CHAPTER 6

TRIM PROTEINS IN CANCER

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Abstract: Some members of the tripartite motif (TRIM/RBCC) protein family are thought to be important regulators of carcinogenesis. This is not surprising as the TRIM proteins are involved in several biological processes, such as cell growth, development and cellular differentiation and alteration of these proteins can affect transcriptional regulation, cell proliferation and apoptosis. In particular, four TRIM family genes are frequently translocated to other genes, generating fusion proteins implicated in cancer initiation and progression. Among these the most famous is the promyelocytic leukaemia gene *PML*, which encodes the protein TRIM19. *PML* is involved in the t(15;17) translocation that specifically occurs in Acute Promyelocytic Leukaemia (APL), resulting in a PML-retinoic acid receptor-α (PML-RARα) fusion protein.

Other members of the TRIM family are linked to cancer development without being involved in chromosomal re-arrangements, possibly through ubiquitination or loss of tumour suppression functions.

This chapter discusses the biological functions of TRIM proteins in cancer.

INTRODUCTION

The RBCC/TRIM (tripartite motif) protein family is involved in a wide range of biological processes, such as cell growth, development and cellular differentiation. Thus, alteration of these proteins has repercussion for several pathological conditions, from hereditary genetic diseases to cancer development and viral infections. Their striking feature is the tripartite or RBCC motif that is invariably present at the N-terminal region of these proteins, while the C-termini can have different domains. The tripartite motif is

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composed of three cysteine-rich zinc-binding domains, a RING-finger and two B-boxes (B1 and B2), followed by a α-helical coiled-coil region.

Within the vast family of TRIM proteins, few have been well characterized or have established physiological roles. Four family members, TRIM19/PML, TRIM24/TIF1α, TRIM33/TIF1γ and TRIM27/RFP, have been described to acquire oncogenic activity upon chromosomal translocations: in these genetic alterations, the RBCC motif is fused to truncated products of other genes, suggesting that the RBCC motif may play a crucial role in cell transformation. Other members of the TRIM family are linked to cancer development without being involved in chromosomal re-arrangements.

Several data indicated that some RBCC/TRIM proteins may be implicated in ubiquitination, probably because the tripartite module represents the ideal scaffold for such a process. Since the conjugation of ubiquitin proteins to target substrates might require higher order structures, it has been proposed that this large protein family represents a novel class of "single protein RING finger" E3-ubiquitin (Ub) ligases. Considering the huge number of substrates that can be targeted by an E3 ligase, it is not surprising that mutations in *RBCC/TRIM* genes can result in several pathological conditions, including cancer.

More recently, it was suggested that some TRIM proteins such as PML, RFP and TRIM32 may be a new class of SUMO protein ligases (SUMO E3), their SUMO activity depending on an intact tripartite motif. Finally, several TRIM proteins are involved in the regulation of p53-pathways.

This chapter focuses on those TRIM proteins whose involvement in cancer development and progression has been more fully described in literature.

TRIM PROTEINS IN CHROMOSOMAL TRANSLOCATIONS

PML

Included in this group is the ProMyelocytic Leukaemia protein PML, probably the best-known among the TRIM proteins, which becomes oncogenic when its RBCC motif recombines with the product of the retinoic acid receptor (*RARα*) gene.[1-3] The *PML* gene (*PML*) fuses to the retinoic acid receptor α (*RARα*) gene, leading to the production of two fusion genes that encode for the chimera proteins PML-RARα and RARα-PML. Both proteins co-exist in leukemic cells, but, to date, work has mainly focused on PML-RARα, since it retains most of the functional domains of the parental proteins, while little is known about RARα-PML.

PML was identified by cloning the breakpoint sites of the t(15:17) chromosome translocation associated with Acute Promyelocytic Leukaemia (APL); the majority (95%) of APL patients present this translocation.[4] RARα is a member of the nuclear hormone receptor superfamily of transcription factors. In physiological conditions, upon retinoic acid (RA) binding, *RARα* transactivates target genes involved in hematopoietic differentiation. In contrast, in the absence of the ligand, transcription is repressed by the recruitment of histone deacetylases (HDACs) through direct interaction between RARα and the corepressors N-CoR and SMRT. In APL, the fusion protein PML-RARα acts as a potent dominant negative inhibitor of the wild-type (WT) RARα, due to its increased affinity for transcriptional corepressors and HDACs. This strong interaction, in turn, leads to repression of transcription and consequent block of differentiation. As a result, in

