MINI-REVIEW

Promyelocytic Leukemia Gene Functions and Roles in Tumorigenesis

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Abstract

The promyelocytic leukemia (*PML*) gene is a gene known to be a tumor suppressor, although recent data suggest that it has a dual function in tumorigenesis. It was initially discovered in acute promyelocytic leukemia (APL) in which a t(15; 17) chromosomal translocation fused it to the retinoic acid receptor alpha (*RARα*). It has been shown to be involved in various types of cancer. It has at least 6 nuclear isoforms and a cytoplasmic type with different characteristics. Its multiple functions in growth inhibition, apoptosis induction, replicative senescence, inhibition of oncogenic transformation, and suppression of migration and angiogenesis have made it a therapeutic target for cancer therapy. However, its dual role in the process of tumorigenesis has made this field challenging. In this review, we discuss *PML* structure, functions and expression in tumors.

Keywords: *PML* - cancers - structure - nuclear bodies - functions and- physiological roles

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Introduction

The promyelocytic leukemia (*PML*) gene was initially discovered in acute promyelocytic leukemia (APL) in which a t(15; 17) chromosomal translocation fused it to the retinoic acid receptor alpha (*RARα*) (de The H, 1990; Kakizuka et al., 1991). The chimeric protein which is synthesized as a result of this translocation (*PML-RARα*) blocks the differentiation of hematopoietic progenitor cells (Nisole et al., 2013). In normal cells, PML forms matrix-associated domains, named PML nuclear bodies (PML-NBs). In APL cells, as a result of expression of the chimeric protein, NBs are scattered as microspeckles, which is thought to contribute to leukemogenesis (Dyck et al., 1994). Treatment of APL patients with arsenic trioxide (As_2O_3) results in the degradation of the fusion protein through its PML moiety and reverses the disease phenotype (Nisole et al., 2013). *PML* has been suggested as an important factor in cell fate decisions, that is, self-renewal *vs*. differentiation, adaptation *vs*. apoptosis or senescence (Mazza and Pelicci, 2013). PML protein has tumor suppressive functions via diverse biologic mechanisms such as growth inhibition, apoptosis induction, replicative senescence, inhibition of oncogenic transformation, and suppression of migration and angiogenesis (Chen et al., 2012). One of the important anticancer functions of PML is to stabilize the tumor suppressor p53 by sequestering Mdm2 to the nucleolus. This permits p53 to activate transcription of growth suppressive targets like p21 (Gamell et al., 2014). In addition, *PML* is a p53 target gene that functions downstream of p53 to facilitate its antiproliferative roles (de Stanchina et al., 2004). *PML* expression has been shown to be downregulated in different types of human tumors with a frequent correlation with tumor progression (Chen et al., 2012). In addition, aberrant degradation of *PML* has been demonstrated in various tumors through ubiquitination-dependent mechanisms (Chen et al., 2012). Recently, there are growing evidences concerning the requirement of *PML* in cancer stem cell (CSC) maintenance in both hematopoietic cancers and solid tumors (Zhou and Bao, 2014). CSCs are a population of cells within tumors capable of self-renewal, either by symmetric or asymmetric cell division, with the exclusive ability to reproduce malignant tumors indefinitely (Tabarestani and Ghafouri-Fard, 2012). As CSCs are appropriate targets for specific anti cancer therapies, elucidation of mechanisms by which *PML* controls CSC pathways are important.

PML Structure

PML gene is located on 15q22. According to the original nomenclature defined by Jensen et al., (Nisole et al., 2013) this gene has nine exons. It has six nuclear isoforms (*PML*I to *PML*VI) and one cytoplasmic isoform (*PML*VII) which are generated by alternative splicing from a single *PML* gene (Jensen et al., 2001). Other splice variants include *PML* VIII (*PML*-IIG), *PML* XI (*PML*-IA), *PML* XII (*PML*-IVA), *PML* XIII (*PML*-IIA) and *PML* XIV (*PML*-VIB) (http://www.uniprot.org/uniprot/P29590). The *PML* protein has three cysteine-rich zinc-binding domains, a RING finger, two B-boxes (B1 and B2) and a predicteda-helical Coiled-Coil domain. These domains together form the RBCC motif (de Visser and Coussens, 2006). All of the mentioned *PML* isoforms

