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REVIEW

Body language: the function of PML nuclear bodies in apoptosis regulation

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Abstract

Promyelocytic leukaemia (PML) nuclear bodies (NBs) are macromolecular nuclear domains present in virtually every mammalian cell. PML nuclear bodies (PML-NBs) were functionally linked to various fundamental cellular processes, including transcriptional control, tumour suppression and apoptosis regulation. Supporting the important function of PML and its associated NBs in apoptosis regulation, several apoptotic regulators localise to PML-NBs, and cells from PML-deficient mice show severe apoptotic defects, including induction of genotoxic stress and death receptor CD95 (Fas/APO-1) activation. Based on the current literature, we hypothesise that PML-NBs regulate apoptosis through different molecular mechanisms, on the one hand by acting as macromolecular scaffolds for recruitment and post-translational modification of the apoptotic key regulator p53, and on the other by regulating the subcellular bioavailability and quality of some apoptotic signal transducers.

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Abbreviations: AIF, apoptosis-inducing factor; APAF-1, apoptosis-activating factor 1; APL, acute promyelocytic leukaemia; ARF, alternative reading frame; As_2O_3, arsenic trioxide; ASK1, apoptosis signal-regulating kinase 1; ATM, ataxia-telangiectasia mutated; CBP, CREB-binding protein; DD, death domain; DED, death-effector domain; DISC, death-inducing signalling complex; HAUSP, herpesvirus-associated ubiquitin-specific protease; HIPK, homeodomain-interacting protein kinase; JNK, c-Jun N-terminal kinase; MAPK, mitogen-activated protein kinase; PIAS, protein inhibitor of activated STAT; PML, promyelocytic leukaemia; SAE, SUMO-1-activating enzyme; SUMO, small ubiquitin-related modifier; TGF, transforming growth factor; TNF-R, tumour necrosis factor receptor; TRADD, TNF-R1-associated death domain protein; TRAIL, tumour necrosis factor-related apoptosis-inducing ligand; UV, ultraviolet

Introduction

The mammalian cell nucleus harbours a number of subnuclear compartments among the family of nuclear bodies (NBs).[1] One particular class of NBs are the promyelocytic leukaemia nuclear bodies (PML-NBs, also termed PML oncogenic domains, PODs, Kremer bodies, nuclear domain 10, ND10, NBs or nuclear dots, NDs). PML-NBs are dynamic macromolecular structures altering their number, size and content in response to diverse stimuli such as viral infections, extracellular signals and genotoxic stress.[2] Under normal growth conditions, almost every mammalian cell usually harbours between 10 and 30 doughnut-like-shaped PML-NBs with a diameter ranging between 0.2 and 1 μm.[3–7] Besides a number of viral proteins, to date more than 40 different cellular proteins have been found in association with PML-NBs (Figure 1). Although the molecular function of PML-NBs is currently not clear, there is accumulating evidence that PML-NBs are regulatory domains involved in various biological processes, including protein degradation,[5] transcriptional regulation,[8,9] cell growth,[10,11] antiviral response,[12,13] cellular senescence,[14–17] tumour suppression,[18] DNA repair[19,20] and apoptosis.[21–25] However, how can one explain that so many different and unrelated processes are linked to these nuclear domains? Since PML-NBs contain several dozens of functionally different residents (Figure 1), the current model is that PML-NBs play an important role in the regulation of these processes by controlling the function of its residents by altering their post-translational modification pattern and regulating their subcellular distribution.