the presence of low or even physiological concentrations of RA ($1 \times 10^{-9} - 1 \times 10^{-7}$ M), RARα is no longer able to release HDACs. However, pharmacological doses of RA ($1 \times 10^{-6} - 2 \times 10^{-5}$ M) cause the dissociation of the PML-RARα/HDAC complex, restoring the normal differentiation pathway.[5] The clinical treatment of APL patients with RA represents the first example of "differentiation therapy": the drug treatment degrades PML/RARα and restores the normal function of PML. Nevertheless, treatment of APL with RA as a single agent results in remission only in a small percentage of patients.[6,7] However, addition of the DNA damaging agent arsenic trioxide (As_2O_3) to the RA treatment increases the percentage of APL eradication to more than 90%.[8] As_2O_3 binds directly to cysteine residues in zinc fingers located within the RBCC domain of PML-RARα and PML. This binding induces PML oligomerization with consequent increased affinity for the small ubiquitin-like protein modifier (SUMO)-conjugating enzyme UBC9, resulting in enhanced SUMOylation and degradation. The loss of PML-RARα through degradation enables terminal differentiation of APL cells explaining the efficacy of the combined treatment.[7,8]

PML-RARα associates physically with PML, potentially interfering with its function. Accordingly, transgenic mice expressing PML-RARα in the myeloid compartment develop leukaemia with APL features, while dominant negative RARα mutants do not (as the mutation does not interfere with PML functions), highlighting the importance of PML functional disruption for leukemogenesis.[9]

PML, as most of the TRIM family members, forms high molecular weight complexes in vivo as a consequence of self-association properties of the coiled-coil region.[10] The coiled-coil region is responsible for the formation of stable PML homodimers and for PML/PML-RARα hetero-dimerization,[5] whereas the RING-finger domain is involved in protein-protein interaction.[11] The tripartite motif seems to be essential for the correct localization of PML and, indeed, key to the growth suppressor activity of the protein.[12]

PML genomic locus spans approximately 35 Kb and is subdivided in nine exons. Due to alternative splicing of the gene transcript, which always involves coding exons, PML exists in a number of different isoforms.[13] The N-terminal region is common to all PML isoforms (amino acids 1-394) and contains the region homologous to the DNA-binding finger domain of numerous transcriptional factors. The C-terminus instead differs in several isoforms and it is variably encoded by exons 7, 8 and 9.[13] All PML isoforms are almost equally expressed in the different cell lines and have the potential to act as transcription factors and to form homo- or hetero-dimers.[13] The function of the complex splicing pattern of PML is not known, however, the role of the different PML isoforms might be to provide several distinct surfaces for protein interactions.

In APL, the PML locus on chromosome 15 can be alternatively disrupted at three different sites (breakpoint cluster regions-bcrs): bcr3 in intron 3, bcr1 intron 7 and bcr2 in exon 6. The resulting PML-RARα proteins contain the N-terminal part and the tripartite motif, but invariably lose the C-terminal domain. In this domain resides a putative phosphorylation site with a serine/proline-rich region, potentially involved in the regulation of the normal protein. Therefore, the PML-RARα chimera protein could be under a different regulatory mechanism and this could contribute to the pathogenesis of APL.

In addition to splicing, posttranslational modifications may increase the level of complexity, either by directly affecting protein-protein interactions (the PML sequence contains SUMO-1 modification sites and phosphorylation sites) or by interfering with the ability of PML to form Nuclear Bodies. In fact, physiological localization of PML

is observed both in the nucleoplasm and in the discrete subnuclear matrix-associated compartments known as PML-Nuclear Bodies (PML-NBs).[15] In APL cells, instead, the fusion protein PML/RARα disrupts PML-NBs and relocalises PML into hundreds of microspeckles in the nucleus and in the cytoplasm. This aberrant localization results from a coiled-coil domain interaction between PML and PML/RARα heterodimers.[15-17] Treatment with RA leads to clinical remission associated with NB re-organization, strongly suggesting that PML-NB integrity could be critical for normal cellular functions. Genetic evidence indicates that the structure of PML-NBs depends on the presence of PML.[18] In cells derived from PML-knockout mice, PML-NBs do not exist and all the proteins normally targeted to these organelles are nuclear diffused or concentrated in distinct domains. The product of the *BLM* gene, for instance, which is inactivated in the Bloom syndrome, normally colocalizes with PML in the NBs, but it is no longer found in these structures in PML-null cells.[19]

