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Review

New insights into the cytoplasmic function of PML

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Summary. PML is a tumour suppressor inactivated in Acute Promyelocytic Leukaemia (APL). PML is the essential component of a subnuclear structure called the PML nuclear body (PML-NB), which is disrupted in APL. By targeting different cellular proteins to this structure, PML can either hamper or potentiate their functions. The PML transcript undergoes alternative splicing to generate both nuclear and cytoplasmic isoforms. Most of the research in this field has focused its attention on studying nuclear PML. Nevertheless, new exciting studies show that cytoplasmic PML may control essential cellular functions, thus opening new avenues for investigation.

Key words: TRIM family, TGFß pathway, Viral infection, Tumours

Introduction

Since its discovery, the promyelocytic leukaemia protein (PML) has been the object of intense research, which revealed its important role in a variety of cellular functions (Zhong et al., 2000b; Jensen et al., 2001; Salomoni and Pandolfi, 2002). The *PML* gene was originally identified in acute promyelocytic leukaemia (APL), a disease characterized by accumulation in the bone marrow and blood of blasts arrested at the promyelocytic stage of the differentiation (de The et al., 1991; Goddard et al., 1991; Kakizuka et al., 1991). The majority of APL cases (more that 90%) are associated with a reciprocal and balanced translocation, t(15;17)(q21;q11.2-12), which fuses the PML gene with the retinoic acid receptor alpha gene (RARα) (de The et al., 1991; Goddard et al., 1991; Kakizuka et al., 1991).

PML was found to be part of sub-nuclear multi-protein complexes referred to as PML-nuclear bodies (PML-NB), ND10 or PML oncogenic domains (PODs) (Jensen et al., 2001; and references cited therein). In $Pml^{-/-}$ cells, all PML-NB components tested to date assume a disperse localization, thus suggesting that PML is essential for the formation and/or the integrity of these structures (Zhong et al., 2000a; Lallemand-Breitenbach et al., 2001). Although $Pml^{-/-}$ mice are viable, they develop a larger number and a different spectrum of tumours upon carcinogenic treatment and are more susceptible to PML-RARα-mediated leukaemogenesis, thus suggesting that PML acts as a tumour suppressor (Wang et al., 1998; Rego et al., 2001). In APL, PML-RARα delocalizes PML from the PML-NBs into aberrant nuclear microspeckles and inhibits its functions. APL cells are uniquely sensitive to treatments with pharmacological concentrations of all trans-retinoic acid (ATRA), which induces the degradation of the fusion protein, thus promoting remission of the disease in patients (Grignani et al., 1993; Zhu et al., 2001).

The fact that a large number of important cellular players have been found to either stably or transiently associate with the PML-NB, such as pRB, p53 and eIF4E, suggests that PML, which is the essential component of the PML-NB, could in theory modulate their function by targeting them to this structure. This has been indeed demonstrated for several PML-NB components (Guo et al., 2000; Pearson et al., 2000; Jensen et al., 2001; Bernardi et al., 2006; Trotman et al., 2006).

Gene, isoforms and TRIM family

Gene and isoforms

The PML gene is located on chromosome 15 and consists of nine exons spread along a locus of 35 Kb. A number of nuclear and cytoplasmic isoforms are originated by alternative splicing of the primary transcript (Fagioli et al., 1992; Jensen et al., 2001). Notably, all isoforms share the N-terminal region (exon 1-3), which encodes the RBCC domain, whereas they differ in their COOH-termini. A nuclear localization sequence (NLS), encoded by exon 6, governs PML nuclear localization. It is conceivable that different COOH-termini could confer isoform-specific functions to PML. In this regard, a nuclear export sequence (NES),

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present in exon 9, is uniquely retained in the C-terminal portion of PML1, thus allowing it to shuttle between the nucleus and the cytoplasm (Condemine et al., 2006). PML1 could have different functions depending on its cellular localization and specific interactions. Despite initial studies suggested that all isoforms were expressed at comparable levels (Fagioli et al., 1992; Jensen et al., 2001), a recent analysis performed on a number of different primary and immortalized cell types revealed that PML isoforms are differentially expressed (Condemine et al., 2006). In particular, PML1 appears to be the isoform expressed at highest levels and, according to its DNA sequence, the one that displayed the highest similarity between humans and mice (Condemine et al., 2006). Furthermore, the expression analysis of different PML isoforms in $Pml^{-/-}$ cells by using isoform-specific antibodies revealed that PML1 accumulated in both nucleus and cytoplasm (Condemine et al., 2006; and our unpublished observation). Interestingly, PML1 mRNA levels were abundant in primary cells but rather low in transformed cells, thus suggesting that PML1 expression may inversely correlate with the transformation status of the cell (Condemine et al., 2006). Notably, several primary tumour samples displayed a PML cytoplasmic staining (Condemine et al., 2006). This could be due either to cytoplasmic sequestration of nuclear PML or to increased nuclear export of PML1 and/or induction of PML cytoplasmic isoforms. It remains to be established what are the consequences of PML cytoplasmic localization on transformation and tumourigenesis. Answering this question will be critical to understand the role of different PML isoforms in transformation and cancer.

TRIM family

PML is the most well characterized member of a growing class of proteins referred to as tripartite motif family of proteins (TRIM). TRIM proteins are invariably characterized by the presence at their N-terminus of a tripartite domain named the RBCC, which consists of a RING (R), one or two B-boxes (B) and a predicted Coiled-Coil region (CC). Remarkably, RBCC domains account for important features such as protein-protein interaction, homo-oligomerization and sub-cellular localization. Although, heterodimerization events between different TRIM members have been suggested to be a rare at least in yeast two hybrid experiments, this has not been formally excluded in mammalian cellular models (Reymond et al., 2001). For instance, PML has been shown to heterodimerize with the Ret Finger Protein (RFP or TRIM27) in mammalian cells (Cao et al., 1998; Morris-Desbois et al., 1999). Therefore, it is conceivable that other TRIMs could heterodimerize, thus affecting their reciprocal localization and/or functions. This is an area of research worth investigating in the near future.

