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Functions and dys-functions of promyelocytic leukemia protein PML

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Abstract

The promyelocytic leukemia protein PML has been previously recognized as a critical and essential regulator of a broad number of cellular functions. At nuclear level PML forms the PML-nuclear bodies, where it can sequester and influence the post-translational modification of a wide number of proteins, ultimately affecting their regulative role in DNA transcription. In such a way, PML acts as a key player in strategic cellular activities like as the antiviral defense, in the regulation of the cell cycle, in senescence and programmed cell death. In addition, PML can redistribute also at cytoplasmic level, where it associates to the endoplasmic reticulum or is recruited to mitochondrial-associated membranes. Here it can interact with key cellular proteins like as p53 and influence cell metabolism, mitochondrial calcium upload and autophagy. Altogether, all these findings depict PML as a protein able to exert a widespread action mainly focused on pro-apoptotic and cytostatic activities. Anyway, presence of "Janus-like" pro-tumoral behaviors have been reported, prompting for further investigation to better dissect and highlight all the possible roles that PML can assume in the different physiological or pathological environments. In this review, we discuss the role of PML in multiple cellular functions and pathologic scenarios and summarize the players that control PML protein both at nuclear and at cytoplasmic level.

1 History/involvement in pathology

PML acquired relevance when it was recognized to be involved as a causative genetic lesion in the etiology of acute promyelocytic leukemia (APL). In this leukemia, the differentiation of promyelocyte is stopped by the inactivation of the nuclear receptor for retinoic acid (retinoic acid receptor, RARα): the gene for this key transcription factor undergoes a reciprocal and balanced t(15;17) chromosomal translocation (de The et al. 1991; Goddard et al. 1991; Kakizuka et al. 1991). This event in over 95% of cases produces two fusion genes, PML-RARα and RARα-PML, while in a spare number of cases the translocation involves other genes such as the promyelocytic leukemia zinc finger gene, the nucleophosmin gene and others.

In APL, and in the presence of physiological concentrations of all-trans retinoic acid (ATRA), these chimeric proteins behave as dominant negative genes, stopping the terminal differentiation of promyelocytes. On the other hand, the pharmacological concentration of ATRA can reactivate the RARα-dependent transcription, strongly improving the prognosis of a previously fatal illness. Interestingly, the structure of the different fusion proteins predicts the response to therapy with ATRA: the not-RARα-part of the chimeric proteins influences the binding and dimerization of the RARα moiety, so the PML-RARα translocation are associated with positive response to ATRA therapy, and

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favorable prognosis, while translocations involving different genes lead to little or no response (Grisolano et al. 1997; He et al. 1998).

Although the interference with RARα dependent transcription is the main strategy used by t(15;17) chromosomal translocation to promote tumorigenesis, the disruption of *PML* gene furnished the first evidence, further reinforced by the use of Pml⁻/⁻ mice, that PML can exert an important role in controlling several key cell functions (Fig. 1).

2 Protein structure

Located on the q arm of chromosome 15, the 53 kbp *PML* gene is a single gene member of the tripartite motif (TRIM) family. It is organized into 9 exons, and through alternative splicing of its C-terminal exons, it can give rise up to 7 known PML protein isoforms (Fig. 2). All the isoforms share the same N terminal part that spans 418 amino acids, where the domains that characterize the PML protein are located. In particular, at the N-terminus of the protein the RBCC/TRIM motif is present, which, following a scheme quite common in the majority of TRIM proteins, includes three zinc-binding domains, a RING (really interesting new gene)

finger, a B-box type 1 and a B-box type 2, and a coiled-coil region (Jensen et al. 2001). The RBCC domain controls not only the capability of PML to dimerize and multimerize (Reymond et al. 2001) but also its interaction with a huge number of partners.

