation	Carratozzolo *et al.*[54]
TRIM13	Mediates polyubiquitination and degradation of Mdm2	Joo *et al.*[57]
TRIM19	Protects p53 from Mdm2-mediated ubiquitination and degradation Enhances p53 phosphorylation and acetylation	Bischof *et al.*[16] Hofmann *et al.*[17]
Decrease in p53 activity		
TRIM21	Ubiquitination of GMPS that culminates in the association of HAUSP with p53 and Mdm2 and destabilization of p53	Reddy *et al.*[69]
TRIM24	Mediates polyubiquitination and degradation of p53	Allton *et al.*[28]
TRIM25	Reduces acetylation of p53	Zhang *et al.*[34]
TRIM28	By using Mdm2 as an adaptor molecule, TRIM28 brings HDAC1 to the vicinity of p53 resulting in deacetylation. TRIM28 furthermore stimulates Mdm2-mediated ubiquitination and degradation of p53	Wang *et al.*[62]
TRIM29	Associates with p53 and causes mislocalisation of p53 in the cytoplasm	Yuan *et al.*[41]
TRIM32	Mediates polyubiquitination and degradation of p53	Liu *et al.*[46]
TRIM39	Mediates polyubiquitination and degradation of p53	Zhang *et al.*[50]
TRIM59	Mediates p53 polyubiquitination and degradation	Zhou *et al.*[51]
TRIM66	Mediates p53 polyubiquitination and degradation	Chen *et al.*[52]

p53-activating complex in response to DNA damage.[19] TRIM19 furthermore increases and prolongs phosphorylation of p53 at serine 18 and serine 20 and acetylation at lysine 382 in response to DNA damage by recruiting Chk2 (checkpoint kinase 2), CK1 (casein kinase 1) and the monocytic leukaemia zinc finger MOZ/KAT6A to p53-containing nuclear bodies.[13,20] In addition to promoting phosphorylation and acetylation of p53, TRIM19 protects p53 from Mdm2-mediated ubiquitination and degradation.[13,20] Some of TRIM19 activities towards p53 after DNA damage are regulated by posttranslational modifications of TRIM19. Upon DNA damage, Chk2 phosphorylates TRIM19 at serine 117 and phosphomimetic mutations of these sites induce apoptosis even without DNA damage.[15] TRIM19 furthermore interacts with mutant p53 and enhances its proliferation and colony-forming ability.[21] As *trim19* is a target gene of p53, both proteins are connected by an autoregulatory loop.[22]

Interestingly, the TRIM19 fusion protein promyelocytic leukaemia protein–retinoic acid receptor-α induces deacetylation and degradation of p53 instead of protecting it from degradation.[23]

In addition to p53, TRIM19 recruits Mdm2 to PODs while Mdm2 directs TRIM19 to the cytoplasm. Sumoylation of TRIM19 reduces its interaction with Mdm2 leading to its return into PODs.[24,25] Upon DNA damage, TRIM19 sequesters Mdm2 in the nucleolus, which prevents Mdm2-mediated degradation of p53.[26] According to its tumour-suppressive function, loss of TRIM19 is frequently observed in human tumours.[27]

OTHER TRIM PROTEINS ACTING ON p53

Several additional TRIM proteins interact with p53 and control p53 activity (Figure 4).