The canonical RBCC architecture is conserved in other mammals and lower organisms, suggesting its importance in the modulation of protein function (Nisole et al., 2005), and references cited therein). The RING is a cysteine-rich zinc finger that binds two atoms of zinc mainly involved in the regulation of protein-protein interactions. In addition, it has been shown that some TRIM proteins possess RING domains capable of intrinsic E3-ubiquitin ligase activity (Meroni and Diez-Roux, 2005). The function of B-boxes domains B1 and B2, which consist of cysteine/hystidine-rich zinc finger motifs found exclusively in TRIM proteins, is still unclear. Finally, the predicted α-helical CC domain has a tri-dimensional conformation that allows both homo-oligomeric and hetero-oligomeric interactions. Indeed, TRIM proteins, apart from few exceptions, generally homodimerize, thus resulting in the formation of nuclear and cytoplasmic aggregates, which, in turn, may function as scaffold for higher-order protein complexes (Reymond et al., 2001; and Table 1). Interestingly, members of the TRIM family are involved in a variety of physiological and pathological conditions (Meroni and Diez-Roux, 2005). A number of mutations that affect TRIM proteins have been described and are associated with genetic inherited disorders (Meroni and Diez-Roux, 2005). Interestingly, some of these TRIM mutant proteins display an aberrant localization pattern (i.e. mutant MID1 in Opitz Syndrome) (Quaderi et al., 1997; Cainarca et al., 1999; Schweiger et al., 1999). Furthermore, in addition to TRIM19/PML other family members, such as TRIM27/RFP and TRIM24/TIF1-α, have been shown to acquire oncogenic activity when involved in chromosomal translocations (Takahashi et al., 1988; Le Douarin et al., 1995; Klugbauer and Rabes, 1999).

Table 1. Human cytoplasmic TRIMs divided based on their ability to form cytoplasmic structures: filaments, ribbon-like or bodies.

CYTOPLASMIC TRIM PROTEINS		
Filaments	Ribbon-like	Bodies
TRIM1/MID2	TRIM29/ATDC	TRIM4
TRIM2/NARF		TRIM5
TRIM3/BERP		TRIM6
TRIM18/MID1		TRIM9/SPRING
		TRIM10/HERF1
		TRIM12
		TRIM14/Pub
		TRIM19/PML(*)
		TRIM21/RO52
		TRIM22/STAF50
		TRIM23/ARD1
		TRIM26
		TRIM27/Rfp(*)
		TRIM30/RPT-1
		TRIM32/HT2A(*)

(*) Cytoplasmic TRIMs potentially implicated in cancer (Klugbauer and Rabes, 1999; Salomoni and Pandolfi, 2002; Horn et al., 2004; Lin et al., 2004).

Initial observations

Despite the existence of a number of PML cytoplasmic isoforms was reported many years ago (Fagioli et al., 1992; Jensen et al., 2001), their functional characterization has been somewhat limited until recently. Initial observations conducted by using a mutant of PML4 lacking the nuclear localization signal (ΔNLS PML), which resulted in cytoplasmic accumulation of the protein, revealed that this mutant was profoundly impaired in its ability of suppressing neu-mediated oncogenic transformation of NIH3T3 cells (Le et al., 1996). ΔNLS PML expression also led to a significant reduction of PML-NBs, thus suggesting a potential dominant negative function over wild-type nuclear PML (Le et al., 1996). Subsequently, Fagioli et al analyzed the effect of different PML splice variants on growth suppression and found that the expression of a cytoplasmic isoform, referred to as PML 3-4-7 based on its exon composition, failed to induce growth suppression in colony forming assay (Fagioli et al., 1998). Hence, they concluded that the presence of the NLS was indispensable for the growth suppressive function of PML (Fagioli et al., 1998). Because of the greater interest on nuclear PML and its growth suppressive and pro-apoptotic functions, the role of cytoplasmic isoforms has been overlooked for a long time. Nevertheless, it is becoming clear that also cytoplasmic PML plays important cellular functions, and this will be the main subject of this review (Lin et al., 2004; Bellodi et al., 2006; Seo et al., 2006).

Role of cytoplasmic PML in the TGF-ß pathway

TGF-ß is a pleiotropic cytokine that is crucially implicated in a variety of cellular processes such as proliferation, differentiation and apoptosis (Siegel and Massague, 2003). This pathway is tightly regulated in physiological conditions (Siegel and Massague, 2003). By contrast, alterations of the TGF-ß pathway occur in cancer and has been strongly linked to the pathogenesis of several human malignancies, encompassing solid as well as haematopoietic tumours (Derynck et al., 2001; Shi and Massague, 2003; Siegel and Massague, 2003; Lin et al., 2005). The TGF-ß pathway and its alterations in cancer have been extensively studied and well characterized (Siegel and Massague, 2003), and references cited therein). The signal is originated at the level of the cell membrane by two serine-threonine kinase receptors: TGF-ß receptor I and II (TßRI and TßRII). TGF-ß binds to the TßRII that associate to and activates the TßRI. Subsequently, the receptor complex is internalized through the chlatrin/early endosome pathway, and the signal is propagated to the nucleus through TßRI mediated-phosphorylation of the transcriptional factors Smad2 and Smad3. In this context, SARA (Smad Anchor for Receptor Activator) promotes the internalization of the receptors into early endosomes, thus facilitating Smad2/3 activation (Tsukazaki et al., 1998; Shi and Massague, 2003). Once activated, Smad2/3 associate with Smad4 and the complex translocates to the nucleus where it coordinates the expression of TGF-ß-responsive genes (Wu et al., 2001; Inman and Hill, 2002).

PML and TGF-ß

In a recent work, the tumour suppressor PML has been implicated in the modulation of TGF-ß signalling (Lin et al., 2004). Remarkably, primary $Pml^{-/-}$ mouse embryo fibroblasts (MEFs) appeared to be insensitive to TGF-ß-induced growth suppression and apoptosis (Lin et al., 2004). Surprisingly, reintroduction of the nuclear, tumour suppressive isoform PML-IV failed to rescue these defects. In contrast, full restoration of TGF-ß responsiveness was achieved by expressing a PML cytoplasmic isoform (cPML; see Fig. 1), thus suggesting a pivotal role of cPML in this pathway (Lin et al., 2004). It is presently unclear whether other PML nuclear isoforms could play any role in rescuing TGF-ß-dependent senescence and cell death. Interestingly, mRNA levels of cPML were induced by TGF-ß in

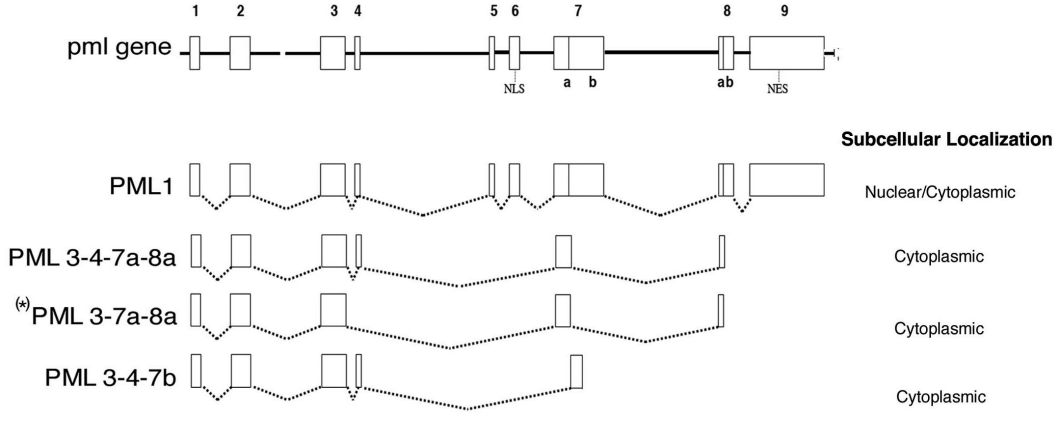

Fig. 1. Exon arrangement of *Pml* gene and cytoplasmic PML isoforms. Cellular localization is governed by the presence or absence of nuclear localization signals (NLS) and nuclear export sequence (NES) encoded by exon 6 and 9, respectively. Notably, PML1 possesses both domains. (*) PML3-7a-8a is the cytoplasmic isoform implicated in the regulation of TGF-ß signalling (Lin et al., 2004).