In addition to the N-terminus, which is common to all the isoforms, PML protein harbors other domains that can be present only in some of the isoforms. They include nuclear localization signal (NLS), nuclear export signal (NES) and several sites for post-translational modifications, such as phosphorylation, ubiquitination, and SUMO-interacting motifs (SIM) for SUMOylation (Schmitz and Grishina 2012). PML isoforms are distinguished into seven classes on the bases of their length, and numbered accordingly, where PML I is the longest and PML VII the shortest. Typically, NLS, located on exon 6, is expressed in all the isoforms from I to VI, (with the exception of PML IVc). These isoforms are also termed as nuclear isoforms and can localize to the nucleus and organize multiprotein complexes termed PML-nuclear bodies (PML-NB). Also, PML I is the only nuclear PML that contains a NES and consequently is expressed both at cytoplasmic and nuclear level. On the other hand, the cytoplasmic isoforms PML VII and PML IVc are devoid of NLS and expressed only at cytoplasmic level. It is to note
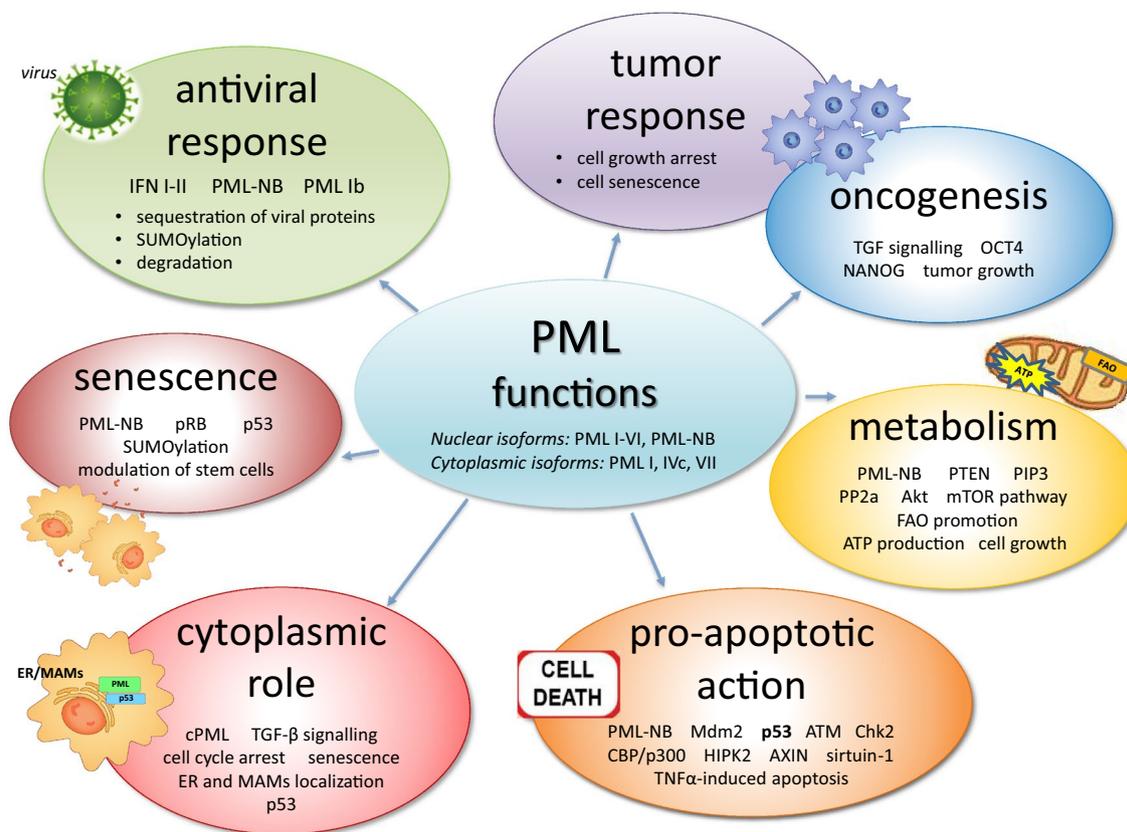
Fig. 1 Overall representation of PML functions at cellular level