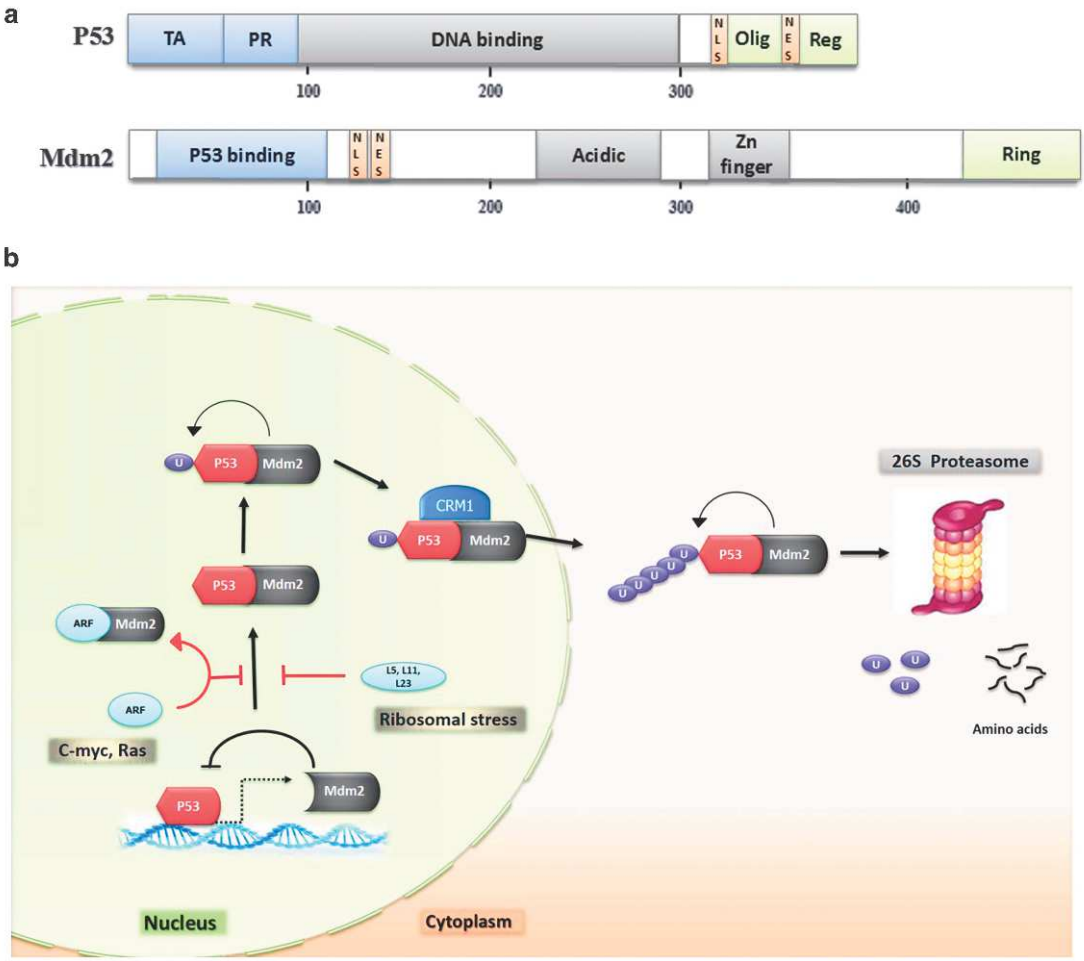

Figure 2. Mdm2 controls the p53 tumour-suppressor protein. (**a**) Schematic drawing of p53 and Mdm2. TA: transactivation domain; PR: proline-rich domain. DNA binding: DNA-binding domain; NLS: nuclear localization signal; Olig: oligomerization domain; NES: nuclear export signal; Reg: regulatory domain; P53 binding: p53-binding domain; acidic: acidic domain; Zn: zinc; Ring: RING domain. (**b**) Mdm2 controls p53 activity by binding to the N-terminal transactivation domain, by mediating its monoubiquitination and fostering CRM1-mediated export and by mediating p53 polyubiquitination and proteasome-mediated degradation. Overexpression of proto-oncogenes such as c-Myc or RAS leads to the sequestration of Mdm2 by ARF (alternative reading frame) and releases p53 from its control while ribosomal stress leads to the release of ribosomal proteins and inactivation of Mdm2.

The interaction of p53 with TRIM24, also called transcription intermediary factor 1 alpha, was identified in a search for proteins that interact with TRIM24.[28] Further investigations showed that TRIM24 increased p53 ubiquitination and reduced p53 protein levels and activity in a RING-dependent manner.[28] Upon DNA damage, ATM phosphorylates TRIM24 at serine 768 leading to TRIM24 degradation and to the release of p53 from TRIM24-mediated control. As TRIM24 is a transcriptional target of p53, TRIM24 abundance is increased after DNA damage leading to p53 degradation and to the shut-off of the DNA damage response.[29] Aberrant expression of TRIM24 is positively correlated with several malignancies and poor prognosis of the patients.[30,31]

Many TRIM family members are implicated in very diverse pathological conditions. TRIM25, also known as Efp (oestrogen-responsive finger protein), is another example of this paradigm. TRIM25 was originally identified in a screen for genes that are regulated by oestrogen.[32] Fibroblasts from TRIM25$^{-/-}$ mice grow significantly slower and show an altered phenotype (CB, unpublished observation).[33] One way by which this is achieved is by ubiquitinating 14-3-3σ.[33] In addition, TRIM25 reduces p53 and Mdm2 ubiquitination and degradation.[34] Surprisingly, whereas p53 levels are elevated after overexpression of TRIM25, its transcriptional activity is reduced. This decrease in p53's activity is accompanied by decreased acetylation of p53.[34] Thus TRIM25 has obviously two distinct activities with regard to p53: (i) regulation of p53 abundance and (ii) control of its transcriptional activity.

Consistent with the inhibition of p53 function, TRIM25 overexpression has been observed in ovarian, breast and lung tumours.[35–37]

TRIM29, also known as the ataxia telangiectasia group D complementing gene product, is another TRIM protein that affects p53 activity. TRIM29 was cloned in an attempt to identify the gene responsible for the autosomal-recessive disorder Ataxia telangiectasia.[38] TRIM29 binds to histones and DNA repair proteins, including ATM, Msh3, RING finger protein 8 (RNF8) and the Tat-interactive protein-60 (TIP60) complex, and is required for efficient phosphorylation of H2AX.[39,40] It does not have a RING domain and controls p53 by binding to its C-terminal domain leading to mislocalization of p53 in the cytoplasm and inhibition of its nuclear activities.[41] The interaction between p53 and TRIM29 is fine-tuned by modification of lysine 116 of TRIM29. Although p300 acetylates TRIM29,

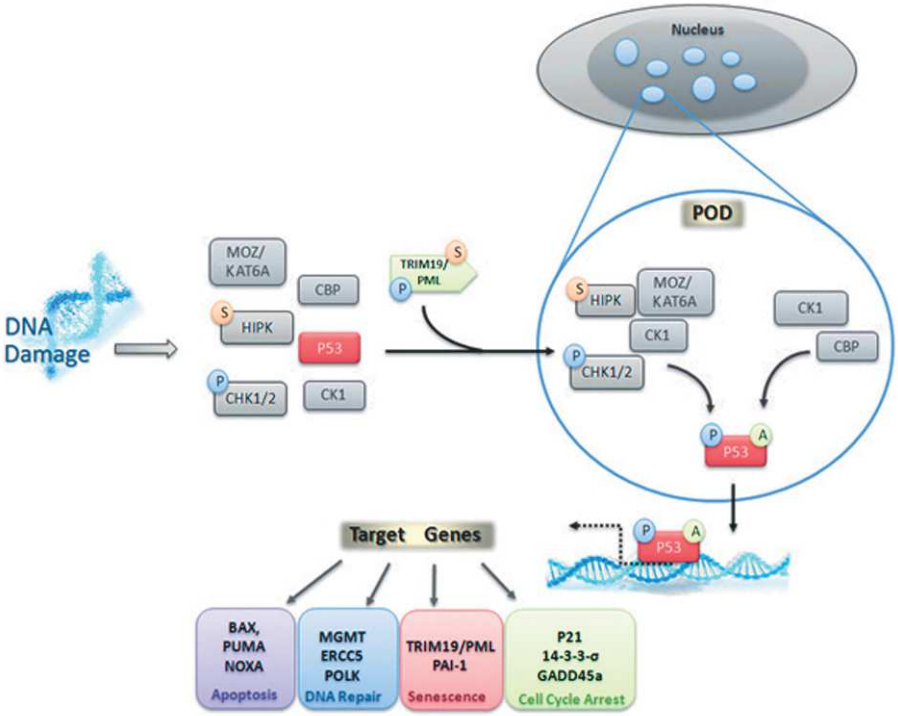

Figure 3. TRIM19/PML (promyelocytic leukaemia protein) stimulates p53's transcriptional activity in response to DNA damage. TRIM19/PML recruits p53, Chk1 and 2 (checkpoint kinase 1 and 2), HIPK2 (homeodomain interacting protein kinase 2), CK1 (casein kinase 1), MOZ/KAT6A (monocytic leukaemia zinc finger/K(lysine) acetyltransferase 6A) and CBP (CREB-binding protein) into PODs, which facilitates phosphorylation and acetylation of p53. These modifications lead to the activation of p53 and transcription of its target genes, including p21, 14-3-3 s, GADD45 (growth arrest and DNA damage 45), PML, PAI-1 (plasminogen-activator inhibitor type 1), MGMT (O^6-alkylguanine DNA alkyltransferase), ERCC5 (excision repair cross-complementing rodent repair deficiency, complementation group 5), POLK (DNA polymerase kappa), BAX (Bcl-2-associated X protein), PUMA (p53 upregulated modulator of apoptosis) and NOXA (phorbol-12-myristate-13-acetate-induced protein 1). P, phosphorylation; S, sumoylation; A, acetylation.