different cell types, thus suggesting that TGF-ß may control PML expression and/or splicing (Lin et al., 2004). Importantly, *Pml*$^{-/-}$ MEFs are defective in TGF-ß-mediated phosphorylation and nuclear translocation of Smad2/3, and these defects could be fully rescued by cPML expression (Lin et al., 2004). Nevertheless, it remains to be established whether depletion of endogenous cPML only in *Pml*$^{+/+}$ MEFs would result in impaired TGF-ß signalling. Interestingly, cPML physically associates with Smad2/3 and SARA (Lin et al., 2004). Furthermore, immunofluoresce and sucrose gradient-mediated fractionation revealed that cPML localized to early endosomes and that the TbRI/II-SARA localization to this subcellular compartment was compromised in Pml$^{-/-}$ cells. Similarly, PML-RARα is able to disrupt this complex, thus interfering with TGF-ß tumour suppressive signalling (Lin et al., 2004). Based on these observations, the authors concluded that cPML is essential for the efficient recruitment and assembly of the TGF-ß receptors/SARA/Smads complex. Altogether, this work has revealed an unexpected role for cPML in the modulation of the TGF-ß signalling. Nevertheless, further efforts are needed to answer several outstanding questions. For example, the status of the TGF-ß/cPML pathway in non-haematopoietic cancers, and in particular in metastatic solid tumours is still unknown. Finally, the absence of phenotypic overlapping between animal models lacking different components of the TGF-ß pathway and *Pml*$^{-/-}$ mice suggests that the role of cPML could be confined to specific tissues or pathological conditions (Shull et al., 1992; Kulkarni et al., 1993; Nomura and Li, 1998; Yang et al., 1998, 1999).

Another recent work (Seo et al., 2006) has demonstrated that the TG-interacting factor (TGIF), a negative regulator of the TGF-ß pathway, blocks cPML function and this results in inhibition of Smad2 phosphorylation and activation. Consistent with previous reports showing that the interaction between c-jun and TGIF is essential to inhibit TGF-ß-activated pathways (Pessah et al., 2001), c-jun$^{-/-}$ fibroblasts are impaired in TGIF-dependent inhibition of Smad2 phosphorylation. The authors also demonstrate that TGIF-dependent effects do not rely on its binding to Smad2 or on c-jun transcriptional activation. As PML physically interacts with c-jun (Salomoni et al., 2005) and modulates the TGF-ß pathway (Lin et al., 2004), the authors hypothesized that PML was involved in TGIF inhibitory activity. Indeed, cPML and TGIF interact in the nucleus, and this interaction is favoured by c-jun (Seo et al., 2006). The presence of a cPML-TGIF-c-jun trimeric complex inversely correlates with the sensitivity of cells to TGF-ß. TGIF appears to inhibit the formation of the cPML-SARA complex, which is required for efficient transduction of TGF-ß signalling. The authors conclude that TGIF-dependent nuclear sequestration of cPML might represent a possible mechanistic explanation for the observed inhibition. Indeed, cPML nuclear sequestration resulted in the destabilization of cPML-SARA complex and impairment of TGF-ß signalling (Seo et al., 2006). It remains to be established whether nuclear PML isoforms play any role in nuclear sequestration of cPML.

The importance of cytoplasmic PML within the TGF-ß pathway rise the intriguing and, so far, unexplored possibility that, from the cytoplasm, PML

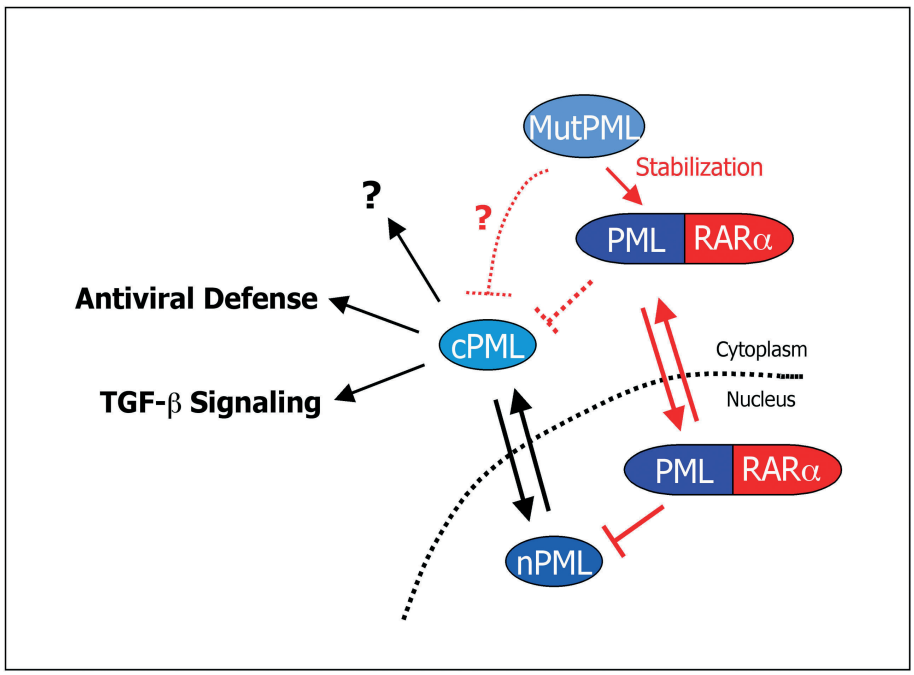

Fig. 2. cPML functions. cPML proteins may consist of cytoplasmic isoforms or nuclear isoforms exported to the cytoplasm. cPML has been implicated in the modulation of TGF-ß signalling and in the antiviral defence mechanism. It is however unclear if it can affect other cytoplasmic pathways. The APL-associated PML mutant MutPML stabilizes and potentiates PML-RARα, which on its own can be nuclear or cytoplasmic. PML in this scenario, it is plausible that both cytoplasmic PML-RARα and MutPML could influence cPML activity.

may directly or indirectly modulate signal transduction. In this regard, as components of the TGF-ß receptor complex are regulated through ubiquitylation (Attisano and Wrana, 2002), it would be interesting to determine whether cPML RING finger could act as an E3 ligase and which are its substrates.