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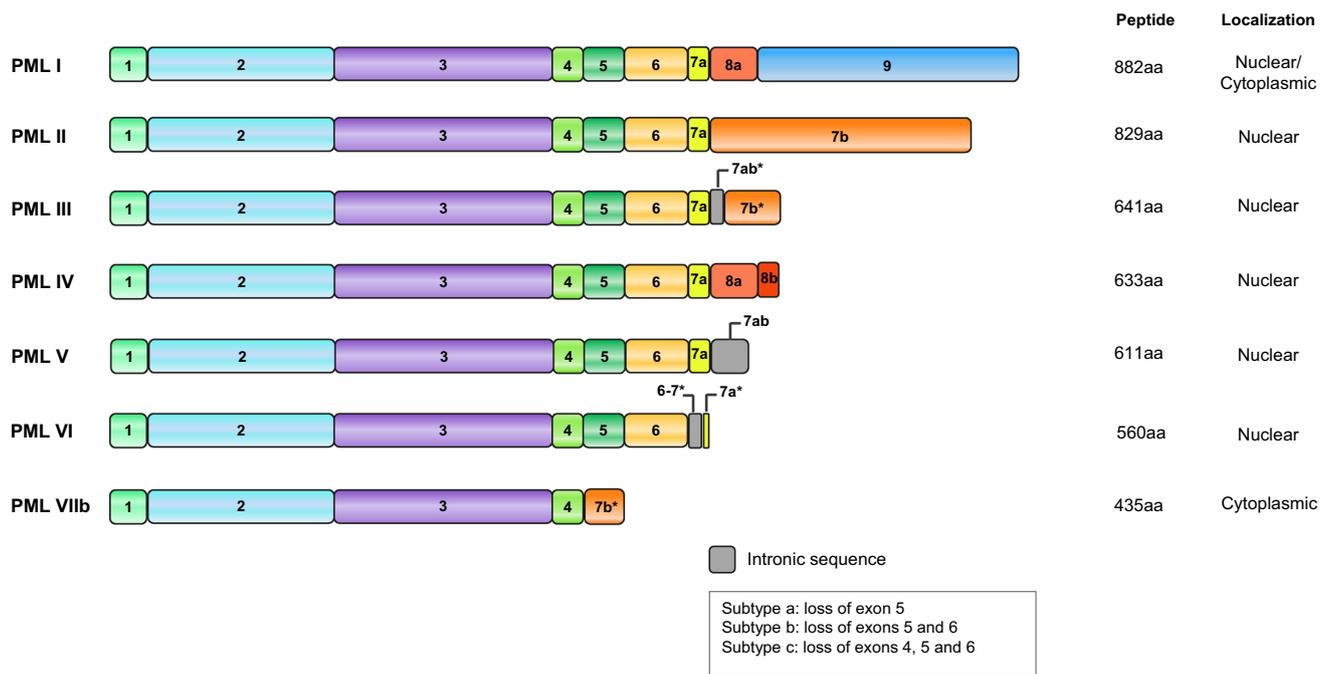
Fig. 2 Schematic of PML isoforms and their localizations. The primary PML transcript is organized into 9 exons and can be alternatively spliced to generate up to seven known class of isoforms that share exons 1–4. It is to note that for each class up to three variants (a, b and c) may be described. The exons are shown as colored boxes. Isoforms III, V and VI contain introns colored in gray that are spliced out in other nuclear isoforms. The asterisks mark the regions from partial exons or introns

that for each class up to three variants (a, b and c) may be described, each one missing specific parts of exons, with the potential generation of new cytoplasmic forms (Jul-Larsen et al. 2010).

3 PML-nuclear bodies

PML isoforms containing the NLS enter into the nucleus and distribute between the nucleoplasm and the nuclear matrix. The presence of RBCC/TRIM motifs allows PML proteins to form homo- and hetero multimers, creating a physical partition of the nucleoplasm that assumes the shape of spheroid, and donut-like bodies, showing variable diameters (0.1–1 µm) and numbers (5–30) according to the cell state. These PML-nuclear bodies (PML-NB) behave as a scaffold able to recruit a wide number of partner proteins, which quickly and transiently associate with and dissociate from PML-NB scaffold, which on the contrary represents a stable structure, with a renewal rate in the range of minutes (Weidtkamp-Peters et al. 2008). PML represents the main contributor of PML-NB, which can be disrupted by PML loss or presence of PML-RARα, while ATRA treatment contributes to their reconstitution (Koken et al. 1994). For a long time, PML-NB assembly seemed to depend upon SUMOylation at SIM domain followed by non-covalent interaction between the SUMO moiety of SUMOylated PML and the

SIM sequence of other PML partners (Shen et al. 2006). A recent evidence indicates that the requirement for an initial ROS-mediated oxidative step resulted in covalent bonds between PML monomers and occurred also in the absence of SUMOylation (Sahin et al. 2014).

PML-NB can interact with a wide number and variety of proteins that up to now can account for more than 200 interactors (which can be found at https://thebiogrid.org/). These interactions permit PML-NB to function according to a sponge-like mechanism, which allows for temporary sequester numerous nuclear transcription factors and other nuclear proteins. This represents the molecular base that allows PML to influence several key cellular pathways like apoptosis, senescence, autophagy, cell division, and behave as a tumor suppressor (Lallemand-Breitenbach and de The 2010). The recruited partner proteins principally depend on two factors: the nature of stress experienced by the cell and the protein SUMOylation. At PML-NB level, the interaction with the recruited proteins can occur in several different ways. This can imply merely protein inactivation through sequestration, or more complex mechanism like facilitation of protein–protein reciprocal interaction often associated with post-translational modifications followed by activation or inactivation. These interactions lead to PML regulation of transcriptional activity and maintenance of genome integrity, acting, for example, at DNA damage repair complexes or at telomere endings. PML-NB can also interact with other

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nuclear components: they can hold mRNA molecules and associate to be telomeric or centromeric chromatin, and with histones or specific genomic loci (Ching et al. 2013).