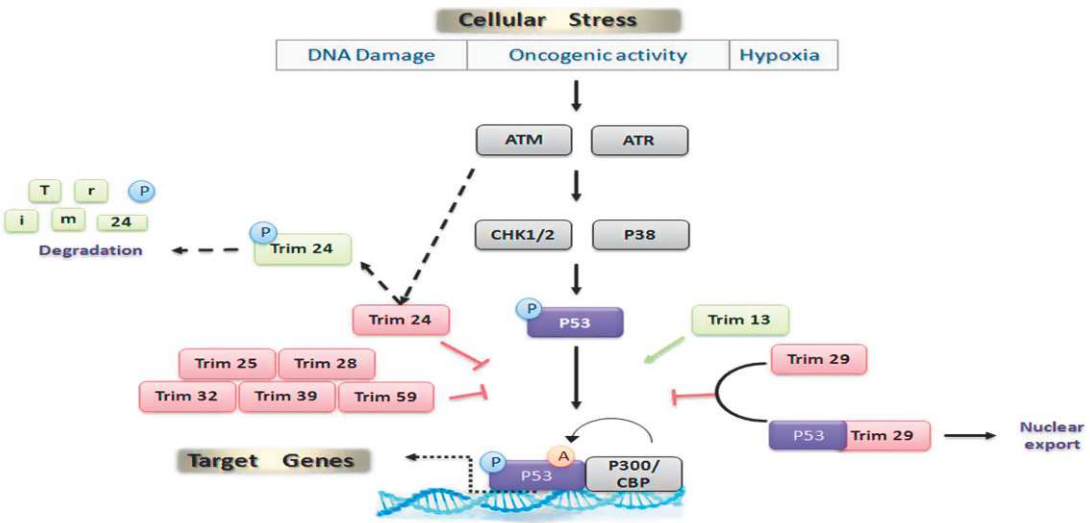

Figure 4. TRIM proteins interact with p53. TRIM19, TRIM24, TRIM25, TRIM29, TRIM32, TRIM39 and TRIM59 associate with p53. Whereas TRIM19 activates p53, TRIM25 and TRIM29 inhibit p53 activity by reducing its acetylation and by fostering nuclear export, respectively. TRIM24, TRIM32, TRIM39 and TRIM59 abolish p53's activity by mediating polyubiquitination and degradation. Cellular stress directs phosphorylation of TRIM24, resulting in its degradation and the release of p53 from its control. ATM: ataxia telangiectasia mutated, CHK1/2: checkpoint kinase 1/2, p38: p38-mitogenactivated kinase, ATR: taxia telangiectasia and Rad3-related protein.

which increases its association with p53, HDAC9 (histone deacetylase 9) deacetylates TRIM29 resulting in increased p53 activity and reduced cell survival.[41] After ultraviolet-irradiation, TRIM29 furthermore binds to TIP60, a transcriptional co-activator for p53, translocates the protein from the nucleus to the cytoplasm and fosters its degradation.[42]

In line with the inhibition of p53 activity, TRIM29 is over-expressed in several human tumours, including pancreatic and

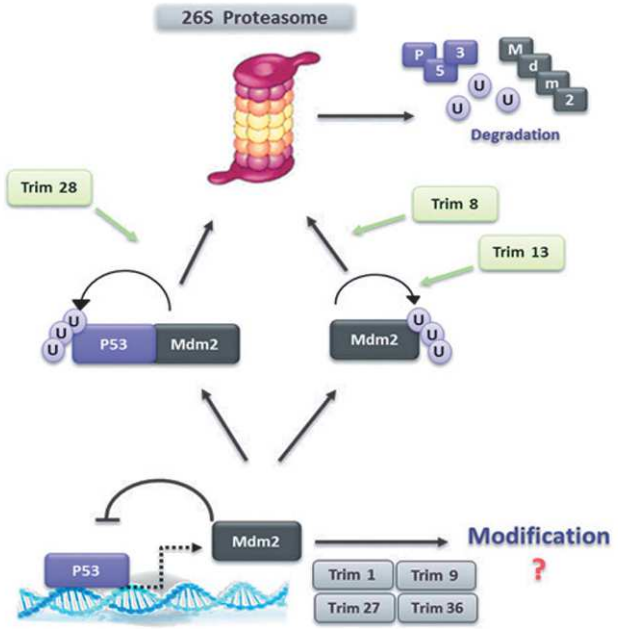

Figure 5. Several TRIM proteins regulate p53 by controlling Mdm2. The Mdm2 protein controls p53 abundance by mediating its polyubiquitination and proteasomal degradation. TRIM8 and TRIM13 increase p53 abundance and activity by inducing the degradation of Mdm2, whereas TRIM28 decreases p53 activity by stimulating Mdm2-mediated ubiquitination and degradation of p53.

lung cancer.[43,44] Yet, as TRIM29 is also able to activate nuclear factor-κB and Wnt signalling, not all tumour-promoting activities of TRIM29 may be ascribed to the regulation of p53.[43,44]

TRIM32, also called HT2A (HIV–Tat-interacting protein 2A), was identified in a yeast two-hybrid screen for HIV-1 Tat-interacting proteins.[45] The relation between TRIM32 and p53 has been found when TRIM32 popped up in a search for p53 target genes.[46,47] Further analysis showed that p53 binds to the promoter of *trim32* and activates its transcription.[46] Yet, while induction of Mdm2 can be seen already 3–5 h after DNA damage, induction of TRIM32 requires up to 15 h. TRIM32 is, however, not only a target gene of p53. It can also bind to p53 and target it for proteasome-mediated degradation.[46] In line with this activity, TRIM32 is frequently overexpressed in tumours.[46,48] Although a significant part of the tumour-promoting activity of TRIM32 is brought about by the regulation of p53, TRIM32-mediated ubiquitination of the Abl-interactor 2 tumour-suppressor protein may also contribute to its oncogenic activities.[49]

Another TRIM protein that regulates p53 directly is TRIM39, also known as RNF23. TRIM39 binds to and ubiquitinates p53 and targets it for degradation in a RING-dependent manner,[50] indicating that TRIM39 may act as an ubiquitin ligase for p53. Downregulation of TRIM39 markedly reduced proliferation of several cell lines with wild-type p53 and increased expression of p53 target genes.[50] In addition to ubiquitinating p53, TRIM39 modifies Mdm2 with SUMO.[6] Whether this modification of Mdm2 contributes to the inactivation of p53 by TRIM39 awaits further investigation.

