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Review

Role of the promyelocytic leukaemia protein in cell death regulation

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The promyelocytic leukaemia gene *PML* was originally identified at the t(15;17) translocation of acute promyelocytic leukaemia, which generates the oncogene PML-retinoic acid receptor α. PML epitomises a subnuclear structure called PML nuclear body. Current models propose that PML through its scaffold properties is able to control cell growth and survival at many different levels. Here we discuss the current literature and propose new avenues for investigation.
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Promyelocytic Leukaemia (PML) and Cancer

Haematological malignancies. The majority of acute promyelocytic leukaemia (APL) cases are characterised by the t(15;17) chromosomal translocation that juxtaposes the *PML* gene and the retinoic acid receptor α (RARα).[1–5] This translocation is reciprocal and balanced, and produces two fusion genes, PML-RARα and RARα-PML. PML-RARα is the main oncogene of APL and is able to transform haemopoietic precursors.[1–3] On one hand, PML-RARα suppresses RARα transcriptional function, thus blocking differentiation at the promyelocytic stage.[1–3] On the other hand, PML-RARα disrupts the PML nuclear bodies (PML-NBs) through formation of PML-RARα/PML heterodimers.[1–3] It is still debated whether this interaction is crucial for leukaemogenesis *in vivo*. At least *in vitro*, the PML moiety within the oncogenic fusion protein has been proposed to only function to promote formation of multimers that cause transcriptional repression and ultimately transformation.[6] However, loss of PML increases the incidence and accelerates the onset of leukaemia in a mouse model of APL,[7] thus suggesting that its inactivation is important for promoting neoplastic transformation *in vivo*. Interestingly, two mutations of the remaining *PML* allele have been found in APL, which generate a premature stop codon and are predicted to encode truncated cytoplasmic proteins.[8,9] PML has been found translocated to the PAX5 locus to generate a PAX5-PML fusion gene in childhood acute lymphoblastic leukaemia,[10] which disrupts both PML and Pax5 function, thus suggesting that disruption of PML function could have a role in non-APL tumours. Indeed, it has been reported that PML expression is lost in other haematological malignancies, such as in 83% diffuse large cell lymphomas (DLCL) and 77% follicular lymphomas.[11] It would be very important to address the impact of PML loss in non-APL tumours. Overall, these studies suggest that PML works as tumour suppressor in the haemopoietic system. However, chronic myeloid leukaemia (CML) represents a notable exception. PML expression has been shown to act as positive regulator of self-renewal in CML-initiating cells.[12] As a result, PML loss leads to exhaustion of the leukaemic stem cell pool and reduced disease progression. Accordingly, PML expression correlates with poor overall survival in CML patients. This report constitutes a change in paradigm in the field of PML research, as it suggests a potential oncogenic role of PML. This is reminiscent of the role played by the growth suppressor p21 in leukaemic stem cells.[13] Further research is needed to fully dissect the mechanisms underlying PML function in cancer stem cells.

Solid tumours. It is becoming clear that PML expression is altered in many solid tumours. In this respect, the study by Gurrieri *et al.*[11] showed that PML expression is absent in 17% of colon adenocarcinomas, 21% of lung tumours, 27% of prostate adenocarcinomas, 31% of breast adenocarcinomas, 49% of CNS tumours (100% medulloblastomas and over 90% oligodendroglial tumours), 49% of germ cell tumours and 68% of non-Hodgkin's lymphomas (83% DLCL and 77% follicular lymphomas). Other studies have shown that PML expression