A cytoplasmic PML isoform, in which a nuclear export sequence (NES) in exon 9 is retained, is also generated as a consequence of alternative splicing. This isoform can shuttle between nucleus and cytoplasm and it is not localized at NBs. It has been implicated in the modulation of TGF-β signalling[20] and calcium metabolism regulation at the endoplasmic reticulum (ER).[21]

A number of tumours and tumour-derived cell lines are characterized by a novel form of PML-NBs, termed ALT-associated PML-Bodies (APBs), in which PML localizes at the telomeres, together with other telomere binding proteins, to maintain telomere length.[22]

The study of mice in which PML has been inactivated by homologous recombination has led to the conclusion that the *PML* gene is not required for viability, at least in the mouse. PML- knockout mice develop normally and do not get spontaneous cancers but are more sensitive to tumour promoting agents.[23] PML can therefore antagonize the initiation, promotion and progression of tumours of different histological origins, behaving in vivo as a tumour suppressor. Consistent with these findings, the overexpression of PML inhibits the transformation of rat embryo fibroblasts induced by Ha-Ras expression in combination with oncogenic mutants of p53 or c-Myc,[24] and causes a dramatic drop in the capacity of cell lines to form colonies in a typical colony formation assay.[12]

PML can exert its growth suppression activity either through the induction of p53-dependent/independent apoptosis or replicative senescence.

The first evidence that PML is involved in the p53-dependent apoptotic pathway came from experiments carried out in PML-knockout mice. These animals are resistant to the lethal effects of ionizing radiations.[25] Similarly, PML –/– splenocytes are resistant to γ-irradiation-induced apoptosis.[26] In these cells, DNA-damage induced apoptosis depends entirely on the presence of a normal p53 function. P53 –/– cells, in fact, are completely insensitive to irradiation, while PML –/– cells display an intermediate phenotype, suggesting that PML may be only required to support p53 pro-apoptotic functions. Upon γ-irradiation p53 becomes stabilized and is activated by posttranslational modifications that take place at several residues. The DNA-damage checkpoint kinase Chk2 is responsible for serine 20 modification. PML recruits Chk2 into PML-NBs and enhances p53 phosphorylation.[27] Similarly, the homeodomain-interacting protein kinase-2 (HIPK2) was found to colocalize with PML within the NBs.[28,29] HIPK2 is responsible for phosphorylating p53 on serine 46 upon ultraviolet irradiation, an event that has been linked to induction of apoptosis.[30] The herpes virus associated ubiquitin-specific protease (HAUSP) is another enzyme affecting p53 activity and localizing at the NBs.

MDM2, the main regulator of p53, was also found to be associated with PML-NBs, in particular after the inhibition of the nuclear export.[31] More recently, it was shown that PML and MDM2 interact[32] and that upon DNA-damage PML protects p53 from MDM2-mediated ubiquitination and degradation.[33] In fact, it appears that following UV irradiation two different complexes, PML/p53 and PML/MDM2, are formed, leading to p53 stabilization.[34]

As regards the role of PML in p53-independent apoptosis, it was reported that splenocytes and hepatocytes from PML-knockout mice have defective Fas- and TNF-induced apoptosis. These two agents induce activation of extrinsic apoptotic pathways, considered p53-independent.[25] The Fas-interacting protein DAXX was found to act as a positive mediator of Fas- and TNF-induced apoptosis. Two models have been proposed to explain the co-operation between PML and DAXX in regulating apoptosis.[35] According to the first one, upon Fas-ligand binding, DAXX moves to the nucleus where it directly interacts with PML. DAXX is a transcriptional repressor and PML is thought to antagonize its transcriptional function by sequestering it into PML-NBs. The second model proposes that the PML/DAXX complex is released from the nuclear bodies upon PML deSUMOylation by Supr-1, or upon DAXX phosphorylation by the homeodomain-interacting protein kinase-1 (HIPK-1). The PML/DAXX complex would have then transcriptional functions and activate pro-apoptotic genes.