4 PML involvement in immunity functions and antiviral response

The TRIM family has been involved in the control of viral infection, and consistently, the PML gene promoter contains target sequences for interferon regulatory factors (El Bougrini et al. 2011; Scherer and Stamminger 2016). After viral aggression, cells produce and release interferons, which trigger cellular antiviral response upon binding to specific receptors and activation of DNA transcription. Interferons type I and II strongly increase transcription of PML and SUMO proteins and also of several NB-associated proteins, like Sp100 (Speckled protein of 100 kDa), Sp140, Sp110, ISG20, PA28, and death-associated protein (DAXX) (Xu and Roizman 2017). This allows an interferon-dependent increase of NB number, which in turn consents sequestration of viral proteins with diminution of their activity at the nuclear level, followed by increased SUMOylation and degradation. PML IV can interact with the 3D polymerase of encephalomyocarditis virus (EMCV) and to sequester it into PML-NB, with the ultimate effect of reducing the viral replication effort (El McHichi et al. 2010; Maroui et al. 2011). This suggests that PML-NB complex can behave as defense station able to slow down and adsorb the viral assault to nuclear structures, with the goal of reducing the viral particles production. Consistently, *Pml* KO mice, which not express PML protein, show a reduced level of apoptosis induced by interferon I and II, and by cytokines like as interleukin 6 (Lunardi et al. 2011).

Also, several viruses, such as the herpes simplex virus type 1 (HSV-1), oppose the PML-NB sequestration: the immediate-early protein of HSV-1 can induce the disassembly of NBs. In turn, a cytoplasmic variant of PML lacking exons 5 and 6 (PML Ib) can bind the infected cell protein 0 of HSV-1 directly in the cytoplasm, preventing its nuclear action and greatly reducing HSV-1 replication (McNally et al. 2008). The preventive block of viral proteins at cytoplasmic level seems to be a more general strategy for PML. Given the relocation of nuclear PML isoforms in the cytoplasm operated by the respiratory syncytial virus, the lymphocytic choriomeningitis virus (LCMV) and the human immunodeficiency virus type 1 (HIV-1), PML can inhibit the activity of viral proteins also in the cytoplasm. As an example, PML and the integrase interactor 1 of HIV-1 redistribute from nucleus to cytoplasm, in a manner dependent upon exportin-1. At cytoplasm level, PML is again able to show an antiviral activity, binding and interfering with the HIV-1 preintegration complex (Turelli et al. 2001).

PML also cooperates with p53 during viral infections. Infact, poliovirus infection induces PML phosphorylation through a mitogen-activated protein kinase (MAPK) pathway and this triggers an increased PML SUMOylation and p53 recruitment within the NBs, leading to p53 activation and induction of p53 target genes, Mdm2 and Nox. This event induces the process of apoptosis in poliovirus-infected cells and the inhibition of viral replication (Pampin et al. 2006).

5 PML involvement in tumor response

In APL, the t(15;17) chromosomal translocation leads to the expression of a PML-RARα chimeric protein which can translocate into the nucleus, originate abnormal heterodimers, suppress the formation of PML-NB, contributing to cell transformation. While the t(15;17) chromosomal translocation is characteristic of APL, the downregulation of PML protein, followed by reduction of expression of PML-NB, occurs with the different extent and variable incidence in a wide number of tumors. This includes hematological tumors different from APL like diffuse large cell lymphoma and follicular lymphoma, or solid tumors, which include high percentages of lung, colon, breast, prostate, CNS and others cancers (Gurrieri et al. 2004). The major mechanism relies not on genetic alteration or reduction of transcription, but on increase of PML proteolytic inactivation. Proteolysis occurs through SUMOylation, but interestingly this step has been proposed to be after PML oxidative damage. Furthermore, several studies, using both overexpression of PML and $pml^{-/-}$ genetic models, showed that while overexpression of PML was joined to cell growth arrest and cell senescence, absence or functional knock out of the protein enhanced cell growth and tumorigenesis (Wang et al. 1998). The importance of PML functional inactivation is related to the cytostatic pathways controlled by PML, in particular pathways involved in senescence, apoptosis and cell replication (Bernardi and Pandolfi 2003; Giorgi et al. 2010).