Nuclear sequestration of cytoplasmic PML is emerging as an important regulatory mechanism (Seo et al., 2006). *Vice versa*, it would be of extreme interest to determine whether nuclear isoforms can be regulated through nuclear exclusion and if cytoplasmic localization of nuclear isoforms could affect TGF-ß-dependent signalling as well. Albeit important results have been achieved, a more substantial effort is needed in the future to gain more insights into this fascinating new research area.

Redistribution of PML to the cytoplasm during the cell cycle

PML-NBs undergo to a dramatic re-organization during the cell cycle: the number, the shape and the composition of nuclear bodies are profoundly altered during S and M phases (Koken et al., 1995; Terris et al., 1995; Everett et al., 1999; Dellaire et al., 2006a). A recent study reported that during mitosis PML redistributes to cytoplasmic domains called mitotic accumulation of PML proteins (MAPPs), which diverge for structure and composition from PML-NBs (Dellaire et al., 2006b). Notably, even in the early G1 phase of cell cycle a large portion of PML is found to reside in cytoplasmic MAPPs. This phenomenon is very likely due to relocalization of nuclear isoforms to the cytoplasm. It is however still unclear whether the NES-containing isoform, PML1, or cytoplasmic isoforms are functionally involved in this process. Interestingly, the co-localization between PML and Daxx, a well-known interphase PML-NB component, is lost during cell cycle. However, the exact function of cytoplasmic MAPPs and their contribution to cell cycle progression need to be further analyzed. Specifically, it is presently unclear whether MAPPs can bear cytoplasmic functions, for instance regulating translation (Cohen et al., 2001) or modulating TGF-ß signalling (Lin et al., 2004), or whether they simply represent a transient depot for the recycling of PML proteins until the mid-G1 reorganization of the PML-NB is completed.

Role of cytoplasmic PML in the cellular defence against viral infection

Pioneering studies conducted by different groups showed that PML levels were induced in response to antiviral interferon treatments (IFN) (Chelbi-Alix et al., 1995; Lavau et al., 1995), revealing that PML was a primary target of IFN (Stadler et al., 1995). As a matter of fact, IFN treatment caused the increase in both size and number of PML-NBs (Chelbi-Alix et al., 1995; Lavau et al., 1995; Stadler et al., 1995). The importance of PML in the viral response is outlined by the fact that many viruses have evolved different strategies in order to disrupt the PML-NB. Interestingly, arenaviruses encode a RING protein, Z protein, which binds PML and promotes its cytoplasmic redistribution. Once in the cytoplasm, PML and protein Z interfere with the function of the eukaryotic translation initiation factor eIF4E by reducing its affinity for 5' cap of mRNAs and, thereby, inhibiting translation (Kentsis et al., 2001). Interestingly, nuclear PML was previously shown to inhibit eIF4E by targeting to the PML-NB, thus suggesting that different PML isoforms could interfere with mRNA transport and translation (Cohen et al., 2001).

An elegant work by Turelli et al. demonstrated that cytoplasmic PML is part of the anti-viral cellular response during the early events of the retroviral life cycle, which spans from the cellular entry of the viral particles to the integration of the viral genome into the host genome. This part of the infection cycle is usually inefficient, as only a small portion of the viral particles entering the cells are able to successfully integrate. The integrase interactor 1 (INI-1) interacts with the HIV-1 integrase and is an essential subunit of the human SWI/SNF chromatin-remodelling complex (Turelli et al., 2001). At steady state, INI-1 presents a nuclear diffuse localization, while PML lies in punctuated PML-NBs. Subsequently, PML and INI-1 undergo a rapid but transient cytoplasmic relocation and accumulation in dense cytoplasmic bodies. The nucleus-cytoplasmic export was demonstrated to be exportin-dependent (Turelli et al., 2001). Importantly, it was found that PML/INI-1 colocalize in the cytoplasm with the incoming retroviral preintegration complex. This event appeared to be crucial for the anti-viral response mediated by PML. Indeed, nuclear sequestration of PML induced by using leptomycin B or arsenic trioxide, As_2O_3, greatly increased viral transduction efficiency (Turelli et al., 2001).

Altogether these lines of evidence suggest that PML is implicated in the cellular defence against viral infections. Interestingly, this is a property that appears common to other TRIM family members such as TRIM1, TRIM5 and TRIM22, thus suggesting that PML could interplay with other TRIMs during viral infection (Nisole et al., 2005; and references cited therein). Alternatively, there could be a degree of redundancy between different TRIMs, and this could explain the contradictory results obtained by testing viral infection efficiency in PML-deficient cells (Nisole et al., 2005; Everett et al., 2006). Finally, what remains unclear is how PML interferes with viruses that possess different replicative strategies. This awaits further investigation.

Cytoplasmic PML in tumours

cPML in solid tumours

Loss of PML expression is detected in tumours of both haematopoietic and epithelial origin (Gurrieri et al.,

2004a). In addition to this, old and new evidence indicates that PML could be found in the cytoplasm in human cancers, suggesting that its cytoplasmic localization may play a role in transformation. For instance, a number of studies revealed that PML is found in cytoplasmic granules in large number of hepatocellular carcinomas, thus suggesting a role for cytoplasmic PML in this disease (Terris et al., 1995; Chan et al., 1998). In addition, PML is found in the cytoplasm also in carcinomas of the skin (Condemine et al., 2006). It is still unclear whether this is due to changes in PML splicing towards cytoplasmic isoforms, mutations or whether nuclear isoforms could be excluded from the nucleus. Interestingly, PML was found mutated in a plasmacytoma cell line to generate a truncated protein, which accumulates in the cytoplasm and is able to delocalize nuclear PML (Zheng et al., 1998). In this respect, we have recently found that cytoplasmic forms of PML can induce the delocalization of nuclear PML to the cytoplasm and impair its ability to activate p53 (Bellodi et al., 2006a). It remains to be determined whether cPML-dependent delocalization of nuclear PML has an impact on p53 function in human cancer as well. This awaits further investigation.

cPML in APL

In a recent study conducted on a cohort of seventeen RA-resistant APL cases, two missense mutations were identified in the remaining *Pml* allele (Gurrieri et al., 2004b). The first DNA variation, Mut1, was a deletion, 1272delAG, in exon 5, identified in a 9-year-old female. The second mutation, Mut2, was a splice site mutation IVSG→A identified in a 19-year-old male that causes a frameshift in the coding frame and splices out exon 4 from the mature transcript. Interestingly, both mutations introduce a premature stop codon upstream the nuclear localization signal (NLS) sequence present in exon 6, so that, the resulting mutant PML proteins (Mut PML) accumulate in the cytoplasm (Gurrieri et al., 2004b). Notably, the mutations are associated with a very aggressive progression of the disease, and poor prognosis, indicating that MutPML proteins could contribute to leukaemogenesis. We showed that Mut PML does not cause cell death or block cell proliferation and accumulate in discrete cytoplasmic foci, referred to as PML cytoplasmic bodies (PML-CBs), which do not overlap with any other cytosolic organelle. Electron microscopy analysis revealed a close structural homology between PML-NB and PML-CB, suggesting that at least some components could be shared between the two structures. We also found that Mut PML colocalizes with PML-RARα in PML-CBs and is able to protect PML-RARα from RA-dependent degradation (Bellodi et al., 2006). More importantly, Mut PML can delay differentiation induced by pharmacological concentrations of RA in APL cells, thus suggesting it can play a role in transformation and in resistance to therapy (Bellodi et al., 2006).