Recently, PML was found to be a critical factor for mitochondrial damage linked to ER stress-induced cell death.[36] In the cytoplasm, in fact, PML was found to localize to the ER and specific membrane structures (mitochondrial-associated membranes, MAMs) involved in ER-to-mitochondria calcium ion (Ca^{2+}) transport.

The existence of a link between premature senescence, PML-NBs and p53 is now generally accepted. In primary fibroblasts, PML overexpression induces premature senescence in a p53-dependent manner, since p53-knockout mouse embryo fibroblasts (MEFs) are protected from this effect. PML itself is upregulated during replicative or Ras-induced senescence.[37] The resulting increase in PML levels leads to a concomitant increase in number and size of PML-NBs. Expression of the activated form of Ras (Ras^{VAL12}) also induces relocalization of p53 within the PML-NBs and promotes its acetylation at lysine 382. Acetylation is essential for p53 biological function and is profoundly impaired in PML –/– MEFs. Although PML does not possess intrinsic acetyltransferase activity, it directly interacts with the acetyltransferase CBP/p300, recruiting it to the NBs. Therefore, PML, p53 and CBP/p300 form a three-complex that localizes to PML-NBs, facilitating p53 acetylation by CBP/p300. Collectively, these observations suggest that PML is required for p53 acetylation and senescence upon oncogene expression. However, conclusive evidence that PML, like p53, is essential for the induction of cellular senescence upon oncogenic transformation comes from the observation that Ras^{VAL12}-induced senescence is drastically impaired in PML –/– cells.

The NAD-dependent deacetylase SIRT1, a negative regulator of p53, has also been found in NBs. SIRT1 binds to p53 and specifically deacetylases lysine 382, resulting in decreased p53-dependent transcription and PML-induced senescence.[38]

In conclusion, PML is involved in a great variety of processes, most of them closely linked to its primary localization into the NBs, where a plethora of different proteins are also assembled and anchored. Thus, the oncogenicity of PML/RARα, which appears to involve the RBCC motif, may result from an abnormal subnuclear organization that diverts subsets of proteins from their natural functions.

TIF1α

Like PML, the Transcriptional Intermediary Factor 1 α (TIF1α), or TRIM24, seems to be involved in the RA-dependent activation pathway.[3]

TIF1α is a nonhistone chromosomal protein (E. Remboutsika, R. Losson and P. Chambon, unpublished results) found to interact specifically and in a ligand-dependent manner with several nuclear receptors, including RARs, the Retinoic X Receptors, the vitamin D3 receptor (VDR), and the oestrogen (ER) and progesterone receptors (PR), and to positively or negatively regulate their transcriptional activity.[39] TIF1α is maintained at high levels in myeloid progenitor cells when granulocyte differentiation is induced by RA, indicating that RARα-mediated TIF1α expression is required for myeloid differentiation.[40] TIF1α can also function as a liver-specific tumour suppressor by attenuating RARα-mediated transcription in mice. TRIM24-knockout mice are viable and fertile, but homozygous deletion of TRIM24 induces a high incidence of hepatic tumour development by 9 months of age. Deletion of a single RARα allele in a *Trim24*-null background suppresses hepatocellular carcinoma (HCC) development and restores wild-type expression of RA-responsive genes in the liver.[41] TIF1α, moreover, can repress transcription in transient transfections when tethered to template DNA through chromatin association, which is mediated by its peculiar C-terminal domain. This domain, called PHD-BROMO domain, contains a polycomb homology domain (PHD) finger and a bromodomain,[42-44] which are characteristic motifs of proteins known to function as transcriptional coregulators at chromatin level.[45,46] Recently, it has been demonstrated that TIF1α recognizes acetylated histone 3 at K23 (h2K23) and unmodified histone H3K4 through a noncanonical histone signature and that its regulation is associated with cellular proliferation and tumour development.[47]

TIF1α, like PML, can operate as a transcriptional intermediary factor, regulating the ability of RXRα/RARα to activate transcription.[3] A putative functional interaction between these two proteins has been suggested in PML –/– cells, where the ability of TIF1α to act as a transcriptional co-activator upon RA is impaired. Moreover, PML and TIF1α co-immunoprecipitate when they are both transiently transfected, but no binding is observed when assayed in yeast two-hybrid assays, suggesting that no direct interaction occurs between them. Recently, Tisserand and colleagues have associated *Trim24* with a negative regulation of the IFN/STAT pathway through RARα inhibition, suggesting that this repression may prevent liver cancer.[48]