6 PML and pro-apoptotic signaling

Many molecular pathways regulated by PML are involved in the apoptotic process. PML and PML-NB control the levels of nuclear p53 by interacting with and sequestering Mdm2, which is the major p53 E3 ubiquitin ligase and acts decreasing p53 levels by targeting it to proteasomal degradation. Also, DAXX, which is a principal PML interactor (Khelifi et al. 2005; Salomoni and Khelifi 2006), has been shown to control p53 ubiquitylation by inhibiting MDM2 degradation (Tang et al. 2006; Song et al. 2008). Furthermore, PML controls genotoxic-induced cell death via ataxia-telangiectasia

mutated (ATM) and checkpoint kinase 2 (Chk2). ATM is a serine-threonine kinase, activated by DNA double-strand breaks, which has among its targets Chk2, while Chk2 is a DNA damage-induced kinase which, recruited at PML-NB, phosphorylates p53 preventing its Mdm2-mediated proteolysis (Yang et al. 2002).

After UV treatment, and more in general after DNA damage, several proteins like the acetyltransferases CBP/p300 and the homeodomain-interacting protein kinase-2 (HIPK2), along with the tumor suppressor AXIN, relocate into PML-NB where they can acetylate or phosphorylate p53. These post-translational modifications prevent p53 degradation, induce its transcriptional activity, leading to apoptosis or senescence. On the other hand, variation in the amount of PML present into NB remodels the PML-NB composition and can also recruit p53-inactivating enzymes like as the NAD-dependent deacetylase sirtuin-1 (Langley et al. 2002), indicating that the regulative activities of NBs are strongly dependent on their structure and composition, which further strengthen the importance of PML downregulation observed in a wide number of tumors. PML affects cell death induction also through p53-independent pathways. The specific nuclear PML IV sensitizes cells to TNFα-induced apoptosis. This also occurs in the p53-negative Saos-2 cell line like in other cell models, triggering the death receptor-dependent apoptotic pathway which recruits caspase-8, -7, and -3. Consistently, the loss of PML function, which occurs in *Pml* KO mouse embryonic fibroblasts renders cells resistant to TNFα-induced apoptosis (Wu et al. 2003). Furthermore, PML IV acts as a repressor of the transactivation function of NF-kB by interacting with p65/RelA and preventing its binding to the NF-kB target sequence (Kuwayama et al. 2009).

7 PML involvement in senescence

Senescence is a biological process that, at cellular level, involves an irreversible exit from the cell cycle, and linked to morphological alterations including modification of cell size and shape, enlargement and vacuolization of the cytoskeleton organization and nuclear morphology. This cytostatic mechanism frequently occurs because of telomere shortening, which ultimately triggers a DNA damage response. It is not surprising that senescence shares some pathways involved in the regulation of programmed cell death, although being very different from it. Consistently, tumor cells must alter a common set of genes involved in both the mechanisms to acquire an unlimited replicative potential. The main proteins involved in senescence are pRB and p53, recruited as pivotal protagonists of DNA damage response and both SUMOylated at PML-NB level. In particular, at PML-NB, in addition to SUMO-1 SUMOylation of p53, a specific ROS-dependent SUMOylation operated on lysine

K386 by SUMO-2/3 can occur (Li et al. 2006). While these activities lead to p53 stabilization and senescence triggering, they require different cofactors, so that it can also elicit diverse p53 transcription patterns, suggesting that the PML-NB can display elevate substrate specificity and finely regulate a wide subset of different nuclear and cellular responses (Stindt et al. 2011).

Another interesting aspect of the regulation of senescence operated by PML is the modulation of stem cell compartment. Among the distinctive features of aging, there is the loss of renewal of different tissues, which in turn contributes to a progressive reduction of function in several organs. This fall in tissue renewal is often due to a negative regulation in the number of the staminal precursor in different cell populations. Accordingly, the influence exerted by PML on cell death regulation can also play a role in tissue development, through the modulation of the size of stem cell compartments. Although *Pml* knockout animals show an almost regular development and life span, several pieces of evidence based on *Pml* knockout animals and cells showed that brain cortex development (Regad et al. 2009), hematopoietic compartment homeostasis (Ito et al. 2008) and mammary gland development (Li et al. 2009) can be affected by PML deficiency. The different regulation of cell death, at the level of stem cell compartment, could explain, respectively, the reduction of neuron number at cortical level and the increased amounts of hematopoietic or mammary committed progenitors (Pan et al. 2009) in bone marrow or mammary glands. In particular, in hematopoietic system (Ito et al. 2012), an unbalancing of the equilibrium between lymphoid versus myeloid compartments, can be attributed to the diminution of lymphoid precursors in favour of myeloid ones (Rossi et al. 2008).