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contain the N-terminal region including the RBCC/TRIM motif. This motif has been shown to be crucial for PML nuclear body (NB) formation and PML homodimerization via the coiled-coiled domain. PML isoforms differences are either in the central region or in the C-terminal region, as a result of alternative splicing in 4-9 exones (Nisole et al., 2013). Different partners and specific role of each isoform is determined by the differences in the C terminal region of each isoform (Tamura, 2006). Among six isoforms, only *PML*I has the nuclear export signal (NES) (amino acids 704-713/exon 9), compatible with the nuclear and cytoplasmic distribution of this isoform. *PML*I to *PML*V contain the small ubiquitin modifier (SUMO) Interacting Motif (SIM). The SIM hydrophobic core is near to specific serines which are substrates for the Casein Kinase-2 (CK2). Numerous stress circumstances that activate CK2, for instance osmotic shock and UV irradiation, make PML prone to proteasomal degradation. Consequently, inhibition of CK2 prohibits stress-induced PML degradation. In addition, a CK2-activating signal (i.e., osmotic shock) can stimulate PML ubiquitination. So it has been hypothesized that CK2 phosphorylation causes PML ubiquitination via an unidentified ubiquitin ligase. It has been shown that the CK2 phosphorylation residues are within a large SIM and phosphorylation of these residues inhibits PML binding to sumoylated proteins (Stehmeier and Muller, 2009; Chang et al., 2011). *PML* phosphomimetic mutant has been shown to have an extended half-life so acts as a super tumor suppressor. In addition, cell based assays have shown that this mutant induces stronger senescence and apoptotic effects compared with wild type *PML*. Aberrant activation of CK2 has been demonstrated in some non-small cell lung cancer (NSCLC) cell lines and patient samples, which is associated with decreased PML protein levels (Shen et al., 2006; Scaglioni et al., 2008).

Post-translational Modification in PML

Ubiquitination

Ubiquitination causes subsequent proteasomal degradation. It is mediated by several molecules named RNF4, UHRF1, UBE3A/E6AP, KLHL20-based E3 ligase complex, SIAH1 or SIAH2. Ubiquitination by KLHL20-based E3 ligase complex needs CDK1/2-mediated phosphorylation at Ser-518 which is recognized by prolyl-isopeptidase PIN1. PIN1-catalyzed isomerization facilitates PML interaction with KLHL20. RNF4 catalyses 'Lys-6'-, 'Lys-11'-, 'Lys-48'- and 'Lys-63'-linked polyubiquitination in a manner which is polysumoylation-dependent (Fanelli et al., 2004; Tatham et al., 2008; Geoffroy et al., 2010; Guan et al., 2013).

Sumoylation

Sumoylation is important in regulation of PML stability and many PML functions including dissociation of the transcription factors from PML-NBs, PML ability to regulate apoptosis and its anti-viral activities. It is also indispensable for maintaining proper PML-NBs structure, integrity and normal function, recruitment of components of PML-NBs, the turnover and maintenance of PML in PML-NBs. Sumoylation on Lys-65, Lys-160 and Lys-490 residues is necessary for nuclear body formation. Sumoylation on Lys-160 is a requirement for sumoylation on Lys-65. Lys-65 and Lys-160 sumoylation is mediated by PISA1 and PIAS2. PIAS1-mediated sumoylation of PML promotes its interaction with CSNK2A1/CK2 and phosphorylation at Ser-565 which in turn induces its ubiquitin-mediated degradation. Sumoylation at Lys-490 by RANBP2 is necessary for the accurate gathering of PML-NBs. DNA damage induces its sumoylation whereas some but not all viral infections can stop sumoylation (Kamitani et al., 1998; Best et al., 2002; Shen et al., 2006; Maiuri et al., 2009; Hattersley et al., 2011; Kim et al., 2011; Rabellino et al., 2012; Satow et al., 2012). In addition to the covalent conjugation of SUMO to PML, the non-covalent interaction of SUMO with the SUMO Interacting Motif (SIM) of PML is necessary for the integrity and normal function of PML-NBs (Ishov et al., 1999; Shen et al., 2006).