Similarly to PML, TIF1α also occurs in chromosomal re-arrangements leading to the formation of the oncoprotein T18, which retains the N-terminal moiety of TIF1α fused to a truncated C-terminal portion of the mouse homologue B-raf proto-oncogene.[3,49] By analogy with PML/RARα, the RBCC domains of TIF1α may potentiate the oncogenicity of B-raf C-terminal through two different mechanisms, not mutually exclusive. In the first, formation of TIFα/TIF-B-raf heterodimers involving the coiled coil domain may have a dominant negative effect, relocating TIF1α and/or TIF1α-associated proteins from the nucleus to the cytoplasm. TIF1α delocalization inactivates its transcriptional function, lending a selective growth advantage to the transformed cells and abrogating their capacity to respond to RA. In addition, because TIF1α associates with nuclear receptors (ER and VDR),[39] T18 may interfere with other hormone-dependent pathways. In the second, the truncated B-raf, associated with the RBCC motif of TIF1α, may acquire a different intracellular localization, thereby increasing its oncogenic potential.

TIF1α is also found fused with RET (Re-arranged during Transfection) receptor tyrosine kinase (tk) in the papillary thyroid carcinomas (PTC). The resulting oncoprotein

displays the C-terminal ret tk domain fused to the RBCC of TIF1α.[50] A modified TIF1α protein, in concert with the altered ret tk activation, might be important for tumour induction and/or progression.

TIF1γ/TRIM33

In contrast with TIF1α, the ubiquitous nuclear protein Transcriptional Intermediary Factor 1 gamma, TIF1γ/TRIM33, does not interact with nuclear receptors but exhibits a strong silencing activity when tethered to a promoter.[51] The *TIF1γ* gene has been mapped to the 1p13 locus. Interestingly, a nonrandom chromosomal translocation t(1;22)(p13;q13) involving this region has been reported in acute megakaryocytic leukaemia [Mitelman F. (1993). In: *Human Gene Mapping*, pp.773-812]. *TIF1γ* was shown involved in the control of haematopoietic cell fate by the TGFβ/Smad pathway.[52] More recently, *TIF1γ* has been proposed as an epigenetically regulated tumour suppressor gene in hematopoietic cells. The authors suggest that in chronic myelomonocytic leukemia changes in *TIF1γ* expression may be considered as biomarkers of response to the demethylating agents and other chromatin structure modifiers (e.g., HDAC inhibitors) currently being developed for the treatment of this disease.[53]

RFP

The last TRIM family member acquiring a transforming activity when its RBCC motif is fused to another protein is the Ret Finger Protein (RFP), also known as TRIM27. Together with other family members, RFP also has, in addition to the tripartite domain, a highly conserved 185 to 195 amino acid motif at the C-terminal region, called the rfp domain or B30.2 motif.[54] Interestingly, *RFP* was originally identified as a gene which becomes oncogenic following its fusion with the *RET* proto-oncogene. This translocation is generated by DNA re-arrangement, which occurs during transfection of the NIH 3T3 cells with human T-cell lymphoma DNA. The resulting transforming protein RFP/RET shows the fusion of the tripartite motif of RFP with the C-terminal tyrosine kinase domain of the RET protein.[55-57] RFP/RET triggers cellular transformation through a mechanism which is still unknown, but two options seem likely: (i) the RFP RBCC motif causes ligand-independent dimerization of the fusion protein resulting in constitutive kinase activation and subsequent cell transformation; (ii) RFP nuclear localization signal leads the fusion protein into the nucleus where it dimerizes with the wild-type RFP, interfering with its normal functions. However, it is also possible that the normal amino-terminal domain of the tyrosine kinase contains regulatory elements that become lost during gene fusion causing abnormal enzymatic activity leading to cellular transformation. Indeed, this mode of activation is frequent with tyrosine kinases.