8 PML involvement in metabolism

Phosphatidylinositol-4,5-bisphosphate 3-kinase (PI-3k) and Akt are kinase enzymes that start crucial pathways promoting cell survival, in particular, coordinating cell growth and proliferation with the availability of energetic resources, like extracellular glucose. Also, the PI-3k/Akt/mammalian target of rapamycin (mTOR) can also regulate cellular autophagy, which is a pivotal mechanism in reestablishing nutrient levels under stress condition or starvation, as it is regarded to be the main pathway in promoting cancer growth (Porta et al. 2014).

PML behaves as an inhibitory mechanism for the main end-points of these pathways, but acting at its very beginning, on the level of the trigger molecule phosphatidylinositol (3, 4, 5) trisphosphate (PIP3). In fact, PML promotes the activity of PTEN, a lipid phosphatase able to downregulate PIP3 levels. In addition, PML also stimulates the protein

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phosphatase PP2a to decrease the extent of Akt activity by colocalizing both into PML-NB (Trotman et al. 2006) and sequestering mTOR protein into NB, reducing the extent of cell autophagic action. This greatly reduces the ability of the cell, in particular of cancer cells, to overpass the stress due to the shortage of essential nutrients. Consistently, in $Pml^{-/-}$ cell lines, autophagy is greatly enhanced (see also below). On the other hand, PML can increase energy production, and consequently in cell survival, through the promotion of fatty acid oxidation (FAO). FAO can provide metabolic substrates which can fuel ATP production, contribute to resistance to metabolic stresses and, ultimately, to healthy and cancer cell growth. Coherently, FAO, ATP production, and cell growth are improved upon PML overexpression, while they are all reduced in $Pml^{-/-}$ models, indicating an unexpected pro-survival role for PML (Carracedo et al. 2012).

Akt, PTEN, and mTOR also exert a strong regulative role on stem cell compartment. In fact, depletion of PTEN leads to enhanced self-renewal without exhaustion in neural stem cells, while absence of PTEN leads to mTOR-dependent exhaustion of hematopoietic stem cells (HSC) (Yilmaz et al. 2006). Being the Akt/mTOR pathway decisive for HSC pool dimension, the negative regulatory role exerted by PML on the Akt/mTOR pathway has a negative effect on neural stem cell pools, and in turn a positive role on HSC maintenance (see above).

9 Is PML involved in oncogenesis?

We have seen above that PML can activate an altered form of TGF-signaling, contributing to tumor growth. Several aspects of PML action have been reported to have a supporting role in cancer. Truncated forms of PML have been identified in plasmacytoma, with possible dominant negative effect, and PML mutations, causing premature protein termination, and it also can interact in APL with PML-RARα and inhibit p53 antitumoral activity (Bellodi et al. 2006). Glycolysis has been reported to be the main method of energy production in long-term HSCs (Simsek et al. 2010), but Ito et al. (2012) showed that the PML-regulated PPAR-δ and FAO profoundly affect quiescence, repopulating capacity and regulates asymmetric division of HSC. In glioblastoma multiforme, arsenic trioxide strongly reduces the number of glioblastoma stem cells and not of non-tumor stem cells. The action of the chemotherapy is mediated by the disruption of PML followed by apoptosis in glioma stem cells (Zhou et al. 2015).

PML is highly expressed in undifferentiated embryonic stem cells (ESC) and preserves ESC naive pluripotency. Its ablation induces significant changes in ESC morphology, global gene expression profile, and lineage specification

decision (Hadjimichael et al. 2017). Besides, PML has been reported to activate Oct4 gene (Chuang et al. 2011) and participate in the regulation of OCT4 and NANOG (Liang et al. 2008). Taken together, these observations suggest that PML can behave as a general regulator of cell fate and stemness, both in normal and tumor tissues.

10 Cytoplasmic PML

Cytoplasmic localization of PML has been described to occur in two different manners, one implying a constitutive association of cytoplasmic isoforms (cPML) to ER or mitochondria, the other seeing a temporary cytoplasmic relocation of nuclear isoforms, due to cell cycle dynamics or viral infection.

During the M-phase of the cell cycle, together with the dissolution of nuclear structure, nuclear PML involved in the structure of PML-NB can find an alternative and temporary localization into specific cytoplasmic complexes, where it can be transiently stocked. During the G1 phase, PML moves to the reassembled nuclear structure where it participates in the reconstitution of NB (Dellaire et al. 2006). Up to now, it is not clear if this temporary cytoplasmic distribution of PML isoforms entails active participation in cell cycle dynamics. Also, during viral infection, nuclear PML can redistribute to the cytoplasm to express its antiviral proficiency binding to viral components at cytoplasm level, to reduce viral protein transduction and translation (Kentsis et al. 2001; Turelli et al. 2001) (see above).