PML Nuclear Bodies

PML nuclear bodies are matrix-associated domains first discovered in the early 1960s. They have been shown to recruit a wide range of proteins in which they may be sequestered, modified or degraded (Lallemand-Breitenbach and de The, 2010). These structures consist of spheres of 0.1-1.0 μm in diameter found in most cell lines and many tissues. They belong to the nuclear matrix, which regulate several nuclear functions, such as DNA replication, transcription, or epigenetic silencing (Bernardi and Pandolfi, 2007). The morphology of PML-NBs is dynamically altered during the cell cycle in response to cellular stresses (Ishov et al., 1999). One of the most important proteins recruited in *PML* bodies is DAXX, which is a strong repressor of transcription and modulator of apoptosis (Lallemand-Breitenbach and de The, 2010). In addition, a ubiquitin-like protein named SUMO is recruited in PML bodies. PML conjugation by SUMO has been shown to be crucial for recruitment of other partners (Koken et al., 1994). It has been demonstrated that the tumor suppressive activity of PML may depend, at least partially, on its capacity to mediate the accurate arrangement of the PML-NBs as mutations in SUMO binding motif or RING domain of PML result in apoptosis defects (Silzle et al., 2004). SUMO binding motif is in *PML* exon 7, which is included in most *PML* isoforms and is always lost in the PML-RARα oncoprotein of APL (Shen et al., 2006). PML-RARα fusion protein fails to aggregate in NB-like structures most probably as a result of loss of SUMO binding motif. It also acts as a dominant negative *PML* mutant in prompting the disruption of the PML-NB in the APL blast (Lallemand-Breitenbach et al., 2008). In most cell lines and also *in vivo*, PML has been demonstrated to predominantly have diffuse nuclear localization, not associated with the nuclear matrix or NBs (Salomoni et al., 2012). Arsenic trioxide as well as many DNA-damage-activated kinases can modulate PML distribution (Lallemand-Breitenbach and de The, 2010). More than 160 proteins have been shown to be associated with PML and the list of them is growing. More than half

of the PML-NB proteins are nuclear, another third of them shuttles actively between nucleus and cytoplasm, whereas other components are mainly cytoplasmatic (Van Damme et al., 2010).

PML Functions

PML functions as the scaffold of PML-NBs to recruit other proteins. This process is regulated by SUMO-mediated modifications. In addition, association of PML with PML-NBs is important in many central cellular processes such as tumor suppression, transcriptional regulation, apoptosis, senescence, DNA damage response, and viral defense mechanisms. The nuclear isoforms in cooperation with SATB1 are play role in local chromatin-loop remodeling and gene expression regulation at the MHC-I locus. Cytoplasmic PML functions in the regulation of the TGF-beta signaling pathway. Table 1 describes specific roles of splice variants.

Induction of Apoptosis

PML is a growth/tumor suppressor crucial for induction of apoptosis by various apoptotic stimuli (Wu et al., 2003). *PML* transduce a range of growth suppressive signals and regulates cellular senescence and programmed cell death. This role has been thought to be mostly mediated by *PML* nuclear splice variants, but it is becoming apparent that cytoplasmic localization of PML can also influence growth suppression and cell death (Salomoni et al., 2012). *PML* has been shown to be required for Fas- and caspase-dependent DNA damage-induced apoptosis in splenocytes. It is essential for the induction of apoptosis by Fas, tumor necrosis factor α (TNFα), ceramide, interferon (INF) α, INFβ, and INFγ (Wang et al., 1998b). In addition, forced expression of *PML* in rat embryo fibroblasts has induced a caspase-independent cell death (Quignon et al., 1998). *PML* is also a transcriptional repressor of NF-κB by interacting with RelA/p65 and prevents its binding to the cognate enhancer. As NF-κB activation is a mechanism to prevent cancer cell death in many tumors, its repression by *PML* contributes to apoptosis (Wu et al., 2003). In brief, *PML* affects important tumor suppressive (pRb) and oncogenic pathways (AKT) via interaction with PP1 and PP2A phosphatases in the nucleus and the cytoplasm (Salomoni et al., 2012). *PML* role in apoptosis is performed via interactions with different proteins as well as cell organelles (Table 2).