Cytoplasmic PML-RARα

As abovementioned, the oncogenic fusion gene PML-RARα is the result of the t15;17 translocation of APL. Two major PML-RARa isoforms can be generated depending on the breakpoint (another one, called variable or bcr-2, is less common): bcr1 and bcr3, where bcr3 is shorter and lacks one of the two NLS (Huang et al., 1993; Vahdat et al., 1994). PML-RARα is able to inactivate the functions of both RARα and PML, thus blocking the differentiation and conferring a growth advantage to the leukemic cells, respectively. While in APL cells the architecture of PML-NB is disrupted, upon retinoic acid treatment, which induces the proteasome-dependent degradation of the fusion protein, PML-NBs are re-established, and this correlates with remission of the disease in patients (Zhu et al., 1999, 2001; Lallemand-Breitenbach et al., 2001; Salomoni and Pandolfi, 2002). PML-RARα ability to interfere with differentiation is believed to occur mainly through its transcriptional inhibitory function, which is exerted directly on promoters of genes mediating haematopoietic maturation. Nevertheless, we and others have shown that PML-RARα also localizes to the cytoplasm in both APL primary patient samples and cell lines (Kastner et al., 1992; Daniel et al., 1993; Koken et al., 1994; Khan et al., 2004; Bellodi et al., 2006b), thus indicating that it could also bear cytoplasmic functions. Furthermore, PML-RARα has been shown to undergo alternative splicing to produce several isoforms, some of which are predicted to localize to the cytoplasm (Pandolfi et al., 1992). In line with these observations, another study reported that proteolytic cleavage of PML-RARα by neutrophyl elastase (NE), an enzyme strongly expressed in promyelocytes, causes cytoplasmic accumulation of the PML portion (Lane and Ley, 2003). Interestingly, NE activity is required for APL leukaemogenesis in mice (Lane and Ley, 2003). However, it is unclear whether cleavage of PML-RARα represents a loss or gain of function event.

We have shown that a ΔNLS PML-RARα mutant (cPML-RARα) is able to inhibit both RA-dependent transcription and differentiation (Bellodi et al., 2006b). Furthermore, cPML-RARα appeared less sensitive to RA-depended proteasomal degradation, indicating that cytoplasmic localization may promote stabilization that, in turn, may possibly lead to increase resistance to RA-based therapies. Previous report showed that RXRα, the transcriptional partner of RARα, colocalizes with PML-RARα in cytoplasmic speckles. However, the importance of this event was not investigated (Perez et al., 1993). In our work, we were able to confirm that RXRα colocalizes with cPML-RARα in the cytoplasm. In addition, a cPML-RARα mutant defective in RXRα binding was partially impaired in its ability to inhibit RA-dependent transcription. This suggests that RXRα binding is important, but does not represent an essential requirement, and other mechanisms are involved. In line with this, another report showed that the binding to

RXRα is not required for full differentiation arrest and immortalization of primary mouse haematopoietic progenitors, at least in the context of full-length bcr1 PML-RARα (Zhu et al., 2005). Interestingly, the bcr3 PML-RARα isoform, which lacks one NLS and is more cytoplasmic compared to bcr1, efficiently delocalizes RXRα in both cell lines and primary patient samples (Bellodi et al., 2006b). It would be very important to test whether transformation induced by bcr3 more greatly relies on its ability to delocalize RXRα.

It is plausible that cPML-RARα may interfere with PML functions. Indeed, APL cells are poorly responsive to TGF-ß treatments and bcr-1 PML-RARα has been shown to interfere with cPML functions (Lin et al., 2004). Consistent with these observations, treatment with As_2O_3, which induces the degradation of the fusion protein, rescues the inhibition of TGF-ß signalling (Lin et al., 2004). Mechanisms underlying the degradation of PML-RARa have not been fully investigated. An elegant work by Scaglioni et al. described a pivotal role of casein kinase 2 (CK2) in nuclear PML turnover. Considering that CK2 is a nuclear-matrix-associated kinase that is frequently activated in human cancer (Scaglioni et al., 2006), it is also conceivable that cytoplasmic variants of PML and PML-RARα may be subjected to a different regulation. Indeed, as aforementioned, we found that cPML-RARα is less sensitive to RA-dependent down-modulation when compared to the wild type form. Furthermore, as bcr3 PML-RARα lacks the CK2 phosphorylation site, it would be very interesting to test whether this has any effect on its stability.

Concluding remarks

Altogether the evidence discussed in this review indicates that both PML and PML-RARα bear cytoplasmic functions, which could possibly modulate the genesis and progression of human malignancies. Nevertheless, several major questions need to be addressed in the future:

1) What is the role of cPML in tumours? In particular, cPML expression will have to be analyzed in a large number of tumour types and to be correlated with survival. Furthermore, a correlation between levels of cPML and the status of TGF-ß signalling in cancer is still missing. Interestingly, cytoplasmic delocalization of another protein involved in APL, NPM, has been found in a large number of myeloid leukaemias and has become a prognostic marker (Grisendi and Pandolfi, 2005).

2) What is the effect of APL-associated Mut PML and cPML-RARα on cPML functions?

3) Can other cytoplasmic TRIMs play a role in modulating the function of cPML and cPML-RARα?

4) Is the balance between different PML isoforms altered in human disease? In this respect, isoform-specific antibodies for cytoplasmic isoforms are urgently needed (see also point 1).

Finally, this field is in extreme need of animal models to determine the function of different PML isoforms *in vivo*.

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References

Attisano L. and Wrana J.L. (2002). Signal transduction by the TGF-beta superfamily. Science 296, 1646-1647.

Bellodi C., Kindle K., Bernassola F., Cossarizza A., Dinsdale D., Melino G., Heery D. and Salomoni P. (2006a). A cytoplasmic PML mutant inhibits p53 function. Cell Cycle 5, 2688-2692.

Bellodi C., Kindle K., Bernassola F., Dinsdale D., Cossarizza A., Melino G., Heery D. and Salomoni P. (2006b). Cytoplasmic function of mutant promyelocytic leukemia (PML) and PML-retinoic acid receptor-alpha. J. Biol. Chem. 281, 14465-14473.