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Abbreviations: APL, acute promyelocytic leukaemia; CML, chronic myeloid leukaemia; DLCL, diffuse large cell lymphomas; ER, endoplasmic reticulum; FL, follicular lymphomas; HAUSP, herpesvirus-associated ubiquitin-specific protease; HSCs, haemopoietic stem cells; IP3R, inositol 1,4,5-trisphosphate receptor; PI3K, phosphoinositide kinase-3; *PML*, promyelocytic leukaemia gene; PML-NB, PML nuclear body; cPML, cytoplasmic PML; PCTA, PML competitor for TGIF association; PP1, protein phosphatase 1; PP2A, protein phosphatase 2A; PTEN, phosphatase and tensin homolog; RARα, retinoic acid receptor alpha; pRb, retinoblastoma protein; SMAD, mothers against decapentaplegic homolog; TGFα, transforming growth factor α; TGIF, TG—interacting factor; TNF, tumour necrosis factor; TRAIL, TNF-related apoptosis-inducing ligand; mTOR, mammalian target of rapamycin; SUMO, small ubiquitine-like modifier
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is lost in breast carcinomas,[14] gastric cancer,[15] small cell lung carcinoma[16] and in invasive epithelial tumours.[17] Interestingly, Gurrieri *et al*.[11] showed that loss of PML expression correlates with a higher tumour grading in breast adenocarcinomas and prostate carcinomas. It has to be noted that this study was conducted using an antibody against a PML epitope reported as fixation-sensitive,[14] thus suggesting that some of the tumours could have been erroneously classified as negative. Despite the lack of expression at protein levels, there is little evidence that PML is regulated at the transcriptional level in cancer.[11] In addition, mutations in non-APL tumours are extremely rare.[11] As a result, research efforts have been focused on studying posttranslational regulation of PML stability. In this respect, PML has been shown to be degraded by the proteasome in lung, colon and gastric cancer cell lines.[11,18] In addition, recent studies have demonstrated that the E3 ligase RNF4 mediates PML ubiquitylation and degradation.[19,20] Interestingly, this process appears to rely on disulphide bonds formation and subsequent SUMOylation of PML in PML-NBs.[21] This work opens a completely new field for the investigation of redox-dependent and SUMO-dependent degradation of nuclear proteins. Given the importance of posttranscriptional/translational regulation of gene expression in cancer, it is conceivable that PML translation could be regulated in cancer cells. In this respect, it is presently unclear whether PML translation is controlled by microRNAs or RNA-binding proteins in normal *versus* cancer cells. This warrants an urgent investigation.

PML Function(s)

PML has been proposed to transduce various growth suppressive signals. Several studies have implicated PML in the regulation of cellular senescence and programmed cell death. Most of them focused on PML nuclear splice variants,[22] but it is becoming clear that cytoplasmic localisation of PML can affect growth suppression and cell death. This review will focus on the role of PML in cell death control and will discuss the impact of most recent discoveries in the field.

PML and death receptors. PML has been shown to regulate apoptosis induced by FAS ligand (FASL) and tumour necrosis factor (TNF) α, which are key regulators of immunity and inflammation.[23–27] In particular, PML-deficient lymphocytes show decreased cell death following treatment with FASL.[28] Furthermore, bone marrow cells from *PML*$-/-$ animals are resistant to TNFα treatment. In both cases, this correlates with decreased caspase activation. It is worth noting that PML has been shown to regulate cell death in a caspase-independent manner, thus suggesting that its role is not confined to caspase-dependent cell death.[29] Finally, PML role is not limited to FASL and TNF, as it has been shown to potentiate interferon α-triggered cell death through induction of TRAIL,[30] a death receptor expressed in cancer cells.[31–37]

PML and pro-apoptotic transcription factors. More recent studies have shown that PML regulates the tumour suppressor p53, a major regulator of apoptosis.[38–40] In particular, it controls p53 degradation through the inhibition of Mdm2, which is the major p53 E3 ubiquitin ligase.[41–45] This function appears to be in part PML-NB independent and occurs through sequestration of Mdm2 into nucleoli, thus promoting p53 activation upon DNA damage.[41] The PML-interacting protein DAXX[46,47] has been shown to control p53 ubiquitylation by inhibiting MDM2 degradation.[48,49] PML can also control p53 by promoting its acetylation[50] and phosphorylation at multiple residues.[33,51–53] cPML isoforms have been shown to negatively regulate PML function,[54] thus suggesting that balance between nuclear and cytoplasmic isoforms could dictate the response to growth suppressive signals. PML can also regulate DNA damage response in a p53-independent manner. In this respect, PML is under the control of the ATM/Chk2 pathway for induction of cell death upon genotoxic stress.[55,56] Finally, PML has been shown to regulate cell death induced by HIV infection.[57] In particular, PML transduces ATM/p53-dependent pro-apoptotic signals in HIV-induced syncytia.[58,59] Overall, these findings indicate that PML can regulate p53 function by acting at different levels of the p53 pathway. It remains to be established whether these different regulatory routes are stimulus- and/or tissue-specific.