Regulation of cellular senescence

Senescence is described as a characteristic feature of primary mammalian cells, in which after a specific number of passages in culture, they experience a permanent growth arrest. Senescence is caused by the progressive erosion of telomeres following each cell division (Cooke and Smith, 1986). Telomere length is preserved by telomerase that is ubiquitously expressed during embryonic development. This enzyme is only expressed in a few adult cell types, mainly stem cells. Alternative lengthening of telomeres (ALT) is another mechanism by which telomere length is maintained by homologous recombination independent of telomerase activity. A hallmark of ALT cell lines and tumors is the presence of specialized PML-NBs, termed ALT-associated PML bodies (APBs) (Yeager et al., 1999). APBs contain telomeric DNA, telomere binding proteins and also a variety of DNA replication, recombination and repair factors. Large APBs are seen in only a minority of cycling ALT cells, perhaps as a result of their enrichment during the G2 phase of the cell cycle (Yeager et al., 1999; Wu et al., 2000). APBs have two types of DNA double-strand break repair and homologous recombination factors, the Rad50/Mre11/NBS1 complex and Rad51/Rad52, in addition to the replication factor A (RPA), the helicase BLM and the telomeric repeat-binding factors TRF1 andTRF2. APBs are considered as a site for actively replicating telomeres in the S/G2 phase of the cell cycle. Nearly all APB proteins are sumoylated. Association of the SUMO ligase SMC5/6 complex with APBs is necessary for TFR1/2 sumoylation and cell survival. *PML*

Table 1. Specific Roles of *PML* Isoforms

Isoforms	Specific Role
PML I	interacts with NLRP3
	interacts with herpes simplex virus-1 (HHV-1) ICP0
PML II	required for efficient IFN-gamma induced MHC II gene transcription via regulation of CIITA
	interacts with human adenovirus 2 E1A
	stimulates E1A-dependent transcriptional activation
PML III	represses human foamy virus (HFV) transcription by complexing the HFV transactivator, bel1/tas
	exhibits antiviral activity against poliovirus
	induces apoptosis in infected cells through the recruitment and the activation of p53 in the *PML*-NBs
PML IV	has a complex role in the regulation of apoptosis and growth suppression
	activates RB1 via interactions with PP1 phosphatase
	inhibits AKT1 via interactions with PP2A phosphatase
	negatively affects the PI3K pathway by inhibiting MTOR and activating PTEN
	positively regulates p53/TP53 by acting at different levels
	acts as a transcriptional repressor of TBX2 during cellular senescence
	regulates double-strand break repair in gamma-irradiation-induced DNA damage responses
	acts as a negative regulator of telomerase by interacting with TERT
	regulates PER2 nuclear localization
	restricts varicella zoster virus (VZV) via sequestration of virion capsids in *PML*-NBs
	restricts rabies virus by inhibiting viral mRNA and protein synthesis
	has antiviral activity against encephalomyocarditis virus (EMCV) by promoting nuclear sequestration of viral polymerase within *PML*-NBs
PML VI	interacts with moloney murine leukemia virus (MoMLV) integrase (IN) and reverse transcriptase (RT)

Table 2. *PML* Role in Apoptosis via Interactions with Proteins/Organelles (Salomoni et al., 2012)

Protein	Evidence
Death Receptors	
FAS Ligand (FASL)	*PML*-deficient lymphocytes show decreased cell death following treatment with FASL.
TNF-a	Bone marrow cells from *PML*-/- animals are resistant to TNFα treatment.
TRAIL	*PML* potentiates interferon a-triggered cell death through induction of TRAIL.
Pro-apoptotic Transcription Factors	
p53	*PML* controls p53 degradation through the inhibition of Mdm2.
	PML controls p53 by promoting DAXX acetylation and phosphorylation at multiple residues.
	PML transduces ATM/p53-dependent pro-apoptotic signals in HIV-induced syncytia.
ATM/Chk2 pathway	*PML* is under the control of the ATM/Chk2 pathway and can regulate DNA damage response.
p63	*PML* regulates p63.
p73	*PML* inhibits the degradation of the p53 family member p73.
c-Jun-N-terminal kinase (JNK)	*PML* modulates its pro-apoptotic function through c-Jun-N-terminal kinase (JNK)-dependent phosphorylation.
PI-3K pathway	
PTEN	*PML* promotes PTEN nuclear localization.
AKT	*PML* inhibits Akt function by promoting its PP2A-dependent dephosphorylation.
phosphatase PP1	*PML* promotes PP1-dependent dephosphorylation of retinoblastoma protein (pRb).
mTOR	*PML* directly interacts with mTOR and induces its localisation to the *PML*-NBs, thus inhibiting its function.
transforming growth factor (TGF) β	In *PML*-deficient fibroblasts the response to TGFβ is impaired, with both senescence and apoptosis being damaged.
Organelle	
Endoplasmic reticulum (ER)	In the absence of *PML*, Ca2+ release from the ER is impaired, and apoptosis is defective.
Mitochondria	*PML* can regulate p53 in mitochondria.