Bernardi R., Guernah I., Jin D., Grisendi S., Alimonti A., Teruya-Feldstein J., Cordon-Cardo C., Simon M.C., Rafii S. and Pandolfi P.P. (2006). PML inhibits HIF-1alpha translation and neoangiogenesis through repression of mTOR. Nature 442, 779-785.

Cainarca S., Messali S., Ballabio A. and Meroni G. (1999). Functional characterization of the Opitz syndrome gene product (midin): evidence for homodimerization and association with microtubules throughout the cell cycle. Hum. Mol. Genet. 8, 1387-1396.

Cao T., Duprez E., Borden K.L., Freemont P.S. and Etkin L.D. (1998). Ret finger protein is a normal component of PML nuclear bodies and interacts directly with PML. J. Cell Sci. 111 (Pt 10), 1319-1329.

Chan J.Y., Chin W., Liew C.T., Chang K.S. and Johnson P.J. (1998). Altered expression of the growth and transformation suppressor PML gene in human hepatocellular carcinomas and in hepatitis tissues. Eur. J. Cancer 34, 1015-1022.

Chelbi-Alix M.K., Pelicano L., Quignon F., Koken M.H., Venturini L., Stadler M., Pavlovic J., Degos L. and de The H. (1995). Induction of the PML protein by interferons in normal and APL cells. Leukemia 9, 2027-2033.

Cohen N., Sharma M., Kentsis A., Perez J.M., Strudwick S. and Borden K.L. (2001). PML RING suppresses oncogenic transformation by reducing the affinity of eIF4E for mRNA. EMBO J. 20, 4547-4559.

Condemine W., Takahashi Y., Zhu J., Puvion-Dutilleul F., Guegan S., Janin A. and de The H. (2006). Characterization of endogenous human promyelocytic leukemia isoforms. Cancer Res. 66, 6192-6198.

Daniel M.T., Koken M., Romagne O., Barbey S., Bazarbachi A., Stadler M., Guillemin M.C., Degos L., Chomienne C. and de The H. (1993). PML protein expression in hematopoietic and acute promyelocytic leukemia cells. Blood 82, 1858-1867.

de The H., Lavau C., Marchio A., Chomienne C., Degos L. and Dejean A. (1991). The PML-RAR alpha fusion mRNA generated by the t(15;17) translocation in acute promyelocytic leukemia encodes a functionally altered RAR. Cell 66, 675-684.

Dellaire G., Ching R.W., Dehghani H., Ren Y. and Bazett-Jones D.P.

(2006a). The number of PML nuclear bodies increases in early S phase by a fission mechanism. J. Cell Sci. 119, 1026-1033.

Dellaire G., Eskiw C.H., Dehghani H., Ching R.W. and Bazett-Jones D.P. (2006b). Mitotic accumulations of PML protein contribute to the re-establishment of PML nuclear bodies in G1. J. Cell Sci. 119, 1034-1042.

Derynck R., Akhurst R.J. and Balmain A. (2001). TGF-beta signaling in tumor suppression and cancer progression. Nat. Genet. 29, 117-129.

Everett R.D., Lomonte P., Sternsdorf T., van Driel R. and Orr A. (1999). Cell cycle regulation of PML modification and ND10 composition. J. Cell Sci. 112 (Pt 24), 4581-4588.

Everett R.D., Rechter S., Papior P., Tavalai N., Stamminger T. and Orr A. (2006). PML contributes to a cellular mechanism of repression of herpes simplex virus type 1 infection that is inactivated by ICP0. J. Virol. 80, 7995-8005.

Fagioli M., Alcalay M., Pandolfi P.P., Venturini L., Mencarelli A., Simeone A., Acampora D., Grignani F. and Pelicci P.G. (1992). Alternative splicing of PML transcripts predicts coexpression of several carboxy-terminally different protein isoforms. Oncogene 7, 1083-1091.

Fagioli M., Alcalay M., Tomassoni L., Ferrucci P.F., Mencarelli A., Riganelli D., Grignani F., Pozzan T., Nicoletti I. and Pelicci P.G. (1998). Cooperation between the RING + B1-B2 and coiled-coil domains of PML is necessary for its effects on cell survival. Oncogene 16, 2905-2913.

Goddard A.D., Borrow J., Freemont P.S. and Solomon E. (1991). Characterization of a zinc finger gene disrupted by the t(15;17) in acute promyelocytic leukemia. Science 254, 1371-1374.

Grignani F., Ferrucci P.F., Testa U., Talamo G., Fagioli M., Alcalay M., Mencarelli A., Peschle C., Nicoletti I. and Pelicci P.G. (1993). The acute promyelocytic leukemia-specific PML-RAR alpha fusion protein inhibits differentiation and promotes survival of myeloid precursor cells. Cell 74, 423-431.

Grisendi S. and Pandolfi P.P. (2005). NPM mutations in acute myelogenous leukemia. N. Engl. J. Med. 352, 291-292.

Guo A., Salomoni P., Luo J., Shih A., Zhong S., Gu W. and Paolo Pandolfi P. (2000). The function of PML in p53-dependent apoptosis. Nat. Cell. Biol. 2, 730-736.

Gurrieri C., Capodieci P., Bernardi R., Scaglioni P.P., Nafa K., Rush L.J., Verbel D.A., Cordon-Cardo C. and Pandolfi P.P. (2004a). Loss of the tumor suppressor PML in human cancers of multiple histologic origins. J. Natl. Cancer Inst. 96, 269-279.

Gurrieri C., Nafa K., Merghoub T., Bernardi R., Capodieci P., Biondi A., Nimer S., Douer D., Cordon-Cardo C., Gallagher R. and Pandolfi P.P. (2004b). Mutations of the PML tumor suppressor gene in acute promyelocytic leukemia. Blood 103, 2358-2362.

Horn E.J., Albor A., Liu Y., El-Hizawi S., Vanderbeek G.E., Babcock M., Bowden G.T., Hennings H., Lozano G., Weinberg W.C. and Kulesz-Martin M. (2004). RING protein Trim32 associated with skin carcinogenesis has anti-apoptotic and E3-ubiquitin ligase properties. Carcinogenesis 25, 157-167.

Huang W., Sun G.L., Li X.S., Cao Q., Lu Y., Jang G.S., Zhang F.Q., Chai J.R., Wang Z.Y. and Waxman S. (1993). Acute promyelocytic leukemia: clinical relevance of two major PML-RAR alpha isoforms and detection of minimal residual disease by retrotranscriptase/polymerase chain reaction to predict relapse. Blood 82, 1264-1269.

Inman G.J. and Hill C.S. (2002). Stoichiometry of active smad-transcription factor complexes on DNA. J. Biol. Chem. 277, 51008-51016.

Jensen K., Shiels C. and Freemont P.S. (2001). PML protein isoforms and the RBCC/TRIM motif. Oncogene 20, 7223-7233.