RFP is a component of PML-NBs through direct interaction with PML, which is mediated by the B-box and the distal α-helical coiled-coil domains of RFP.[58] In APL-derived NB-4 cells, which cannot differentiate, RFP is found dispersed in microgranules, retaining, however, its colocalization with PML and/or PML/RARα. After treatment with All Trans Retinoic Acid (ATRA), the block in differentiation is released and RFP and PML colocalize together with the other components in the reformed PML-NBs. Further studies demonstrated that RFP exhibits transcriptional repressive activity.[59-62] Recently, an interesting study showed evidence for physical and functional interactions between the retinoblastoma tumour suppressor protein (Rb) and RFP. It has been

proposed that RFP may inhibit *Rb* activating function by inducing stabilization of the histone acetyl-transferase (HAT) inhibitor EID-1 (E1A-like inhibitor of differentiation).[63] EID-1 is involved in the transcriptional regulation of genes that are required for cellular differentiation through the block of the activity of the histone acetyltransferase p300.[64] TRIM27 expression also correlates with ERBB2 protein expression and ERBB2 gene amplification in breast cancer.[65]

All these data suggest that RFP may have a role in growth regulation and that, in the appropriate context, could act as an oncogene.[58]

Finally, a recent study suggests that TRIM27/RFP functions as a SUMO E3 ligase toward MDM2 and that TRIM27 acts on MDM2 independently of PML, with whom it directly interacts.[66]

TRIM PROTEINS AS E3-Ub LIGASES

EFP

A striking example of an E3-Ub ligase involved in oncogenesis is EFP (oestrogen-responsive finger protein), also called TRIM25, which was found to be highly expressed in breast cancer. Since the discovery that the ovarian hormone oestrogen stimulates breast tumour growth, many efforts have been directed to determine an endocrine therapy which can inhibit the synthesis or the action of oestrogen.[67] The direct effect of oestrogen on oestrogen-responsive tissues requires two oestrogen receptors (ERα and ERβ): these are ligand-dependent transcription factors found in low levels in the normal mammary gland tissue and in higher concentration in most human breast cancers.[68] Downstream target genes of ERs have an important role in mediating oestrogen action in breast cancer; among these, *EFP* is a target gene of ERα and, besides breast cancer, it is also expressed in a variety of other female organs.[69] Urano et al, analysed the effect of EFP antisense oligonucleotides on tumour formation from breast cancer MCF7 cells implanted in female athymic mice. Administration of antisense EFP oligonucleotides inhibited the tumour growth generated by MCF7 cells. Moreover, EFP-overexpressing MCF7 cells in ovariectomized athymic mice generated tumours in the absence of estrogen.[70] More detailed studies demonstrated that EFP directly degrades the cell cycle regulatory protein 14-3-3σ through an ubiquitin-dependent pathway in which EFP functions as an E3-Ub ligase. 14-3-3σ degradation is followed by dissociation of the protein from the cyclin-Cdk complexes, leading to cell cycle progression and tumour growth. Thus, EFP may contribute to the deregulated proliferation of breast cancer cells *via* the accelerated destruction of the cell cycle regulator 14-3-3σ. In MEFs, loss of TRIM25 causes accumulation of 14-3-3σ, which is responsible for reduced cell proliferation. In agreement with these findings, TRIM25 immunoreactivity is significantly correlated with poor prognosis in patients with breast cancer.[71] It has been reported that even in the absence of hormone-stimulation, TRIM25 physically interacts with ERα leading to ERα ubiquitylation. In the presence of oestrogen, TRIM25 overexpression enhances ERα-mediated transcription, suggesting that TRIM25 overexpression functions as a cofactor for ERα-induced transcription.[72]

Recently, the accumulation of experimental evidences regarding oestrogen-responsive genes such as EFP has opened the way for the development of potential molecular targets of therapeutic intervention. In particular, it has been shown that DNA-modified siEfps

(chimeric siEfps) have good potential in clinical applications inducing fewer off-target effects or immune responses in mammalian cells.[73]

TRIM32

TRIM32, like TRIM25, is linked to cancer without being an oncogenic fusion protein. The C-terminal portion of the protein contains NHL repeats: the domain consists of repeats of a ~44-residue sequence that is rich in glycine and hydrophobic residues, and contains a cluster of charged residues near its C-terminal end. This domain is known by the name of NHL because was first identified in three proteins, NCL-1,[74] HT2A,[75] and LIN-41.[76]

TRIM32 accumulates in characteristic cytoplasmic speckles.[10,77]