PML regulates the function of other members of the p53 family. In this respect, PML has been shown to inhibit the degradation of the p53 family member p73.[60–67] More recent studies have further dissected the functional consequences of PML/p73 interaction.[68–71] Notably, the shorter, growth-promoting isoform ΔN-p73 is regulated by the APL oncogene PML-RARα, thus adding another level of complexity.[72] Finally, PML is known to regulate the remaining member of the family, p63,[73–80] which has a key role in development and homeostasis of different epithelia.[81]

PML interacts also with c-Jun upon UV irradiation, and modulates its pro-apoptotic function through c-Jun-N-terminal kinase (JNK)-dependent phosphorylation,[82] a pathway implicated in the regulation of apoptosis.[67,83–85] UV causes dramatic PML-NB reorganisation, which leads to formation of multiple microspeckles positive for both phosphorylated c-Jun and PML.[82] Interestingly, DAXX has been shown to regulate JNK in human fibroblasts,[46] thus suggesting that PML could regulate the JNK/c-Jun pathway via DAXX.

PML, PTEN and AKT. Recent evidence has implicated PML in the regulation of the PI-3K pathway at multiple levels.[86] This work predominantly comes from the Pandolfi's group. First, PML has been shown to promote PTEN nuclear localisation by affecting its interaction with HAUSP and its ubiquitylation status.[87] Second, PML is able to inhibit Akt function by promoting its PP2A-dependent dephosphorylation.[88] Notably, we have shown that PML interacts with another phosphatase PP1, and promotes PP1-dependent dephosphorylation of retinoblastoma protein (pRb) in neural stem cells.[89] Finally, PML directly interacts with mTOR and induces its localisation to the PML-NBs, thus inhibiting its function. Taken together, these findings indicate that PML has an important role in regulation of the PI-3K pathway. In this respect, PML has been shown to regulate the intracellular degradation mechanism autophagy,[90–103] which is negatively regulated by mTOR, and has been implicated in cancer development and longevity. It is

therefore possible that PML through inhibition of mTOR could promote induction of autophagy. As mentioned above, it is still unclear whether these regulatory nodes exist in the same cell or whether they vary depending on the cell type or extracellular environment.

PML and transforming growth factor (TGF) β. TGFβ is known to control key tumour suppressive functions in normal cells, whereas in cancer cells it has been proposed to bear pro-metastatic functions.[104,105] The group led by Pier Paolo Pandolfi has shown that in PML-deficient fibroblasts the response to TGFβ is blunted, with both senescence and apoptosis being severely impaired.[106] Surprisingly, this effect was mainly caused by loss of PML cytoplasmic isoforms (cPML). In particular, cPML regulates endosomal trafficking of TGFβ receptors by promoting the association of Smad2/3 and Smad anchor for receptor activation. Interestingly, this pathway can be modulated by nuclear retention of cPML via a mechanism involving TG-interacting factor (TGIF-) and c-Jun.[107,108] In turn, TGIF is inhibited by PML competitor for TGIF association (PCTA), thus activating cPML tumour suppressive function.[108] A recent study has demonstrated that the nuclear corepressor SnoN, a known regulator of TGFβ, controls p53 stabilisation via interaction with PML and PML-NBs and independent of Smads.[109] This study suggests that nuclear PML is also involved in regulation of the TGFβ pathway. Further research efforts are needed to fully dissect the role of different PML isoforms in regulation of this pathway.

PML and the endoplasmic reticulum. A very recent study from Pandolfi's group has proposed a novel role for PML in the cytoplasm.[110,111] PML appears enriched at the endoplasmic reticulum and at the mitochondria-associated membranes, which constitute ER-to-mitochondria communication sites involved in transport of Ca^{2+} and induction of apoptosis.[110–112] At these sites, PML interacts with the 1,4,5-triphosphate receptor (IP3R), AKT and PP2A. In the absence of PML, AKT-dependent phosphorylation of IP3R is increased, whereas Ca^{2+} release from the ER is impaired, resulting in blunted apoptosis. These data suggest that PML can affect both nuclear and cytoplasmic functions of AKT through its interaction with PP2A, thus promoting its inactivation and apoptosis induction. Mitochondria act as crucial regulators of cell death through a complex interplay of pro- and anti-apoptotic proteins associated with these organelles. The tumour suppressor p53 has been demonstrated to localise to mitochondria and promote apoptosis via regulation of BCL-2 family members.[113–118] It is conceivable that PML could regulate p53 not only in the nucleus but also in mitochondria. Further studies are needed to address a potential functional interaction between PML and p53 in mitochondria, and the impact of this interaction on BCL-2 family members and apoptosis induction.