PML and its associated NBs

PML was originally identified in leukaemic blasts from patients suffering from acute promyelocytic leukaemia (APL). In APL blasts, PML-NBs are delocalised into multiple tiny micro-speckeled NBs,[26] in most cases due to the expression of the oncogenic PML-RARα fusion protein, which is the product of a reciprocal chromosomal translocation.[27–30] PML-RARα causes APL through a multifunctional mechanism involving RARα target gene repression,[31] inhibition of apoptosis[32] and epigenetic gene silencing.[33] Treatment of APL patients with all-*trans* retinoic acid (ATRA) or the chemotherapeutic drug arsenic trioxide (As_2O_3) leads to PML-RARα degradation, reformation of PML-NBs and terminal differentiation to myelocytes, or in case of As_2O_3 treatment to apoptosis of the APL blasts, which is followed by disease remission.[26,32]

The tumour suppressor protein PML belongs to the family of RING domain containing proteins[34,35] and is expressed in at least seven different isoforms, which differ in their function and subcellular localisation.[36] Unravelling the functional differences of the PML isoforms is currently an intensively

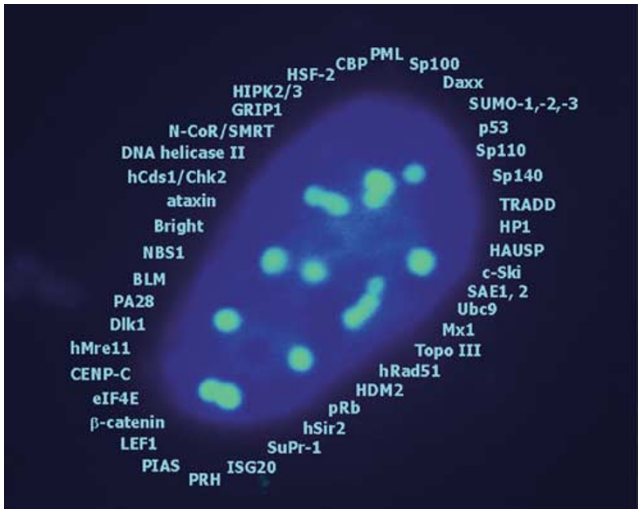

Figure 1 Cellular PML-NB components. A list of cellular proteins identified to localise at PML-NBs. Currently, the PML and Sp100 proteins are the only known constitutive PML-NB residents. All other indicated components either transiently localise to PML-NBs or are recruited to PML-NBs under specific conditions, including treatment with type I and II interferons, genotoxic stress or oncogenic transformation and induction of premature cellular senescence. The various PML-NB components have functions in transcriptional regulation, maintenance of genomic stability, post-translational protein modification, protein degradation, DNA repair and apoptosis

investigated topic in PML research. Cells from PML-deficient mice lack PML-NBs and the PML-NB components Daxx, small ubiquitin-like modifier-1 (SUMO-1) and CREB-binding protein (CBP) are mislocalised throughout the nucleoplasm. The exogenous expression of PML relocalises these factors to PML-NBs,[5,37,38] indicating that PML, although it appears not to bind all its PML-NB residents, establishes a supramolecular scaffold necessary for recruiting diverse proteins to this nuclear domain.

Recently, it has been reported that the PML-NB component eIF4E localises to NBs in PML−/− cells, and exogenously expressed PML distributes to eIF4E bodies in PML−/− cells, demonstrating that eIF4E forms NBs independent of PML.[2,39] This finding suggests that eIF4E bodies might represent the underlying structure where PML-NBs are assembled. However, more detailed investigations using, for example, non-transformed primary human diploid cells are needed to generalise this interesting finding.