By searching for mRNAs that are specifically increased in gastric tumour samples, TRIM59 was identified as a further TRIM protein regulating p53.[51] TRIM59 interacts with p53 and increases p53 ubiquitination and degradation while its downregulation reduced proliferation and migration of gastric cancer cells.[51] Increased levels of TRIM59 have been observed in gastric tumours and they were associated with advanced tumour stage, reduced expression of p53 target genes and reduced patient survival.[51]

A further RING-less TRIM protein that is controlling p53 abundance is TRIM66, also known as transcription intermediate factor 1 delta. Overexpression of TRIM66 was found in osteosarcoma where it was associated with lung metastasis and poor survival. Its downregulation increased the abundance of p53 and the apoptosis markers caspase 7 and caspase 9.[52]

TRIM PROTEINS ACTING VIA MDM2

Although some TRIM proteins control p53 activity by directly acting on p53, others affect p53's activity by interacting with Mdm2 (Figure 5).

One of these proteins is TRIM8, also known as the glioblastoma expressed RING finger protein.[53]

TRIM8 mediates growth suppression and this decrease in proliferation depends on the RING domain and on the presence of p53. Misregulation of TRIM8 has been observed in a wide range of neoplasms.[53,54] It is, however, not clear whether the RING domain is needed because it confers E3 ligase activity or whether it acts as a scaffold. Its antiproliferative activity is based on decreasing Mdm2 protein stability, which leads to increased abundance and activity of p53. Interestingly, TRIM8 decreases Mdm2 protein stability without directly interacting with Mdm2. TRIM8, however, associates with p53 and p53 may bridge TRIM8 and Mdm2 to allow Mdm2 degradation.[54] In addition to stabilizing p53, TRIM8 increases p53 phosphorylation at serine 15 and serine 20 leading to the induction of its target genes *p21* and *gadd45* while expression of the pro-apoptotic target genes *bax* and *puma* and induction of apoptosis is not affected.[54] Thus TRIM8 may not only control p53 activity but also influence the cell fate. Interestingly, TRIM8 transcription is stimulated by p53, which connects the two proteins by a positive feed-forward loop.[54] How this loop is interrupted when p53 activity needs to be shut-off remains to be determined.

TRIM13, also called Ret finger protein 2 or LEU5 (leukaemia-associated gene 5) was identified while searching for genes located at 13q14, a locus that is frequently deleted in B-cell chronic lymphatic leukaemia.[55] TRIM13 is an unstable protein that is involved in endoplasmatic reticulum-associated protein degradation.[56] It co-localizes with Mdm2 in nuclear structures and mediates Mdm2 polyubiquitination and degradation in a RING-domain-dependent manner resulting in increased p53 stability and activity.[56] Accordingly, overexpression of TRIM13 induced apoptosis. Notably, TRIM13 is induced by γ-irradiation and this induction may contribute to the accumulation of p53.[57]

TRIM28, also known as KAP1 (KRAB-associated protein 1) or TIF-1β (transcriptional intermediary factor 1 beta) has been cloned as a co-repressor of the KRAB (Krüppel-associated box)-domain.[58] TRIM28 is part of the N-CoR1 (nuclear receptor co-repressor 1) complex and recruits the HDAC complex NuRD (nucleosome remodeling deacetylase) and the methyltransferase SETDB1 (SET-domain bifurcated 1) to promoters.[59–61]

Mdm2 as well as its close homologue MdmX (Mdm4) co-precipitate with TRIM28, an interaction that involves the coiled-coil region of TRIM28 while the C-terminal PHD and bromodomain are left free for other interactions.[62] By using Mdm2 as an adaptor molecule, TRIM28 is able to bring HDAC1 into the vicinity of p53 resulting in enhanced deacetylation of p53 and reduced transcriptional activity.[62] In addition to inhibiting p53's transcriptional activities, TRIM28 cooperates with Mdm2 to promote p53 ubiquitination and degradation. As this activity requires the RING domain of Mdm2, it is most likely that TRIM28 is not ubiquitinating p53 itself.[62] The regulation of p53 by TRIM28 is further modulated by MAGE (melanoma antigen) proteins.[63] TRIM28 binds to several MAGE proteins and this binding leads to increased ubiquitination and reduced activity of p53.[63] The interaction between Mdm2 and