has role in facilitating these processes (Meyerson et al., 1997; Nakamura et al., 1997; Wu et al., 2000; Potts and Yu, 2007). It has been demonstrated that overexpression of *PML* isoform IV, triggers senescence through an Rb-dependent mechanism. In the process of senescence induction, PML-NBs are colocalized with senescence-associated heterochromatin foci (SAHF), Rb, and E2F. Consequently, PML represses of E2F target genes, which in turn leads to proliferation arrest, DNA damage and senescence. A T-box transcription factor, named TBX2 has been identified as an E2F target necessary for *PML*-induced senescence (Bischof et al., 2002; Mallette et al., 2004; Vernier et al., 2011; Martin et al., 2012).

Regulation of neoangiogenesis

PML recruits PP2a to PML-NBs, thus dephosphorylates and inactivates Akt. Additionally, PML recruits mTOR activator Rheb to the nucleus, so inhibits mTOR. As Akt-mTOR pathway controls the protein synthesis of HIF-1α, downregulation of this pathway by PML inhibits neoangiogenesis (Trotman et al., 2006). As *PML* regulates mTOR/HIF-1α pathway, it is also involved in hypoxia responses (Bernardi et al., 2006). Studies in human and mouse tumors have shown that *PML* deficiency leads to overexpression of pro-angiogenic factors such as HIF-1α and VEGF and increases neoangiogenesis (Chen et al., 2012).

Regulation of cell migration

PML has been shown to inhibit MDA-MB-231 cell migration through downregulation of integrin β1 expression (Reineke et al., 2010). However, another study has shown that mouse embryonic fibroblasts derived from *PML* knockout mice migrate slower than normal fibroblasts (Tang et al., 2013).

Cell adhesion, morphology and proliferation

In a recent study, the proteome of mouse embryonic fibroblasts (MEFs) derived from normal (*PML*+/+) and *PML* knockout (*PML*-/-) mice have been compared. Many of the differentially expressed proteins between *PML*+/+ and *PML*-/- have been those play critical roles in cell adhesion, migration, morphology and cytokinesis. It has been shown that *PML*-/- and *PML*+/+ MEFs are morphologically different. In addition, *PML*-/- MEFs were less adhesive, proliferated more extensively and migrated considerably slower than *PML*+/+ MEFs (Tang et al., 2013).

Regulation of DNA damage responses

The association between PML-NBs and chromatin has implied a possible role for PML-NBs in DNA repair mechanisms. Following DNA damage numerous DNA repair factors shuttle in and out of PML-NBs. In addition, PML-NBs colocalize with sites of unscheduled DNA synthesis in damaged cells (Dellaire et al., 2006). Other evidences supporting the role of *PML* in DNA damage signaling come from the observation that *PML*-null cells can not fully activate p53 in response to DNA damage (Guo et al., 2000) and the PML protein is phosphorylated in response to DNA double-strand breaks (DSBs) by Chk2 (Yang et al., 2002) and ataxia telangiectasia and Rad3-related (ATR) kinase (Dellaire et al., 2006; Bernardi and Pandolfi, 2007). However, it is not obvious whether these modifications of PML or PML-NB composition are prerequisite for DNA repair or are a consequence of ongoing repair (Dellaire et al., 2006). Briefly, PML-NBs have been known as highly sensitive DNA damage sensors involved in preservation of integrity of the DNA repair pathways. Although PML-NB number has been shown to be increased in response to DSBs (Dellaire et al., 2006), the mechanism underlying this increase has not been clarified (Carbone et al., 2002).