In addition to the redistribution of nuclear isoforms, cytoplasmic localization of PML can be supported by specific isoforms, expressing NES or lacking NLS. In the cytoplasm, PML can carry on its tumoral suppressive action operating in association with different subcellular organs. Several reports highlighted as PML can control TGF-β signaling, which can induce cell cycle arrest and senescence, and is a known up-regulator of the two cyclin inhibitors p15 and p21. TGF-β signaling is strongly impaired in Pml KO mice (Lin et al. 2004), but this deficiency can be restored only upon transfection of cytosolic and not with nuclear isoforms of PML, suggesting that cytoplasmic PML can carry on specific antitumor activity. This view is further reinforced by supporting evidence which shows that nuclear sequestration of cPML, operated by TG-interacting factor and c-Jun, inhibits TGF-β signaling (Seo et al. 2006). This interferes with the formation of the complex formed by TGF-β, its receptors, Smad protein and the adaptor protein, SARA, the Smad anchor for receptor activation, whose phosphorylation is regulated by cPML. Paradoxically, in the presence of dysfunctional TGF-signaling like in prostate cancer, the cytoplasmic localization of PML I and its nuclear export carrier, exportin 1, promotes epithelial to mesenchymal transition.

Cytoplasmic PML I localization was found in patients with poor prognosis, strengthening the activator role of PML on TGF-β signaling, up to constituting a pro-tumoral attitude in a pathological environment (Buczek et al. 2016). Presence of cytoplasmic mutants of PML has been reported (Gurrieri et al. 2004). cPML can also have additional regulative roles, and in particular, cPML was shown to be associated to and to be enriched at ER and mitochondria-associated membranes (MAMs) (Pinton et al. 2011). The latter are specialized contact sites between ER and mitochondria (Giorgi et al. 2015), which organize an integrated scaffold of proteins able to warrant not only structural tethering among the vicinal structures, but also integrate mitochondrial and reticular physiology, regulating phospholipids and cholesterol exchange, and being involved in autophagy and mitophagy control (Giorgi et al. 2015). Furthermore, MAMs represent a special regulatory site in calcium transfer from ER and cytosol (Marchi et al. 2017). In fact, MAMs can fulfill the role of specialized buffering sites, relying on an enriched presence of the low-affinity VDAC1 channel (Danese et al. 2017). VDAC can create a calcium microdomain at MAM level, which stimulates the function of the MCU transporter and other related proteins, allowing for fast calcium transfer into mitochondria (Missiroli et al. 2017). Far from being just a mere buffering activity, the calcium transfer operating at MAMs can influence and shape the calcium concentration variations occurring at the perireticulum space, regulating the action of IP3Rs and SERCA pumps, whose activity is influenced by vicinal calcium concentrations. A complex equilibrium, based on the calcium-mediated interplay and feedback loops, occurs between ER and MAMs, which is further tightened by the fact that calcium entry in the mitochondria affects oxidative phosphorylation, which in turn provides ATP to fuel SERCA activity. Notably, the calcium entry in the mitochondria regulates mitochondrial activity only inside a defined physiological range of calcium concentrations. Excess of calcium entry triggers the dissipation of mitochondrial potential, the activation of mitochondrial transition pore and, at the end, the intrinsic pathway of apoptosis (Pedriali et al. 2017). ER calcium pumps assume a central role in programmed cell death. In particular, IP3R3, whose activity results not to be inhibited in a feedback loop by high calcium concentrations, results to be a major player in celcium-dependent apoptosis (Kuchay et al. 2017). Some recent papers of Giorgi's group highlighted the role of PML in the regulation of cell death acting at MAMs level. In particular, Giorgi et al. (2010) identified cPML as a part of a macromolecular complex also including IP3Rs, the pivotal protein kinase Akt and the protein phosphatase PP2a. Here, PML regulates the Akt- and PP2a-dependent IP3R phosphorylation. Tumor-dependent downregulation of PML, or cPML somatic mutations can behave as a brake on calcium release from ER, strongly reducing the cell ability

to undergo apoptosis. In addition, Missiroli et al. (2016) showed that cytoplasmic p53 is also enriched in the MAM fraction and that PML needs the p53 presence to localize at MAMs. In fact, PML was not found at MAMs in *p53* KO MEFs, whereas p53 exhibited a normal subcellular distribution in *Pml*$^{-/-}$ MEFs. In addition, loss of p53 or PML was associated with an increased autophagy, which greatly increased tumor fitness, providing essential metabolic precursors to sustain tumor growth, also in the presence of stress and growth-limiting conditions. Notably, autophagy was turned to basal levels also in *p53* KO MEFs by overexpressing an ER-targeted PML version. These results indicate that cPML can have a tumor suppressive function at MAMs acting as a calcium and autophagy modulator, at least in the latter downstream of p53 (Fig. 3).