Kakizuka A., Miller W.H. Jr., Umesono K., Warrell R.P. Jr, Frankel S.R., Murty V.V., Dmitrovsky E. and Evans R.M. (1991). Chromosomal translocation t(15;17) in human acute promyelocytic leukemia fuses RAR alpha with a novel putative transcription factor, PML. Cell 66, 663-674.

Kastner P., Perez A., Lutz Y., Rochette-Egly C., Gaub M.P., Durand B., Lanotte M., Berger R. and Chambon P. (1992). Structure, localization and transcriptional properties of two classes of retinoic acid receptor alpha fusion proteins in acute promyelocytic leukemia (APL): structural similarities with a new family of oncoproteins. EMBO J. 11, 629-642.

Kentsis A., Dwyer E.C., Perez J.M., Sharma M., Chen A., Pan Z.Q. and Borden K.L. (2001). The RING domains of the promyelocytic leukemia protein PML and the arenaviral protein Z repress translation by directly inhibiting translation initiation factor eIF4E. J Mol. Biol. 312, 609-623.

Khan M.M., Nomura T., Chiba T., Tanaka K., Yoshida H., Mori K. and Ishii S. (2004). The fusion oncoprotein PML-RARalpha induces endoplasmic reticulum (ER)-associated degradation of N-CoR and ER stress. J. Biol. Chem. 279, 11814-11824.

Klugbauer S. and Rabes H.M. (1999). The transcription coactivator HTIF1 and a related protein are fused to the RET receptor tyrosine kinase in childhood papillary thyroid carcinomas. Oncogene 18, 4388-4393.

Koken M.H., Linares-Cruz G., Quignon F., Viron A., Chelbi-Alix M.K., Sobczak-Thepot J., Juhlin L., Degos L., Calvo F. and de The H. (1995). The PML growth-suppressor has an altered expression in human oncogenesis. Oncogene 10, 1315-1324.

Koken M.H., Puvion-Dutilleul F., Guillemin M.C., Viron A., Linares-Cruz G., Stuurman N., de Jong L., Szostecki C., Calvo F., Chomienne C. et al. (1994). The t(15;17) translocation alters a nuclear body in a retinoic acid-reversible fashion. EMBO J. 13, 1073-1083.

Kulkarni A.B., Huh C.G., Becker D., Geiser A., Lyght M., Flanders K.C., Roberts A.B., Sporn M.B., Ward J.M. and Karlsson S. (1993). Transforming growth factor beta 1 null mutation in mice causes excessive inflammatory response and early death. Proc. Natl. Acad. Sci. USA 90, 770-774.

Lallemand-Breitenbach V., Zhu J., Puvion F., Koken M., Honore N., Doubeikovsky A., Duprez E., Pandolfi P.P., Puvion E. and Freemont P. (2001). Role of promyelocytic leukemia (PML) sumolation in nuclear body formation, 11S proteasome recruitment, and As_2O_3-induced PML or PML/retinoic acid receptor alpha degradation. J. Exp. Med. 193, 1361-1371.

Lane A.A. and Ley T.J. (2003). Neutrophil elastase cleaves PML-RARalpha and is important for the development of acute promyelocytic leukemia in mice. Cell 115, 305-318.

Lavau C., Marchio A., Fagioli M., Jansen J., Falini B., Lebon P., Grosveld F., Pandolfi P.P., Pelicci P.G. and Dejean A. (1995). The acute promyelocytic leukaemia-associated PML gene is induced by interferon. Oncogene 11, 871-876.

Le Douarin B., Zechel C., Garnier J.M., Lutz Y., Tora L., Pierrat P., Heery D., Gronemeyer H., Chambon P. and Losson R. (1995). The N-terminal part of TIF1, a putative mediator of the ligand-dependent activation function (AF-2) of nuclear receptors, is fused to B-raf in the oncogenic protein T18. EMBO J. 14, 2020-2033.

Le X.F., Yang P. and Chang K.S. (1996). Analysis of the growth and transformation suppressor domains of promyelocytic leukemia gene, PML. J. Biol. Chem. 271, 130-135.

Lin H.K., Bergmann S. and Pandolfi P.P. (2004). Cytoplasmic PML function in TGF-beta signalling. Nature 431, 205-211.

Lin H.K., Bergmann S. and Pandolfi P.P. (2005). Deregulated TGF-beta signaling in leukemogenesis. Oncogene 24, 5693-5700.

Meroni G. and Diez-Roux G. (2005). TRIM/RBCC, a novel class of 'single protein RING finger' E3 ubiquitin ligases. Bioessays 27, 1147-1157.

Morris-Desbois C., Bochard V., Reynaud C. and Jalinot P. (1999). Interaction between the Ret finger protein and the Int-6 gene product and co-localisation into nuclear bodies. J. Cell Sci. 112 (Pt 19), 3331-3342.

Nisole S., Stoye J.P. and Saib A. (2005). TRIM family proteins: retroviral restriction and antiviral defence. Nat. Rev. Microbiol. 3, 799-808.

Nomura M. and Li E. (1998). Smad2 role in mesoderm formation, left-right patterning and craniofacial development. Nature 393, 786-790.

Pandolfi P.P., Alcalay M., Fagioli M., Zangrilli D., Mencarelli A., Diverio D., Biondi A., Lo Coco F., Rambaldi A., Grignani F., Rochette-Egly C., Gaube M.P., Chambon P. and Pelici P.G. (1992). Genomic variability and alternative splicing generate multiple PML/RAR alpha transcripts that encode aberrant PML proteins and PML/RAR alpha isoforms in acute promyelocytic leukaemia. EMBO J. 11, 1397-1407.

Pearson M., Carbone R., Sebastiani C., Cioce M., Fagioli M., Saito S., Higashimoto Y., Appella E., Minucci S. and Pandolfi P.P. and Pelici P.G. (2000). PML regulates p53 acetylation and premature senescence induced by oncogenic Ras. Nature 406, 207-210.

Perez A., Kastner P., Sethi S., Lutz Y., Reibel C. and Chambon P. (1993). PMLRAR homodimers: distinct DNA binding properties and heteromeric interactions with RXR. EMBO J. 12, 3171-3182.

Pessah M., Prunier C., Marais J., Ferrand N., Mazars A., Lallemand F., Gauthier J.M. and Atfi A. (2001). c-Jun interacts with the corepressor TG-interacting factor (TGIF) to suppress Smad2 transcriptional activity. Proc. Natl. Acad. Sci. USA 98, 6198-6203.

Quaderi N.A., Schweiger S., Gaudenz K., Franco B., Rugarli E.I., Berger W., Feldman G.J., Volta M., Andolfi G., Gilgenkrantz S., Marion R.W., Hennekam R.C., Opitz J.M., Muenke M., Ropers H.K. and Ballabio A. (1997). Opitz G/BBB syndrome, a defect of midline development, is due to mutations in a new RING finger gene on Xp22. Nat. Genet. 17, 285-291.