Overall, studies in the last few years indicate that PML, through interaction with PP1 and PP2A phosphatases in the nucleus and the cytoplasm, could affect key tumour suppressive (pRb, see below) and oncogenic pathways (AKT). It remains to be established whether the function of p53 and c-Jun could also be modulated by PML-mediated regulation of PP1 and/or PP2A (Figure 1). Finally, it is conceivable that PML itself could be a target for PP1 and/or PP2A-mediated dephosphorylation as part of a positive or negative feedback loop.

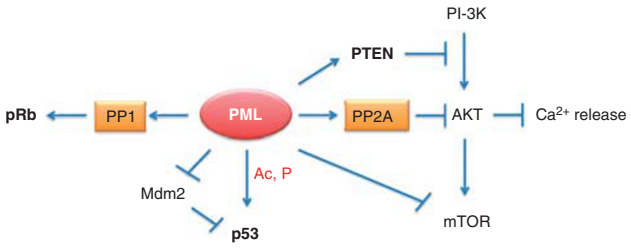

Figure 1 Multifaceted role of PML in regulation of apoptosis and growth suppression. PML activates pRb and inhibits AKT via interactions with PP1 and PP2A phosphatases, respectively. In addition, it negatively affects the PI-3K pathway by inhibiting mTOR and activating PTEN. cPML retains the ability to promote PP2A-dependent AKT inhibition, thus causing Ca^{2+} release at contact sites between the mitochondria and the ER. Finally, PML positively regulates p53 by acting at different levels (that is, acetylation, phosphorylation and Mdm2-dependent degradation). It is presently unclear whether sequestration of PP1 and PP2A into PML-NBs can inhibit their function

PML Function in Stem Cells: what is the Contribution of Cell Death Regulation?

PML has emerged as an important factor regulating stem cell function within multiple tissues. In particular, our work has shown that PML regulates neural stem cell (NSC) function during corticogenesis by a mechanism involving PP1 and pRb.[89] In the bone marrow, PML loss affects self-renewal in haemopoietic stem cells (HSCs) potentially through its action on the mTOR pathway.[12] Finally, PML regulates mammary gland development and its loss results in skewing of mammary progenitor subtypes.[119,120] It is presently unclear whether the phenotypes caused by PML loss in these different tissues is in part due to alterations of cell death. For instance, increased NSC number in PML−/− cortices during development could be because of increased cycling as well as decreased cell death. Vice versa, reduction in neuronal numbers in PML-deficient cortices could be because of increased cell death following commitment of neural progenitors to neuronal fate. In the haemopoietic system, the increased proliferation of PML−/− committed progenitors could be caused by impaired cell death (Figure 2). Finally, altered cell death pathways could explain the increased generation of ERα^+ luminal progenitors in PML-deficient mammary glands.[108,109]

The last few years of PML research have produced fascinating results. However, the increasing complexity of PML function and its promiscuous interactions raise a number of key questions: (i) Are these interactions tissue- or context-specific? (ii) Does PML work differently in normal cells *versus* immortalised or transformed cells? (iii) How is the interplay between cPML and nuclear functions regulated? The field is in need of more refined mouse models, such as knockin and conditional knockouts, which will help addressing these important points.

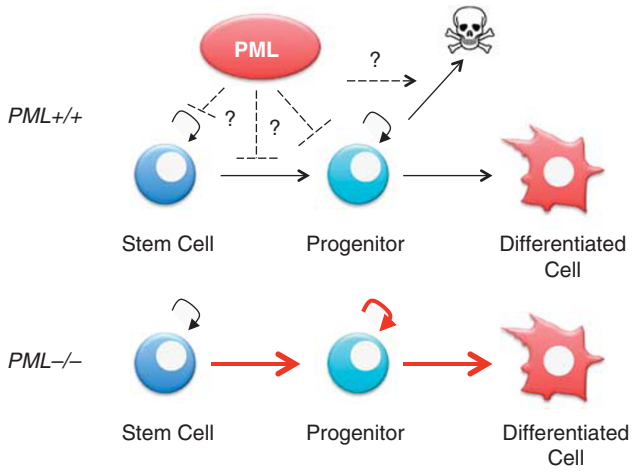

Figure 2 Role of PML in stem/progenitor cells. PML regulates stem cell function in different tissues. In the haematopoietic system, PML loss leads to expansion of the progenitor pool and increased entry into differentiation, accompanied by reduction in the stem cell pool. This phenotype could be explained by increased transition to committed progenitors, augmented proliferation and/or decreased cell death. Further research is needed to determine whether PML can promote cell death in committed progenitors, and by which mechanism(s)

Conflict of Interest

The authors declare no conflict of interest.

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