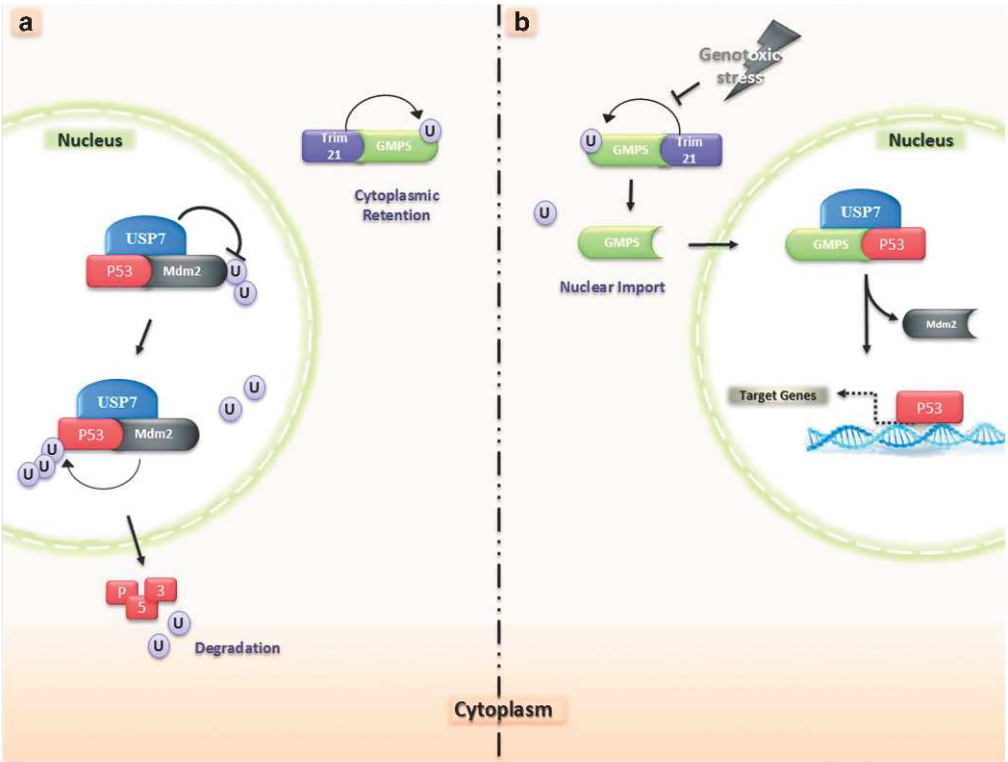

Figure 6. TRIM21 controls p53 activity by ubiquitinating GMPS. Normally, GMPS is sequestered in the cytoplasm owing to TRIM21-mediated monoubiquitination while USP7 (ubiquitin-specific-processing protease 7)/HAUSP is associated with p53 and Mdm2 resulting in Mdm2 de-ubiquitination and maintenance of sufficient Mdm2 levels to target p53 for degradation. Upon genotoxic stress, TRIM21 is separated from GMPS and non-ubiquitinated GMPS can enter the nucleus and displace Mdm2 from the interaction with p53 resulting in its stabilization.

TRIM28 is counteracted by p19ARF. As p19ARF has a higher affinity for Mdm2 than for TRIM28, it releases Mdm2 from the interaction with TRIM28 and shifts the balance towards an interaction of Mdm2 and p19ARF.[62] In line with its role as a suppressor of p53 activity, expression of TRIM28 is elevated in lung and breast cancer.[64,65] Expression of TRIM28 was furthermore strongly correlated with the metastatic potential and its inactivation in hepatocytes promoted hepatocellular cancer in mice.[66,67]

Several other TRIM proteins have been reported to modify Mdm2 through SUMOylation.[6] Whether this activity also occurs *in vivo* and whether it is relevant for p53's anticancer activity remains to be determined.

TRIM PROTEINS ACTING ON p53 VIA OTHER PARTNERS

TRIM21, also known as Ro52, an autoantigen that is commonly found in patients with Sjögren's syndrome and systemic lupus erythematosus, regulates p53 in a very elegant way involving the herpesvirus-associated ubiquitin-specific protease (HAUSP) and the guanosine 5′monophosphate synthase (GMPS).[68,69]

HAUSP can associate with GMPS, which stimulates HAUSP activity.[70] GMPS is usually kept in the cytoplasm due to interaction with and monoubiquitination by TRIM21 (Figure 6). This way, HAUSP is free to associate with p53 and Mdm2 in the nucleus and to deubiquitinate Mdm2 culminating in p53 degradation. Upon genotoxic stress, GMPS is released from the interaction with TRIM21 leading to the accumulation of GMPS in the nucleus and the displacement of Mdm2 from the complex with p53 and HAUSP followed by p53 stabilization.[69] According to its regulatory role for p53, higher amounts of antibodies against TRIM21 have been found in cancer patients.[71]

TRIM PROTEINS REGULATED BY p53

As described above, several TRIM proteins that regulate p53 itself or Mdm2 are, in turn, transcriptionally regulated by p53. Additional transcriptional targets of p53 are TRIM22 and TRIML2.

TRIM22, also called Staf50 (stimulated transactivated factor of 50 kDa) has first been identified as an interferon-inducible gene. TRIM22 is transcriptionally activated by p53 in several cancer cell lines owing to a p53-responsive enhancer-like element in the first intron.[72] Ectopic expression of TRIM22 reduced clonogenic growth, suggesting that it may contribute to the growth-suppressive effect of p53. Breast tumour cells frequently show downregulation of TRIM22, which correlates with p53 activity. Low levels of TRIM22 are, furthermore, associated with a higher risk of relapse and a higher mortality rate of patients with Wilms tumour.[73,74]

TRIML2 (tripartite motif family-like 2), a protein without the full RBCC motif, has been identified by searching for p53 targets whose induction depends on the polymorphism at amino acid 72 of p53. TRIML2 was specifically upregulated by the arginine variant owing to enhanced promoter binding. Although downregulation of TRIML2 reduced p53 protein levels, transcription of most genes was not affected. Induction of some pro-apoptotic genes was, however, reduced while transcription of some cell cycle arrest-inducing genes was enhanced, indicating that TRIML2 may affect the decision between life and death.[75] In line with the regulation of p53, according to the ONCOMINE database, TRIML2 is down-regulated in several cancers.[75]

CONCLUSIONS AND OUTLOOK

In summary, the involvement of TRIM proteins in the control of p53 anticancer activity is broad and not only exploits their ability

to act as E3 ubiquitin ligases but also their scaffolding and delocalizing features. The majority of TRIM proteins implicated in p53 regulation use their E3 ubiquitin ligase activity to counteract p53's anticancer role while others promote or prevent other types of posttranslational modifications. As the TRIM proteins are implicated in several processes and signalling pathways, it is conceivable that they might come into play upon different stimuli and may participate in independent pathways converging on p53 activity. Is the growing number of TRIM proteins implicated in p53 function just a reflection of their huge numbers? It is possible that subgroups of TRIM proteins might participate within the same p53 pathway. In particular, as many TRIM proteins have an important role in innate immunity,[76] one possibility is that their participation in p53 regulation established a direct regulatory relationship of p53 with the innate immune pathway. Along the same line, the TRIM family has been more recently implicated in the regulation of autophagy. Although the mechanistic insights are still incomplete, p53 is reported to inhibit autophagy and this may be another court where TRIM and p53 may fight or collaborate.