Rego E.M., Wang Z.G., Peruzzi D., He L.Z., Cordon-Cardo C. and Pandolfi P.P. (2001). Role of promyelocytic leukemia (PML) protein in tumor suppression. J. Exp. Med. 193, 521-529.

Reymond A., Meroni G., Fantozzi A., Merla G., Cairo S., Luzi L., Riganelli D., Zanaria E., Messali S., Cainarca S., Guffanti A., Minucci S., Pelicci P.G. and Ballabio A. (2001). The tripartite motif family identifies cell compartments. EMBO J. 20, 2140-2151.

Salomoni P. and Pandolfi P.P. (2002). The role of PML in tumor suppression. Cell 108, 165-170.

Salomoni P., Bernardi R., Bergmann S., Changou A., Tuttle S. and Pandolfi P.P. (2005). The promyelocytic leukemia protein PML regulates c-Jun function in response to DNA damage. Blood 105, 3686-3690.

Scaglioni P.P., Yung T.M., Cai L.F., Erdjument-Bromage H., Kaufman A.J., Singh B., Teruya-Feldstein J., Tempst P. and Pandolfi P.P. (2006). A CK2-dependent mechanism for degradation of the PML tumor suppressor. Cell 126, 269-283.

Schweiger S., Foerster J., Lehmann T., Suckow V., Muller Y.A., Walter G., Davies T., Porter H., van Bokhoven H., Lunt P.W., Traub P. and Ropers H.H. (1999). The Opitz syndrome gene product, MID1, associates with microtubules. Proc. Natl. Acad. Sci. USA 96, 2794-2799.

Seo S.R., Ferrand N., Faresse N., Prunier C., Abecassis L., Pessah M., Bourgeade M.F. and Atfi A. (2006). Nuclear retention of the tumor suppressor cPML by the homeodomain protein TGIF restricts TGF-beta signaling. Mol Cell 23, 547-559.

Shi Y. and Massague J. (2003). Mechanisms of TGF-beta signaling from cell membrane to the nucleus. Cell 113, 685-700.

Shull M.M., Ormsby I., Kier A.B., Pawlowski S., Diebold R.J., Yin M., Allen R., Sidman C., Proetzel G., Calvin D., Annunciata N. and Doetschman T. (1992). Targeted disruption of the mouse transforming growth factor-beta 1 gene results in multifocal inflammatory disease. Nature 359, 693-699.

Siegel P.M. and Massague J. (2003). Cytostatic and apoptotic actions of TGF-beta in homeostasis and cancer. Nat. Rev. Cancer 3, 807-821.

Stadler M., Chelbi-Alix M.K., Koken M.H., Venturini L., Lee C., Saib A., Quignon F., Pelicano L., Guillemin M.C., Schindler C. et al. (1995). Transcriptional induction of the PML growth suppressor gene by interferons is mediated through an ISRE and a GAS element. Oncogene 11, 2565-2573.

Takahashi M., Inaguma Y., Hiai H. and Hirose F. (1988). Developmentally regulated expression of a human "finger"-containing gene encoded by the 5' half of the ret transforming gene. Mol. Cell Biol. 8, 1853-1856.

Terris B., Baldin V., Dubois S., Degott C., Flejou J.F., Henin D. and Dejean A. (1995). PML nuclear bodies are general targets for inflammation and cell proliferation. Cancer Res. 55, 1590-1597.

Trotman L.C., Alimonti A., Scaglioni P.P., Koutcher J.A., Cordon-Cardo C. and Pandolfi P.P. (2006). Identification of a tumour suppressor network opposing nuclear Akt function. Nature 441, 523-527.

Tsukazaki T., Chiang T.A., Davison A.F., Attisano L. and Wrana J.L. (1998). SARA, a FYVE domain protein that recruits Smad2 to the TGFbeta receptor. Cell 95, 779-791.

Turelli P., Doucas V., Craig E., Mangeat B., Klages N., Evans R., Kalpana G. and Trono D. (2001). Cytoplasmic recruitment of INI1 and PML on incoming HIV preintegration complexes: interference with early steps of viral replication. Mol. Cell. 7, 1245-1254.

Vahdat L., Maslak P., Miller W.H. Jr, Eardley A., Heller G., Scheinberg D.A. and Warrell R.P. Jr (1994). Early mortality and the retinoic acid syndrome in acute promyelocytic leukemia: impact of leukocytosis, low-dose chemotherapy, PMN/RAR-alpha isoform, and CD13 expression in patients treated with all-trans retinoic acid. Blood 84, 3843-3849.

Wang Z.G., Delva L., Gaboli M., Rivi R., Giorgio M., Cordon-Cardo C., Grosveld F. and Pandolfi P.P. (1998). Role of PML in cell growth and the retinoic acid pathway. Science 279, 1547-1551.

Wu J.W., Fairman R., Penry J. and Shi Y. (2001). Formation of a stable heterodimer between Smad2 and Smad4. J. Biol. Chem. 276, 20688-20694.

Yang X., Letterio J.J., Lechleider R.J., Chen L., Hayman R., Gu H., Roberts A.B. and Deng C. (1999). Targeted disruption of SMAD3 results in impaired mucosal immunity and diminished T cell responsiveness to TGF-beta. EMBO J. 18, 1280-1291.

Yang X., Li C., Xu X. and Deng C. (1998). The tumor suppressor SMAD4/DPC4 is essential for epiblast proliferation and mesoderm induction in mice. Proc. Natl. Acad. Sci. USA 95, 3667-3672.

Zheng P., Guo Y., Niu Q., Levy D.E., Dyck J.A., Lu S., Sheiman L.A. and Liu Y. (1998). Proto-oncogene PML controls genes devoted to MHC class I antigen presentation. Nature 396, 373-376.

Zhong S., Muller S., Ronchetti S., Freemont P.S., Dejean A. and Pandolfi P.P. (2000a). Role of SUMO-1-modified PML in nuclear body formation. Blood 95, 2748-2752.

Zhong S., Salomoni P. and Pandolfi P.P. (2000b). The transcriptional role of PML and the nuclear body. Nat. Cell Biol. 2, E85-90.

Zhu J., Gianni M., Kopf E., Honore N., Chelbi-Alix M., Koken M., Quignon F., Rochette-Egly C. and de The H. (1999). Retinoic acid induces proteasome-dependent degradation of retinoic acid receptor alpha (RARalpha) and oncogenic RARalpha fusion proteins. Proc. Natl. Acad. Sci. USA 96, 14807-14812.

Zhu J., Lallemand-Breitenbach V. and de The H. (2001). Pathways of retinoic acid- or arsenic trioxide-induced PML/RARalpha catabolism, role of oncogene degradation in disease remission. Oncogene 20, 7257-7265.

Zhu J., Zhou J., Peres L., Riaucoux F., Honore N., Kogan S. and de The H. (2005). A sumoylation site in PML/RARA is essential for leukemic transformation. Cancer Cell 7, 143-153.

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