Viral infection

It has been demonstrated that the genome of nuclear-replicating DNA viruses preferentially become associated with PML-NBs. In addition, their initial sites of transcription and DNA replication sites are often next to PML-NBs or their remnants (Everett, 2001). Sumoylation seems to be involved in the mechanisms that cause the association of viral genomes and proteins with PML-NBs (Everett, 2001). Herpes simplex virus 1(HSV) ubiquitin ligase ICP0 has been demonstrated to target *PML* via

two distinctive mechanisms: one dependent on SUMO modification and the other via SUMO-independent interaction with *PML* I (Cuchet-Lourenco et al., 2012). In addition, it has been shown that PML-NBs have role in the repression of HSV-1 infection in the absence of functional ICP0 and they may be involved in regulating lytic and latent infection of HSV-1(Wang et al., 2012). PML-NB protein has been shown to interact with the EBV protein SM and increase the stability of lytic EBV transcripts (Nicewonger et al., 2004). In addition, *PML* has been among three hub genes identified by Network analysis showing significant differential expression between EBV positive and negative post-transplant lymphoproliferative disorder (PTLD). Consequently, this gene has been suggested to be involved in the molecular mechanism of EBV positive lymphoma (Wu et al., 2013).

There are some evidences supporting the hypothesis of a negative effect of PML-NBs on viral gene expression such as the role of ICP0 family of proteins in HSV infection. However, many studies have suggested a positive role of PML-NBs in viral transcription. For instance, PML-NBs can act as sites for active transcription. In addition, PML seems to have interaction with transcription factors involved in viral gene expression. Consequently, splitting of PML-NBs can release stored factors required for viral gene expression (Everett, 2001).

Control of cytokine signaling

All IFNs significantly increase *PML* expression at mRNA and protein levels leading to increase in the number and size of PML-NBs (Chelbi-Alix et al., 1995). Jak/ STAT pathway is responsible for induction of *PML* gene expression by IFNs and interleukin 6 (IL-6) (Stadler et al., 1995; Hubackova et al., 2012). On the other hand, analysis of *PML*-/- mice has demonstrated that these cells are resistant to apoptotic stimuli induced by cytokines (Wang et al., 1998a; Lin et al., 2004). All nuclear *PML* isoforms (*PML*I to *PML*VI), but not the cytoplasmic one (*PML*VIIb), have been shown to be positive regulators of IFN-γ signaling (Maarifi et al., 2014). *PML* has been shown to play an important role in TGF-β signaling in cytoplasm (Jin et al., 2013).

Granting a selective advantage for tumor cells

Recent data have shown that *PML* does not always function as a tumor suppressor. For instance, in triple negative and basal high tumor grade breast cancers, high levels of *PML* expression correlates with early tumor recurrence, and is a characteristic of poor prognosis. The capacity for *PML* to influence fatty acid oxidation is involved in the mechanism by which its overexpression provides selective advantage for breast tumor cells (Carracedo et al., 2012). In addition, *PML* has been shown to have pro-survival function in hematopoietic stem cells and this function is implicated in leukemia (Ito et al., 2008). Another evidence for oncogenic role of *PML* has come from the findings that low levels of *PML* correlate with better overall survival for CML patients (Ito et al., 2008). In brief, it seems that *PML* has a dual role in tumorigenesis which may depend on tumor microenvironment.

Gastric Carcinoma

An example for involvement of PML in carcinogenesis

Gastric carcinoma is among cancers in which *PML* expression has been shown to be decreased or abolished. Loss of PML expression in gastric carcinoma has been shown to be associated with increased level of lymphatic invasion, higher stages, and unfavorable prognosis (Lee et al., 2007). In addition, it has been demonstrated that loss of PML expression in gastric carcinoma tissues is associated with higher numbers of infiltrating T-cells, compared to tissues positive for PML (Kim et al., 2011). Infiltrating T-cells have role in tumor rejection as well as tumor immune evasion according to their subtype classifications. It has been demonstrated that CD4+ CD25+ regulatory T cells can suppress tumor-specific T cell responses thus inhibiting tumor rejection (Ghafouri-Fard et al., 2014).