The fact that several of the above-mentioned TRIM proteins are also transcriptional p53 targets makes the story more intriguing and further supports the idea about a complex fine-regulation of p53. It is now time to start considering the p53 control by TRIM proteins as a whole, taking into account both positive and negative p53 regulators of the TRIM family and consider that several of them are also able to heterodimerize and form different complexes.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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REFERENCES

1 Riley T, Sontag E, Chen P, Levine A. Transcriptional control of human p53-regulated genes. *Nat Rev Mol Cell Biol* 2008; **9**: 402–412.
2 Vaseva AV, Marchenko ND, Moll UM. The transcription-independent mitochondrial p53 program is a major contributor to nutlin-induced apoptosis in tumor cells. *Cell Cycle* 2009; **8**: 1711–1719.
3 Dahm-Daphi J, Hubbe P, Horvath F, El-Awady RA, Bouffard KE, Powell SE *et al.* Nonhomologous end-joining of site-specific but not of radiation-induced DNA double-strand breaks is reduced in the presence of wild-type p53. *Oncogene* 2005; **24**: 1663–1672.
4 Boehme KA, Blattner C. Regulation of p53--insights into a complex process. *Crit Rev Biochem Mol Biol* 2009; **44**: 367–392.
5 Meroni G, Diez-Roux G. TRIM/RBCC, a novel class of 'single protein RING finger' E3 ubiquitin ligases. *Bioessays* 2005; **27**: 1147–1157.
6 Chu Y, Yang X. SUMO E3 ligase activity of TRIM proteins. *Oncogene* 2011; **30**: 1108–1116.
7 Zou W, Zhang DE. The interferon-inducible ubiquitin-protein isopeptide ligase (E3) EFP also functions as an ISG15 E3 ligase. *J Biol Chem* 2006; **281**: 3989–3994.
8 Battivelli EJ, Migraine D, Lecossier S, Matsuoka D, Perez-Bercoff S, Saragosti F *et al.* Modulation of TRIM5alpha activity in human cells by alternatively spliced TRIM5 isoforms. *J Virol* 2011; **85**: 7828–7835.
9 Nisole S, Maroui MA, Mascle XH, Aubry M, Chelbi-Alix MK. Differential roles of PML isoforms. *Front Oncol* 2013; **3**: 125.
10 Cambiaghi V, Giuliani V, Lombardi S, Marinelli C, Toffalorio F, Pellici PG. TRIM proteins in cancer. *Adv Exp Med Biol* 2012; **770**: 77–91.
11 Mu ZM, Chin KV, Liu JH, Lozano G, Chang KS. PML, a growth suppressor disrupted in acute promyelocytic leukemia. *Mol Cell Biol* 1994; **14**: 6858–6867.
12 Le XF, Vallian S, Mu ZM, HungMC, Chang KS. Recombinant PML adenovirus suppresses growth and tumorigenicity of human breast cancer cells by inducing G1 cell cycle arrest and apoptosis. *Oncogene* 1998; **16**: 1839–1849.
13 Louria-Hayon I, Grossman T, Sionov RV, Alsheich O, Pandolfi PP, Haupt Y. The promyelocytic leukemia protein protects p53 from Mdm2-mediated inhibition and degradation. *J Biol Chem* 2003; **278**: 33134–33141.
14 Yang S, Kuo C, Bisi JE, Kim MK. PML-dependent apoptosis after DNA damage is regulated by the checkpoint kinase hCds1/Chk2. *Nat Cell Biol* 2002; **4**: 865–870.
15 Guo A, Salomoni P, Luo J, Shih A, Zhong S, Gu W *et al.* The function of PML in p53-dependent apoptosis. *Nat Cell Biol* 2000; **2**: 730–736.
16 Bischof O, Kirsh O, Pearson M, Itahana K, Pelicci PG, Dejean A. Deconstructing PML-induced premature senescence. *EMBO J* 2002; **21**: 3358–3369.
17 Hofmann T, Moller A, Sirma H, Zentgraf H, Taya Y, Droge W *et al.* Regulation of p53 activity by its interaction with homeodomain-interacting protein kinase-2. *Nat Cell Biol* 2002; **4**: 1–10.
18 Alsheich-Bartok O, Haupt S, Alkalay-Snir I, Saito S, Appella E, Haupt Y. PML enhances the regulation of p53 by CK1 in response to DNA damage. *Oncogene* 2008; **27**: 3653–3661.
19 Li Q, He Y, Wei L, Wu X, Wu D, Lin S *et al.* AXIN is an essential co-activator for the promyelocytic leukemia protein in p53 activation. *Oncogene* 2011; **30**: 1194–1204.
20 Rokudai S, Laptenko O, Arnal SM, Taya Y, Kitabayashi I, Prives C. MOZ increases p53 acetylation and premature senescence through its complex formation with PML. *Proc Natl Acad Sci USA* 2013; **110**: 3895–3900.
21 Haupt S, di Agostino S, Mizrahi I, Alsheich-Bartok O, Voorhoeve M, Damalas A *et al.* Promyelocytic leukemia protein is required for gain of function by mutant p53. *Cancer Res* 2009; **69**: 4818–4826.
22 de Stanchina E, Querido E, Narita M, Davuluri RV, Pandolfi PP, Ferbeyre G *et al.* PML is a direct p53 target that modulates p53 effector functions. *Mol Cell* 2004; **13**: 523–535.
23 Insinga A, Monestiroli S, Ronzoni S, Carbone R, Pearson M, Pruneri G *et al.* Impairment of p53 acetylation, stability and function by an oncogenic transcription factor. *EMBO J* 2004; **23**: 1144–1154.
24 Zhu H, Wu L, Maki CG. MDM2 and promyelocytic leukemia antagonize each other through their direct interaction with p53. *J Biol Chem* 2003; **278**: 49286–49292.
25 Wei X, Yu ZK, Ramalingam A, Yu JH, Bloch DB *et al.* Physical and functional interactions between PML and MDM2. *J Biol Chem* 2003; **278**: 29288–29297.
26 Bernardi R, Scaglioni PP, Bergmann S, Horn HF, Vousden KF, Pandolfi PP. PML regulates p53 stability by sequestering Mdm2 to the nucleolus. *Nat Cell Biol* 2004; **6**: 665–672.
27 Gurrieri C, Capodieci P, Bernardi R, Scaglioni PP, Nafa K, Rush LJ *et al.* Loss of the tumor suppressor PML in human cancers of multiple histologic origins. *J Natl Cancer Inst* 2004; **96**: 269–279.
28 Allton K, Jain AK, Herz HM, Tsai WW, Jung SY, Qin J *et al.* Trim24 targets endogenous p53 for degradation. *Proc Natl Acad Sci USA* 2009; **106**: 11612–11616.
29 Jain AK, Allton K, Duncan AD, Barton MC. TRIM24 is a p53-induced E3-ubiquitin ligase that undergoes ATM-mediated phosphorylation and autodegradation during DNA damage. *Mol Cell Biol* 2014; **34**: 2695–2709.
30 Tsai WW, Wang Z, Yiu TT, Akdemir KC, Xia W, Winter S *et al.* TRIM24 links a non-canonical histone signature to breast cancer. *Nature* 2010; **468**: 927–932.
31 Cui Z, Cao W, Li J, Song X, Mao L, Chen W. TRIM24 overexpression is common in locally advanced head and neck squamous cell carcinoma and correlates with aggressive malignant phenotypes. *PLoS One* 2013; **8**: e63887.
32 Inoue S, Orimo A, Hosoi T, Kondo S, Toyoshima H, Kondo T *et al.* Genomic binding-site cloning reveals an estrogen-responsive gene that encodes a RING finger protein. *Proc Natl Acad Sci USA* 1993; **90**: 11117–11121.
33 Urano T, Saito T, Tsukui T, Fujita M, Hosoi T, Muramatsu M *et al.* Efp targets 14-3-3 sigma for proteolysis and promotes breast tumour growth. *Nature* 2002; **417**: 871–875.
34 Zhang P, Elabd S, Hammer S, Solozobova V, Yan H, Bartel F *et al.* TRIM25 has a dual function in the p53/Mdm2 circuit. *Oncogene* 2015; **34**: 5729–5738.
35 Sakuma M, Akahira J, Suzuki T, Inoue S, Ito K, Moriya T *et al.* Expression of estrogen-responsive finger protein (Efp) is associated with advanced disease in human epithelial ovarian cancer. *Gynecol Oncol* 2005; **99**: 664–670.
36 Suzuki T, Urano T, Tsukui T, Horie-Inoue K, Moriya T, Ishida T *et al.* Estrogen-responsive finger protein as a new potential biomarker for breast cancer. *Clin Cancer Res* 2005; **11**: 6148–6154.
37 Qin Y, Cui H, Zhang H. Overexpression of TRIM25 in lung cancer regulates tumor cell progression. *Technol Cancer Res Treat* 2015; e-pub ahead of print 25 June 2015.
38 Kapp LN, Painter RB, Yu LC, van Loon N, Richard CW 3rd, James MR *et al.* Cloning of a candidate gene for ataxia-telangiectasia group D. *Am J Hum Genet* 1992; **51**: 45–54.
39 Masuda Y, Takahashi H, Sato S, Tomomori-Sato C, Saraf A, Washburn MP *et al.* TRIM29 regulates the assembly of DNA repair proteins into damaged chromatin. *Nat Commun* 2015; **6**: 7299.
40 Yang H, Palmbos PL, Wang L, Kim E, Ney GM, Liu C *et al.* ATDC (Ataxia Telangiectasia Group D Complementing) promotes radioresistance through an interaction with the RNF8 ubiquitin ligase. *J Biol Chem* 2015; **290**: 27146–27157.