Role of SUMO in PML-NB formation and transcriptional regulation

The assembly of PML into macromolecular PML-NBs depends on its covalent post-translational modification with SUMO-1.[37,38] SUMO-1 (also termed sentrin, GMP1, SMT3C or ULP1) is a small polypeptide that is covalently attached to PML and several other PML-NB components, including Sp100,[40] Daxx,[41] p53[42,43] and CBP,[44] through an enzymatic machinery similar to that of ubiquitin modification.[45] SUMOlation requires an activating E1 SUMO-1 activating enzyme (SAE)1/SAE2 heterodimer,[46] the conjugating E2 enzyme Ubc9[47,48] and the recently identified E3 SUMO ligases.[49–52] In contrast to ubiquitination, SUMOlation usually does not lead to protein degradation but regulates protein–protein interactions, subcellular localisation and activity. Interestingly, SUMO-conjugated PML interacts with Daxx and recruits Daxx from chromatin into the PML-NBs and releases the Daxx repressor function.[8,53] This mechanism allows the regulation of Daxx function via PML SUMO conjugation and deconjugation. Recently, different SUMO peptidases have been identified in mammals, which differ in their subcellular localisation and target protein specificity. Interestingly, a recently identified SUMO peptidase that deconjugates SUMO from PML has been shown to regulate PML-NB assembly and transcription.[54] Similarly, p300/CBP and Sp3 are both regulated in their cofactor function – whether they act as a coactivator or corepressor – via timely controlled SUMO conjugation and deconjugation.[44,55] Thus, SUMO is an important factor to control PML-NB assembly and transcription. For a detailed review about the function of SUMO, we refer to some recently published reviews on this topic.[45,56,57]

Several reports indicate an important function of PML and its NB residents in apoptosis regulation. As discussed below, we hypothesise that PML-NBs regulate apoptosis through different mechanisms, either by serving as transcriptional modification and activation platforms for the tumour suppressor p53, and in addition, by regulating the quality and subcellular bioavailability of apoptotic regulators. As a basis for our discussion of the mechanisms of how PML-NB components participate in apoptosis regulation, we will first briefly outline the different apoptosis pathways.

Apoptosis pathways

Apoptosis is a tightly regulated mechanism to eliminate unwanted or potentially dangerous cells in multicellular organisms during development, tissue homeostasis and immune response.[58,59] The physiological meaning of apoptosis is illustrated by a number of human diseases, including cancer, autoimmune disorders and neurodegenerative diseases, which are associated with apoptosis dysregulation.[60–63]

Two principle apoptotic pathways exist in mammalian cells, the intrinsic and extrinsic pathways. Both pathways converge on the level of caspase activation. Caspases, aspartate-specific proteases, are the main arm of the apoptotic machinery,[64] although caspase-independent apoptosis pathways have also been described.[65,66] Caspases are synthesised as procaspases, catalytically inactive precursors (zymogens), and are proteolytically activated either by other caspases and proteases or by a local increase in caspase concentration.[67] The intrinsic pathway is regulated by the mitochondria and is activated by translocation of proapoptotic members of the Bcl-2 protein family (such as Bax or truncated Bid) into the mitochondrial membrane,[68] which triggers the release of cytochrome *c* into the cytosol. Cytochrom *c* binds the apoptosis-activating factor 1 (APAF-1), an evolutionarily conserved proapoptotic factor, in the cytosol in an energy-dependent manner. APAF-1 then assembles into a multimeric caspase activation platform termed 'apoptosome', which

recruits and activates caspase-9 and thus allows the activation of the downstream effector caspase-3.[69]

Extrinsic apoptosis pathways are elicited by a large number of cell surface receptors, which are capable of inducing apoptosis upon their multimerisation and activation through their respective ligands or agonistic antibodies.[61] The best understood death receptor systems are the tumour necrosis factor receptor (TNF-R)1 and CD95 (Fas/APO-1), members of the death receptor superfamily characterised by an intracellular death domain (DD). Upon their activation, they transmit an apoptotic signal via intracellular recruitment and assembly of a death-inducing signalling complex (DISC).[70,71] The CD95 and TNF-R1 DISCs are multiprotein complexes that consist of the adaptor molecules FADD and TNF-R1-associated death domain protein (TRADD) (only in the TNF-R1 receptor system), which in turn mediate recruitment of the death-effector domain (DED) containing initiator procaspases caspase-8 and -10 (only in humans) to the DISC. The procaspases are then autocatalytically cleaved and processed to active caspases,[67,72] which activate a caspase cascade finally resulting in proteolysis of various death substrate proteins and the execution of cell death.[73]