Besides, gastric carcinoma tissues displaying reduced PML levels have shown increased levels of IFNγ-inducible protein 10 (IP-10/CXCL10) expressions (Kim et al., 2011). IP-10 is one of the chemokines with multiple functions in inflammatory diseases and cancer (Ben-Baruch, 2006; Lee et al., 2009). It has been proposed that loss of PML protein expression in gastric cancer cells results in increased IP-10 transcription through enhancement of STAT-1 activity, which, in turn, increases lymphocyte infiltration within tumor (Kim et al., 2011).

Regulation of *PML* Expression in Human Cancers

The mentioned functions of *PML* in inhibition of tumorigenesis imply that its downregulation may be involved in tumor initiation and/or progression. The first malignancy associated with *PML* has been APL in which *PML* function is disturbed by PML-RARα fusion protein. Downregulation or loss of expression of *PML* has been demonstrated in various types of malignancies such as prostate adenocarcinoma, colon adenocarcinoma, breast carcinoma, lung carcinoma, lymphoma, CNS tumors, and germ cell tumors. It has been demonstrated that decreased expression of *PML* can result in loss of cell cycle control and inhibition of apoptosis and is probably an important event in the process of carcinogenesis (Gurrieri et al., 2004; Reineke and Kao, 2009). A correlation has been demonstrated in some types of tumors, for *PML* loss and tumor invasiveness and metastatic progression. The mechanism of *PML* loss of expression is not fully elucidated. In spite of the recurrent downregulation of PML protein in tumors, its mRNA is expressed in all tumor samples and cell lines tested. Proteasome inhibitors could lead to re-expression of PML protein and re-establishment of PML-NBs in a number of PML negative tumor cells. Consequently, proteasome-dependent degradation which is mediated by ubiquitination is suggested as a mechanism for PML downregulation in tumors (Gurrieri et al., 2004).

PML and Cancer Immunotherapy

Since the role of the immune system to eradicate tumor cells is crucial, immunotherapeutic approaches

have been developed for the treatment of cancer. These approaches aimed to compensate for the drawbacks of conventional cancer treatment options, particularly in the treatment of cancer metastases (Ghafouri-Fard and Ghafouri-Fard, 2012b). Active immunotherapy requires prior identification of tumor-associated antigens (TAAs) to induce long term responses against tumor and terminate immune tolerance (Ghafouri-Fard and Ghafouri-Fard, 2012a; Ghafouri-Fard et al., 2012). *PML-RARα* mutation in PML seemed to be a potential target for immunotherapy at first glance. However, investigation into the antigenicity of the PML-RARα fusion product could not prove immunogenic neoantigen epitope (Dermime et al., 1996). Despite this failure, results from animal models were promising. For instance, a DNA-based vaccine has been developed by fusing the *PML-RARα* oncogene to tetanus fragment C (FrC) sequences. This DNA vaccine showed a significant effect on survival in the mouse model of PML, both alone and when combined with all-trans retinoic acid (ATRA) (Padua et al., 2003).

Conclusions

Although *PML* first has been identified in a subtype of leukemia, it has been shown to be involved in many other malignancies. Despite the fact that it has been historically classified as a tumor suppressor gene, growing evidences implied that it may have a dual function. As discussed in this review, *PML* has different isoforms with different cellular localizations and functions. Although not clarified yet, the dual role of *PML* in tumorigenesis may be at least partly due to its different splicing variants. Consequently, in order to design specific *PML*- based anti cancer therapies, it is necessary to elucidate specific functions of each isoform. In addition, these isoforms may be used as prognostic biomarkers, if the pattern of expression of each of them has been evaluated in each cancer type. The role of *PML* in stem cell maintenance opens a new field for researches aiming at finding novel anti cancer therapies. Besides, as PML-RARα fusion protein has been shown to be presented at the cell surface by MHC molecules and may induce a tumor-specific T-cell response in patients (Osman et al., 1999), future researches can focus in the evaluation of it as a potential target for cancer immunotherapy.

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