Among TRIM family members, TRIM32 is unique in being involved both in hereditary developmental syndromes and in carcinogenesis. In fact, TRIM32 is mutated in the limb-girdle muscular dystrophy Type 2H (LGMD2H) and, more recently, a second mutation has been linked to the Bardet-Biedl syndrome (BBS).[78,79] In addition, deficiency of TRIM32 in mice results in several features observed in human LGMD2H.[80]

TRIM32 was originally identified in the mouse clonal epidermal model of carcinogenesis. In this model, nontransformed keratinocytes, treated with 7,12-dimethylbenz[*a*]anthracene (DMBA), gave rise to three independently initiated clones; these clones, once inoculated in mice, produced papillomas and squamous cell carcinomas (SCCs), used to derive the tumorigenic cell lines.[81] The clonality of the model permits to dissect the process of carcinogenesis in normal, initiated and tumorigenic cells. Initiated and tumorigenic cells from this model exhibit a 2-5 fold increase in TRIM32 expression compared to normal cells, indicating a role for TRIM32 in tumour initiation but also during progression and transformation.[77]

TRIM32 level is also elevated in a fraction of human head and neck squamous cell carcinoma (HNSCC) samples and in the adjacent mucosa. HNSCC represents the sixth most common cancer in the world and can affect different anatomical regions: the oral cavity, oropharynx, larynx and hypopharinx. The prognosis of HNSCC is influenced by many factors: tumour, node, metastasis staging and pathologic grading of differentiation. TRIM32 expression is elevated early in HNSCC development and, as in the clonal keratinocyte model, maintained in malignant progression. Interestingly, in vitro ectopic expression of TRIM32, at levels comparable to those detected in sporadic cancer, increases transformation frequency and is able to block UV-induced apoptosis in keratinocytes.[77] Increased TRIM32 expression has also been detected in murine skin tumours, induced after exposure to UVB or to a chemical [12-*O*-tetradecanoylphorbol-13-acetate (TPA) + DMBA] two-step carcinogenesis protocol.[77] TRIM32 imparts a survival phenotype to epidermal cells responding to UVB/TNFα-induced stress, whereby these epidermal cells persist and can accumulate additional UVB-induced DNA damage or other oncogenic events, leading to cancer development.

TRIM32 has the hallmarks of an E3-Ub ligase, including self-ubiquitination and interaction with ubiquitinated proteins in response to combined UVB/TNFα (Tumour Necrosis Factor α).[77] A direct target of TRIM32 degradation through ubiquitylation is Piasy, a member of the PIAS (protein inhibitors of activated STATs) family: this protein is a E3-SUMO ligase, involved in the control of apoptosis, senescence and Nuclear Factor-Kappa β (NF-Kβ) activation.[82] Piasy promotes keratinocyte apoptosis in response to UVB and TNFβ-induced stress through the inhibition of NF-Kβ survival function. The interaction between TRIM32 and Piasy, therefore, appears to modulate the balance

between survival and apoptosis in response to specific stress conditions. An interesting open question is whether this interaction is also important in the development of human HNSCC since this mechanism may have important clinical implications.

A role for TRIM32 as tumour suppressor has been also suggested. In mammalian cells, TRIM32 induces tumour necrosis factor (TNF)-mediated apoptosis through its interaction and ubiquitylation of XIAP (X-linked inhibitor of apoptosis). Degradation of XIAP prevents its inhibition of pro-apoptotic caspases.[83]

Recently, TRIM32 was found to interact with RARα enhancing its transcriptional activity in the presence of RA. TRIM32 overexpression in mouse neuroblastoma cells and embryonal carcinoma cells promoted stability of RARα, resulting in enhancement of neural differentiation. These findings suggest that TRIM32 could be a therapeutic target for developmental disorders and RA-dependent leukaemia.[84]

TRIM PROTEINS WITH TUMOUR SUPPRESSOR FUNCTIONS OR INVOLVEMENT IN p53 PATHWAYS

TRIM8/GERP

TRIM8/GERP (glioblastoma expressed RING finger protein) has been implicated in a variety of neoplastic transformations. TRIM8 is highly conserved between human and mouse and contains two B-box domains. The gene is expressed in several tumours, including anaplastic oligodendroglioma, and maps to chromosome 10q24.3, a region showing frequent deletions or loss of heterozygosity in glioblastomas,[85] which suggests the presence of a tumour suppressor gene at that site.