In addition to receptor-induced pathways, nuclear apoptosis pathways also exist. For example, several transcription factors, including p53 or forkhead transcription factors[74,75] can induce the expression of proapoptotic target genes that either directly trigger apoptosis via the intrinsic, mitochondrial pathway (e.g. by upregulating proapoptotic Bcl-2 family members[76–78]) or by transcriptional upregulation of death receptors such as CD95[79] or p53RDL1,[80] which results in extrinsic receptor-mediated apoptosis pathways. Moreover, apoptosis sensitivity can be altered by repressing the target gene expression of antiapoptotic transcription factors, such as NF-κB, which leads to an increased sensitivity towards TNF-α-induced apoptosis.[81–83]

PML-NB components in receptor-mediated apoptosis pathways

PML

Cells derived from PML$-/-$ mice show, in addition to defects in ceramide-induced, γ-irradiation-induced and type I and type II interferon-induced apoptosis, a strongly decreased sensitivity against death receptor-mediated apoptosis through stimulation with CD95L and TNF-α.[21] Moreover, injection of CD95-activating antibodies in PML$-/-$ mice resulted in a markedly decreased apoptosis-derived liver failure and increased survival time. Despite the defects in multiple apoptosis pathways, PML$-/-$ mice are viable and show no obvious abnormalities.[21] This might reflect the particular importance of PML in apoptosis pathways that are not essential for normal development, reminiscent of the phenotype of the tumour suppressor p53, which also plays a pivotal role only in stress-induced apoptosis pathways.[84]

Although a role of PML in death receptor-induced apoptosis is likely, there is currently no evidence that PML exerts a direct function in death receptor signalling by participating in receptor proximal events. Thus, one might assume that PML-NBs regulate the bioavailability of signal transduction components participating in death receptor-induced apoptosis pathways either by regulating their expression and/or by controlling their subcellular distribution. In agreement with this assumption, it was recently reported that APL cells respond to ATRA treatment by upregulation of the death ligand tumour necrosis factor-related apoptosis-inducing ligand (TRAIL) leading to paracrine apoptosis of the APL blasts, indicating that PML-RARα suppresses the normal regulation of TRAIL in these cells.[85]

The CD95 and TNF-R1 can signal cell death via caspase-dependent and -independent pathways.[61,65,66] Although the DISC components and caspase-1, -3 and -8 are properly expressed, caspase activation is markedly inhibited upon CD95L or TNF-α stimulation in PML-deficient cells.[21] The underlying molecular defect explaining this interesting phenotype is presently unknown. Currently available evidence does not fully exclude the fact that nucleoplasmic or cytoplasmic PML can also directly regulate the apoptotic machinery NB-independently. Taking the defects in PML-deficient cells in various apoptosis pathways into account, this either might be explained by a defect in a general downstream death signalling component, or by various pathway-specific upstream defects. The detailed role of PML in CD95-mediated apoptosis and other death receptor pathways is far from being understood and awaits further investigations. PML also regulates a caspase-independent apoptosis pathway.[86] Using an inducible overexpression system, it has been shown that PML can trigger a death pathway independent from *de novo* protein synthesis that is not sensitive to synthetic caspase inhibitors.[86] However, this finding remains to be confirmed under more physiological conditions. An overview of currently known PML-NB-associated proteins and their function in cell surface receptor-mediated or nuclear apoptosis pathways are given in Table 1 and in Figure 2.

TRADD

Recently, the TNF-R1-associated DD protein TRADD was found to shuttle between the cytoplasm and the nucleus.[87] In the cytoplasm, TRADD serves as an adaptor molecule for the recruitment of FADD and caspase-8 to the TNF-R1 DISC.[70] In the nuclear compartment, TRADD is found in association with PML-NBs.[87] The isolated DD of TRADD, which also mediates the interaction with the TNF-R1 DD,[70,88] is responsible for PML-NB localisation and is sufficient to initiate a caspase-independent form of cell death. Apoptosis through TRADD-DD overexpression is in part inhibited by Bcl-X_L, a mitochondria-localised antiapoptotic member of the Bcl-2 protein family, and relies on p53 and PML expression.[87] These results imply that a pathway targeting the mitochondria is involved in this death mechanism. The physiological relevance and the exact molecular mechanism of TRADD-DD-induced apoptosis remain to be determined in the future.