11 Conclusions

The protein PML is involved in a wide number of cellular functions. Its nuclear forms can exert a fundamental role in the different key activities, like antiviral response, DNA damage response, and control of senescence and cell death. To exert these functions, nuclear PML evolved the capacity to regulate the activity of a surprisingly high number of proteins acting at the nuclear level, recruiting them inside specialized multiprotein complexes, and ultimately modulating their regulatory activity on DNA transcription. More recent researches highlighted that PML protein can also be present at the cytoplasmic level, on the surface of endoplasmic reticulum and at the MAMs. Cytoplasmic PML can again have antiviral functions or can modulate cell growth and death. In particular, influencing the activity of a high number of proteins joined to MAMs, PML can deeply alter cell autophagy and metabolism, or the overall mitochondrial activity, up to determining the cell fate. Although there is impressive amount of data on PML action, numerous questions remain to be answered, claiming for further effort in elucidating the dark sides of this protein. First of all, PML showed to be not only an established pro-apoptotic and oncosuppressive player, but also, sometimes, an oncogenic factor. It remains to be clarified in which tissues and under which regulative actions PML can change its role so deeply. Again, information is lacking on the specific role of the different isoforms, and of their post-translational modifications, both in the physiologic activities of PML like in its pro-tumoral shift. In particular, the ability of PML to act, at mitochondrial level, regulating autophagy and calcium entry appears to be a very interesting clue in the regulation of cell survival.

Fig. 3 Representation of PML function at MAM level. PML can regulate Ca^{2+} transfer onto mitochondria through the regulation of IP3R3 phosphorylation level. This in turn influences energy production and several ATP-dependent cellular functions. PML localization and functions at MAM level can be regulated by p53 protein during apoptotic induction
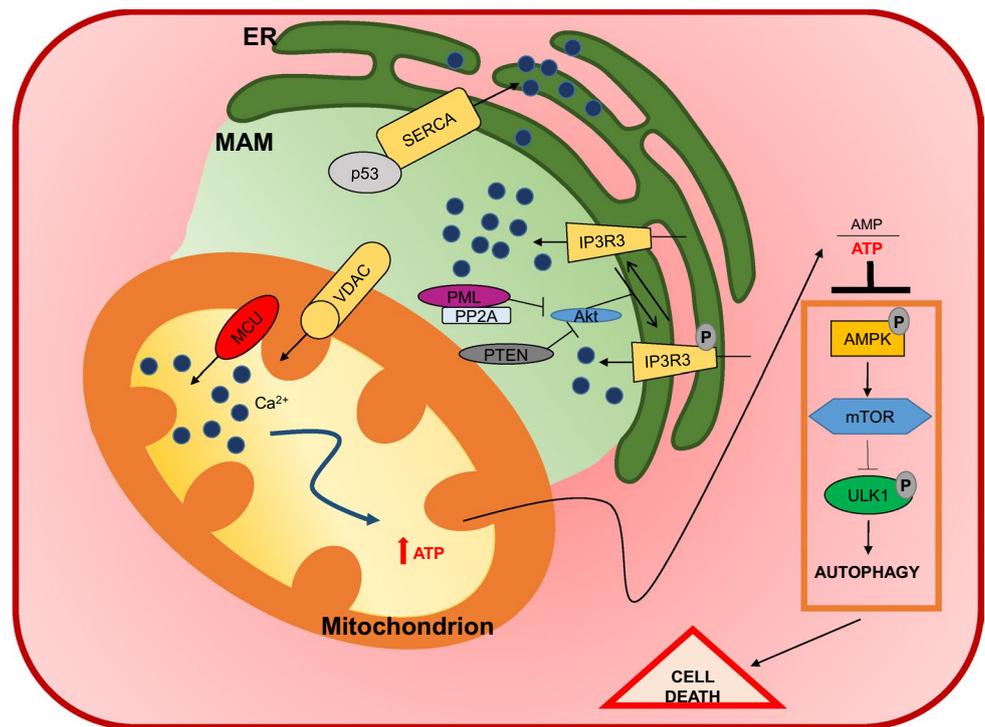
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