A study of primary tumour samples from patients undergoing surgery for laryngeal squamous cell carcinoma (LSCC), the most frequent neoplasia of the head and neck region, supports TRIM8 tumour suppressor role.[86] Microarrays screening, performed to identify genes regulated during tumour progression, showed that TRIM8 inversely correlates with metastatic potential. The anti-metastatic and anti-proliferative involvement of TRIM8 was also tested in an in vitro colony formation assay: human cell lines stably transfected with TRIM8 cDNA produce significantly less colonies than control cells.[86]

TRIM8 is a suppressor of cytokine signalling 1 (SOCS1)-interacting protein that is induced by IFNγ.[87] TRIM8 was also shown to interact with the protein inhibitor of activated STAT3 (PIAS3). PIAS3 inhibits IL-6-dependent activation of STAT3, a signalling pathway that is known to be important for cancer development and progression.[88] Ectopic expression of TRIM8 can block PIAS3 action on STA3 either by inducing its degradation or by excluding PIAS from the nucleus.[88] Moreover, a recent study has shown that the translocation of phosphorylated STAT3 into the nucleus is modulated by the interaction between TRIM8 and the heat shock protein 90β (HSP90β) and consequently regulates transcription of Nanog in embryonic stem cells, suggesting a role for TRIM8 in the regulation of stem cell self-renewal or differentiation.[89]

More recently, TRIM8 has been described as a new modulator of p53-mediated tumour suppression mechanisms.[90] Under stress conditions, such as UV exposure, p53 induces the expression of TRIM8, which in turn impairs p53 interaction with MDM2 leading to p53 stabilization, cell cycle arrest and reduction of cell proliferation. Conversely, silencing of TRIM8 reduces the capacity of p53 to activate genes involved in cell-cycle arrest and DNA-repair.

Other TRIM proteins have been shown involved in p53 regulation, for instance TRIM24/TIF1α and TRIM19/PML which, respectively, target p53 for degradation (TRIM24 deletion in human breast cancers leads to p53-dependent apoptosis, suggesting that TRIM24 could constitute a potential therapeutic target in breast cancer that expresses wild-type p53) or facilitate p53-Thr18 phosphorylation in response to DNA-damage.[91-93]

TRIM28 interacts and co-operates with MDM2 to promote p53 ubiquitylation and degradation;[94] MAGE proteins, which are upregulated in many cancers, were reported to function as cofactors in TRIM28-mediated p53 suppression.[95]

TRIM29/ATDC (ataxia telangiectasia group D-complementing) binds to p53, exporting it out of the nucleus and thus blocking p53-mediated transcription.[96] This interaction is tightly regulated by TRIM29 acetylation.[96] Histone deacetylase 9 deacetylates TRIM29, preventing its association with p53, and inhibiting its cell growth-promoting activity.[97] TRIM29 can also suppress UV-induced apoptosis in HCT116 cells through inhibition of p53acetylation. Thus,TRIM29 might be involved in DNA-damage response and act as an oncogene promoting tumour growth.[98]

Increased levels of **TRIM13**, upon ionizing radiation, can induce the ubiquitylation and degradation of MDM2 with consequent p53 protein stabilization and induction of apoptosis,[99] suggesting that TRIM13 functions as a tumour suppressor.

Finally,**TRIM22** expression is also found increased in response to p53; ectopic expression of TRIM22 in U-937 cells resulted in reduced clonogenic growth, while TRIM22 mRNA levels correlated with ATRA-induced differentiation, suggesting that TRIM22 might be involved in the differentiation of leukemic cells.[100]

CONCLUSION

In summary, TRIM proteins participate in several mechanisms important for the maintenance of cellular homeostasis but acquire oncogenic potential when altered. Some members of this family can be present as fusion proteins due to chromosomal re-arrangements; these chimera proteins have a dominant negative effect on both wild type partners, interfering with their physiological functions. Conversely, other TRIMs, although not involved in genetic aberrations, are linked to oncogenesis either because they alter the ubiquitination pathways of key cell-cycle proteins or because they lose their tumour suppression function. Moreover, a few members of the TRIM family have a dual role in tumour suppression or tumour promotion by modulating p53 activation.

Thus, further understanding of TRIM protein role in tumorigenesis, in particular their involvement in ubiquitination and SUMOylation, may provide novel targets for effective cancer therapies of the future.

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