HIPKs

Homeodomain-interacting protein kinases (HIPKs) belong to a novel family of predominantly nuclear serine/threonine kinases. In humans and mice the family comprises three

Table 1 PML-NB-associated proteins and their function in different apoptosis pathways

	Apoptosis pathway	Interaction partners	Reference
PML	CD95, TNF-R1, ceramide, p53	p53[1,2,3], Daxx[1,3], CBP[1,3], hSir2[1,2,3]	14, 17, 21–23
Daxx	CD95, TGF-β-RI, nuclear	CD95[1,2,3], TGF-β-R1[1,2,4], PML[1,3], CBP[1], HIPK2[1,2,3]	8, 38, 95, 100–103, 105, 108, 113, 114[a]
TRADD	TNF-R1, nuclear	TNF-R1[1–4], HIPK2[3]	70, 87, 88
HIPK2	p53	p53[1–4], CBP[1,3], Daxx[1,2,3], TRADD[3], CD95[4]	24, 25, 94[a]
P53	p53	CBP[1,2,3], Hdm2[1,2,3], PML[1,2,3], HIPK2[1–4], hSir2[1,2,3]	14, 17, 23–25, 120–121, 140–142
CBP	p53	p53[1,2,3], HIPK2[1,3], Daxx[1]	25, 108, 140–142
hSir2	p53	p53[1,2,3]	17, 145, 146
Hdm2	p53	p53[1,2,3], PML[2,3]	120, 121, 134, 148
HIPK3	Unknown	CD95[3,4]	95

Interaction: [1]Endgenous, [2]GST pull down, [3]overexpression and [4]yeast two hybrid Various PML-NB components previously reported to play a role in nuclear and/or surface receptor-induced apoptosis pathways are listed. The interaction partners are given and the different experimental methods used for determining the interaction of the respective proteins are indicated [a]Own unpublished results

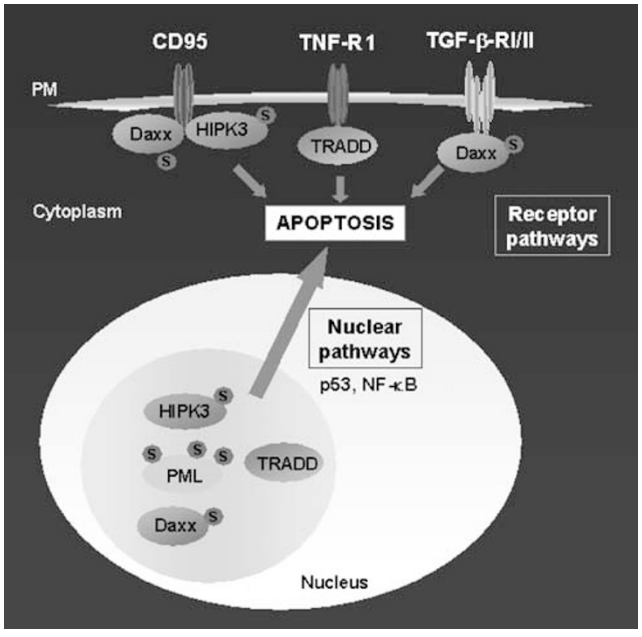

Figure 2 Model of PML-NB components and their function in nuclear- and surface receptor-mediated apoptosis pathways. Fractions of the proteins Daxx, TRADD and HIPK3 were shown to localise both into PML-NBs and to the cytoplasmic compartment. Dependent on their subcellular localisation and their molecular context Daxx, HIPK3 and TRADD can either function directly in cell surface receptor-mediated apoptosis pathways[70,88,95,100,101,113] or in nuclear apoptosis pathways[87,102,105,148] by regulating the target gene expression of proapoptotic transcription factors such as p53 or by suppressing the activity of the antiapoptotic transcription factor NF-B. The regulated PML-NBs recruitment and the release of these proteins may control their bioavailability and might modulate cellular sensitivity towards nuclear or receptor-mediated apoptosis pathways. PM, plasma membrane. For a detailed description, see text