41 Yuan Z, Villagra A, Peng L, Coppola D, Glozak M, Sotomayor EM *et al.* The ATDC (TRIM29) protein binds p53 and antagonizes p53-mediated functions. *Mol Cell Biol* 2010; **30**: 3004–3015.

42 Sho T, Tsukiyama T, Sato T, Kondo T, Cheng J, Saku T *et al.* TRIM29 negatively regulates p53 via inhibition of Tip60. *Biochim Biophys Acta* 2011; **1813**: 1245–1253.

43 Wang L, Heidt DG, Lee CJ, Yang H, Logsdon CD, Zhang L *et al.* Oncogenic function of ATDC in pancreatic cancer through Wnt pathway activation and beta-catenin stabilization. *Cancer Cell* 2009; **15**: 207–219.

44 Tang ZP, Dong QZ, Cui QZ, Papavassiliou P, Wang ED, Wang EH. Ataxia-telangiectasia group D complementing gene (ATDC) promotes lung cancer cell proliferation by activating NF-kappaB pathway. *PLoS One* 2013; **8**: e63676.

45 Fridell RA, Harding LS, Bogerd HP, Cullen BR. Identification of a novel human zinc finger protein that specifically interacts with the activation domain of lentiviral Tat proteins. *Virology* 1995; **209**: 347–357.

46 Liu J, Zhang C, Wang XL, Ly P, Belyi V, Xu-Monette ZY *et al.* E3 ubiquitin ligase TRIM32 negatively regulates tumor suppressor p53 to promote tumorigenesis. *Cell Death Differ* 2014; **21**: 1792–1804.

47 Tebaldi T, Zaccara S, Alessandrini F, Bisio A, Ciribilli Y, Inga A. Whole-genome cartography of p53 response elements ranked on transactivation potential. *BMC Genomics* 2015; **16**: 464.

48 Horn EJ, Albor A, Liu Y, El Hizawi S, Vanderbeek GE, Babcock M *et al.* RING protein Trim32 associated with skin carcinogenesis has anti-apoptotic and E3-ubiquitin ligase properties. *Carcinogenesis* 2004; **25**: 157–167.

49 Kano S, Miyajima N, Fukuda S, Hatakeyama S. Tripartite motif protein 32 facilitates cell growth and migration via degradation of Abl-interactor 2. *Cancer Res* 2008; **68**: 5572–5580.

50 Zhang L, Huang NJ, Chen C, Tang W, Kornbluth S. Ubiquitylation of p53 by the APC/C inhibitor Trim39. *Proc Natl Acad Sci USA* 2012; **109**: 20931–20936.

51 Zhou Z, Ji Z, Wang Y, Li J, Cao H, Zhu HH *et al.* TRIM59 is up-regulated in gastric tumors, promoting ubiquitination and degradation of p53. *Gastroenterology* 2014; **147**: 1043–1054.

52 Chen Y, Guo Y, Yang H, Shi G, Xu G, Shi J *et al.* TRIM66 overexpresssion contributes to osteosarcoma carcinogenesis and indicates poor survival outcome. *Oncotarget* 2015; **6**: 23708–23719.

53 Vincent SR, Kwasnicka DA, Fretier P. A novel RING finger-B box-coiled-coil protein, GERP. *Biochem Biophys Res Commun* 2000; **279**: 482–486.

54 Caratozzolo MF, Valetti A, Gigante M, Aiello I, Mastropasqua F, Marzano F *et al.* TRIM8 anti-proliferative action against chemo-resistant renal cell carcinoma. *Oncotarget* 2014; **5**: 7446–7457.