members, HIPK1-3, which were originally identified as corepressors for homeodomain transcription factors.[89] HIPKs belong to the dual-specificity tyrosine phosphorylation-regulated kinase family of protein kinases[90] and function in transcriptional regulation,[91,92] growth suppression[93] and apoptosis.[24,25] HIPK2, which mediates p53 phosphorylation and ultraviolet (UV)-induced apoptosis[24,25] (for details, see section on 'Regulation of p53 activity in PML-NBs') was found to interact with TRADD when overexpressed in 293T cells.[94] Whether this interaction is of importance for the TRADD-or TRADD-DD-induced cell killing remains to be determined in the future. Murine HIPK3/FIST (Fas-interacting signal transducer), a close relative of HIPK2, has been identified by its interaction with the intracellular domain of CD95 in a yeast two-hybrid screen. Interestingly, ectopically expressed HIPK3 also coprecipitates with FADD and Daxx and a fraction of HIPK3 is found in PML-NBs.[95] Overexpression of HIPK3 induces FADD phosphorylation and inhibits CD95-induced c-Jun N-terminal kinase (JNK) activation. However, HIPK3 expression showed no effect on CD95-induced apoptosis in the cell system used. Unexpectedly, FADD was recently found to localise preferentially to the nucleus in different adherent cell lines[96] and, additionally, murine FADD was demonstrated to be essential for regulating cell proliferation and cell cycle progression in lymphocytes.[97] The latter function depends on its specific phosphorylation at serine, 191,[97] a conserved site equivalent to serine 194 in human FADD.[98] FADD-deficient mice die *in utero*, suggesting that FADD also plays a role in nonlymphoid tissue proliferation.[99] Future studies are required to reveal a more detailed view about the function of HIPKs in death receptor signalling and their role in FADD phosphorylation.

Daxx

Daxx is a multifunctional protein that participates in different apoptosis pathways.[100–103] The protein was originally identified by its interaction with the intracellular domain of CD95 in a yeast two-hybrid screen and described to act as an adaptor protein that activates the MAP3K apoptosis signal-regulating kinase 1 (ASK1) and triggers the activation of the JNK signalling pathway and apoptosis.[100,101] A recent report suggests that the CD95–Daxx–ASK1 signalling route in particular operates in a cell type-specific manner during apoptosis of motoneurons.[104] In addition to its role in the cytoplasm, Daxx also fulfils an important function in the nucleus in transcriptional regulation. Dependent on the cellular and the molecular context, Daxx acts as a transcriptional corepressor[8,105–107] or coactivator.[108] Several laboratories demonstrated that Daxx localises to the nucleus where it is found in association with chromatin, centromeres and PML-NBs.[8,105–107,109] The subcellular localisation of Daxx can be regulated by its interaction with the kinase ASK1, which recruits Daxx independent of its kinase activity to the cytoplasm.[110,111] In the cytoplasm, Daxx and ASK1 can

initiate a caspase-independent apoptosis pathway that is inhibited by the overexpression of the small heat-shock protein Hsp27.[112] Recently, Daxx was shown to function, similar to its role in CD95 signalling, as an adaptor for the transforming growth factor (TGF)-β type II receptor in order to transduce TGF-β-induced JNK activation and apoptosis in hepatocytes and B cells.[113] In this respect, it is interesting to note that HIPK2 interacts with Daxx and releases it from PML-NBs by inducing their disintegration. HIPK2 cooperates with Daxx in TGF-β-induced JNK activation and apoptosis in human hepatoma cells (Hofmann TG, Stollberg N, Schmitz ML, Will H, manuscript submitted). Recently, Daxx has also been demonstrated to interact with other members of the HIPK family of proteins,[95,114] which suggests a redundant function of HIPKs in regulating the subcellular localisation and the function of Daxx.