55 Kapanadze B, Kashuba V, Baranova A, Rasool O, van Everdink W, Liu Y *et al.* A cosmid and cDNA fine physical map of a human chromosome 13q14 region frequently lost in B-cell chronic lymphocytic leukemia and identification of a new putative tumor suppressor gene, Leu5. *FEBS Lett* 1998; **426**: 266–270.

56 Lerner M, Corcoran M, Cepeda D, Nielsen ML, Zubarev R, Ponten F *et al.* The RBCC gene RFP2 (Leu5) encodes a novel transmembrane E3 ubiquitin ligase involved in ERAD. *Mol Biol Cell* 2007; **18**: 1670–1682.

57 Joo HM, Kim JY, Jeong JB, Seong KM, Nam SY, Yang KH *et al.* Ret finger protein 2 enhances ionizing radiation-induced apoptosis via degradation of AKT and MDM2. *Eur J Cell Biol* 2011; **90**: 420–431.

58 Friedman JR, Fredericks WJ, Jensen DE, Speicher DW, Huang XP, Neilson EG *et al.* KAP-1, a novel corepressor for the highly conserved KRAB repression domain. *Genes Dev* 1996; **10**: 2067–2078.

59 Underhill C, Qutob MS, Yee SP, Torchia J. A novel nuclear receptor corepressor complex, N-CoR, contains components of the mammalian SWI/SNF complex and the corepressor KAP-1. *J Biol Chem* 2000; **275**: 40463–40470.

60 Schultz DC, Ayyanathan K, Negorev D, Maul GG, Rauscher FJ 3rd. SETDB1: a novel KAP-1-associated histone H3, lysine 9-specific methyltransferase that contributes to HP1-mediated silencing of euchromatic genes by KRAB zinc-finger proteins. *Genes Dev* 2002; **16**: 919–932.

61 Schultz DC, Friedman JR, Rauscher FJ 3rd. Targeting histone deacetylase complexes via KRAB-zinc finger proteins: the PHD and bromodomains of KAP-1 form a cooperative unit that recruits a novel isoform of the Mi-2alpha subunit of NuRD. *Genes Dev* 2001; **15**: 428–443.

62 Wang C, Ivanov A, Chen L, Fredericks WJ, Seto E, Rauscher FJ 3rd *et al.* MDM2 interaction with nuclear corepressor KAP1 contributes to p53 inactivation. *EMBO J* 2005; **24**: 3279–3290.

63 Yang B, O'Herrin SM, Wu J, Reagan-Shaw S, Ma Y, Bhat KM *et al.* MAGE-A, mMage-b, and MAGE-C proteins form complexes with KAP1 and suppress p53-dependent apoptosis in MAGE-positive cell lines. *Cancer Res* 2007; **67**: 9954–9962.

64 Liu L, Zhao E, Li C, Huang L, Siao L, Cheng L *et al.* TRIM28, a new molecular marker predicting metastasis and survival in early-stage non-small cell lung cancer. *Cancer Epidemiol* 2013; **37**: 71–78.

65 Addison JB, Koontz C, Fugett JH, Creighton CJ, Chen D, Farrugia MK *et al.* KAP1 promotes proliferation and metastatic progression of breast cancer cells. *Cancer Res* 2015; **75**: 344–355.

66 Ho J, Kong JW, Choong LY, Loh MC, Toy W, Chong PK *et al.* Novel breast cancer metastasis-associated proteins. *J Proteome Res* 2009; **8**: 583–594.

67 Herquel B, Ouararhni K, Khetchoumian K, Ignat M, Teletin M, Mark M. Transcription cofactors TRIM24, TRIM28, and TRIM33 associate to form regulatory complexes that suppress murine hepatocellular carcinoma. *Proc Natl Acad Sci USA* 2011; **108**: 8212–8217.

68 Tanaka M, Tanji K, Niida M, Kamitani T. Dynamic movements of Ro52 cytoplasmic bodies along microtubules. *Histochem Cell Biol* 2010; **133**: 273–284.

69 Reddy BA, van der Knaap JA, Bot AG, Mohd-Sarip A, Dekkers DH, Timmermans MA *et al.* Nucleotide biosynthetic enzyme GMP synthase is a TRIM21-controlled relay of p53 stabilization. *Mol Cell* 2014; **53**: 458–470.

70 Faesen AC, Dirac AM, Shanmugham A, Ovaa H, Perrakis A, Sixma TK. Mechanism of USP7/HAUSP activation by its C-terminal ubiquitin-like domain and allosteric regulation by GMP-synthetase. *Mol Cell* 2011; **44**: 147–159.

71 Kuboshima M, Shimada H, Liu TL, Nomura F, Takiguchi M, Hiwasa T *et al.* Presence of serum tripartite motif-containing 21 antibodies in patients with esophageal squamous cell carcinoma. *Cancer Sci* 2006; **97**: 380–386.

72 Obad S, Brunnstrom H, Vallon-Christersson J, Borg A, Drott K, Gullberg U. Staf50 is a novel p53 target gene conferring reduced clonogenic growth of leukemic U-937 cells. *Oncogene* 2004; **23**: 4050–4059.

73 Sun Y, Ho GH, Koong HN, Sivaramakrishnan G, Ang WT, Koh QM *et al.* Down-regulation of tripartite-motif containing 22 expression in breast cancer is associated with a lack of p53-mediated induction. *Biochem Biophys Res Commun* 2013; **441**: 600–606.

74 Wittmann S, Wunder C, Zirn B, Furtwangler R, Wegert J, Graf N *et al.* New prognostic markers revealed by evaluation of genes correlated with clinical parameters in Wilms tumors. *Genes Chromosomes Cancer* 2008; **47**: 386–395.

75 Kung CP, Khaku S, Jennis M, Zhou Y, Murphy ME. Identification of TRIML2, a novel p53 target, that enhances p53 sumoylation and regulates the transactivation of proapoptotic genes. *Mol Cancer Res* 2014; **13**: 250–262.

76 Ozato K, Shin DM, Chang TH, Morse HC 3rd. TRIM family proteins and their emerging roles in innate immunity. *Nat Rev Immunol* 2008; **8**: 849–860.

77 Short KM, Cox TC. Subclassification of the RBCC/TRIM superfamily reveals a novel motif necessary for microtubule binding. *J Biol Chem* 2006; **281**: 8970–8980.