Daxx interacts with SUMOlated PML[8,37] and both proteins cooperate in a nuclear apoptosis pathway in activation-induced cell death in splenocytes that depends on PML expression.[102] These findings indicate an important role of SUMO-1 in apoptosis regulation. In addition, a proapoptotic function of Daxx has also been found in human B progenitor cells, where Daxx is upregulated by interferon treatment and regulates lymphopoiesis through downregulation of the antiapoptotic regulator Bcl-2 followed by the induction of apoptosis.[103]

In contrast to its proapoptotic functions, Daxx also exerts an antiapoptotic function during embryogenesis.[115] Moreover, recent work using siRNA-mediated downregulation of Daxx also demonstrated an antiapoptotic function of Daxx in different cell lines, demonstrating that this effect is not just restricted to embryonic development.[116] Future studies are needed to clarify the discrepancies in the role of Daxx in apoptosis, in particular considering a possible cell type-specific function, which might in part be explained by its molecular context-dependent gene repressive or stimulatory function.[108] Since Daxx also interacts with p53,[117] it would be interesting to study whether the apoptosis in Daxx-deficient mice depends on p53, and whether Daxx might serve as a corepressor for unrestrained p53 activation during development. In this respect, it is interesting to note that Daxx can repress the transactivating potential of the transcription factor Ets-1,[106] which in turn appears to be essential for stress-induced p53 target gene expression as revealed by experiments with Ets-1-deficient embryonic stem cells.[118]

Regulating p53-dependent apoptosis from the PML-NBs

In addition to their regulatory function in receptor-mediated apoptosis pathways, PML and its associated NBs also regulate the activity of a central apoptotic regulator – the tumour suppressor protein p53.

p53 activation and regulation

The tumour suppressor protein p53 is a cellular key player controlling cell cycle arrest, premature cellular senescence and apoptosis.[119] p53 is a modular organised transcription factor, comprising an N-terminal transactivation domain, a central DNA-binding domain and a C-terminal regulatory domain. p53 activation is mainly regulated by its subcellular localisation and on the post-transcriptional level through protein stabilisation and post-translational modification. In normal dividing cells, p53 is kept silent at almost undetectable protein levels through its association with the proto-oncoprotein Mdm2 (or its human homologue Hdm2). Mdm2, a RING finger E3 ubiquitin ligase, binds p53 and mediates its ubiquitination that results in proteasomal degradation of p53.[120,121] In response to a plethora of different stimuli – including genotoxic insult (UV- or γ-irradiation), chemotherapeutics, hypoxia, interference with DNA synthesis by stalling replication forks, oncogenic transformation and replicative senescence – p53 gets stabilised via abolishing its proteolytic degradation.[122,123] p53 mainly acts as a sequence-specific transcription factor that transactivates or represses its multiple target genes, thereby controlling its effector pathways either leading to cell cycle arrest, premature cellular senescence or apoptosis. However, p53 can also induce apoptosis via transcription-independent mechanisms and translocation to the mitochondria triggering cytochrome *c* release and caspase activation.[124–127]

Basically, two mechanistically different pathways mediating p53 activation have been identified. One pathway relies on the activation of a set of protein kinases, which directly phosphorylate serine or threonine residues within the N-terminal transactivation domain and the C-terminal regulatory domain of p53.[128] For example, the kinases ataxia-telangiectasia mutated (ATM) and Chk2 are activated in response to DNA damage induced by UV- or γ-irradiation and mediate the phosphorylation of Serine 15, which triggers disruption of the Mdm2-p53 complex resulting in an increased p53 half-life.[129,130] The second mechanism, which operates during deregulated expression of cellular or viral oncogenes, leads to a phosphorylation-independent stabilisation of p53 involving the INK4a locus encoded protein $p14^{ARF}$. $p14^{ARF}$ – a small protein transcriptionally regulated by the kinase DAPK[131] and the transcription factors and proto-oncogenes E2F-1 and c-myc – binds to MDM2 and inhibits its ubiquitin ligase activity resulting in p53 stabilisation and target gene expression.[132] Thus, hyperproliferative stimuli through the expression of cellular oncogenes, such as oncogenic Ras^{V12}, c-Myc or E2F-1, induce upregulation of $p14^{ARF}$ and trigger a cellular tumour suppressive response that counteracts transformation. In primary cells, the resulting p53 activation triggers premature cellular senescence – a permanent, irreversible cell cycle arrest in the G1 phase.[133]

The different p53 activation mechanisms converge on the level of p53 stabilisation, which is regulated by p53 post-translational modification pattern – including acetylation, phosphorylation, deubiquitination and SUMO-1 conjugation. Several recent findings argue for an important role of PML-NBs and, their components in the control of p53s post-translational modification and activity.

Regulation of p53 activity in PML-NBs

Accumulating experimental evidence provided by several independent groups indicates a role of the PML-NB and its

components in p53 activation in response to different stimuli. Besides p53 itself, multiple factors regulating p53 are found within or in association with PML-NBs, in particular PML,[15,22,23] CBP,[14] Hdm21,[34] HIPK2,[24,25] Chk2,[135,136] hSir2[17] and herpesvirus-associated ubiquitin-specific protease (HAUSP).[137,138] This significant accumulation of factors involved in p53 regulation raises the assumption that PML-NBs form supramolecular scaffolds that establish a peculiar molecular microenvironment enabling efficient post-translational modification of a fraction of p53. Similar to most PML-NB components, p53 is not a constitutive resident and its recruitment to PML-NBs is mediated by all PML isoforms,[16] but only interaction with PML IV regulates p53 activity.[22,23] Genotoxic stress induces PML-NB formation and leads to p53-dependent apoptosis induction, which in part requires PML.[22,23] Upon high-dose UV-radiation, HIPK2 localises together with p53 and CBP in PML-NBs and phosphorylates p53 at serine 46.[24,25] HIPK2 expression results in p53-dependent apoptosis that is associated both with p53 Ser46 phosphorylation and Lys382 acetylation,[24,25] modifications previously shown to trigger p53 activation and apoptosis.[139–142] A recent publication suggests that the nucleoplasmic fraction of PML IV mediates the effect on p53 Ser46 phosphorylation and Lys382 acetylation during premature senescence, indicating that the PML-p53 interaction can also take place in the nucleoplasm.[16] Although these experiments show that PML-NBs are not required for Ser46 phosphorylation, they do not exclude the fact that PML-NBs might favour p53 modification and promote its activation before its release into the nucleoplasm and to its target promoters at the chromatin. PML is required for HIPK2-mediated p53 Ser46 phosphorylation and can be restored in PML−/− cells by ectopically expressed PML IV (Möller A, Sirma H, Hofmann TG, Rueffer S, Klimczak E, Dröge W, Will H, Schmitz ML, manuscript submitted). In addition, the p53 Ser20 kinase Chk2 was recently identified in PML-NBs in response to genotoxic stress.[135] The overexpression of PML prolonged the period of p53 Ser20 phosphorylation and protected it against mdm2-mediated ubiquitination and degradation, thus leading to increased p53 stability and activity.[135]

Several groups reported that p53 is a target protein for SUMO-1.[42,43,143,144] SUMO-1 is covalently attached to specific lysine residues of its target proteins by an enzymatic machinery, which is similar to that of ubiquitin modification.[45] p53 is SUMOlated at Lys386 within its regulatory domain through a complex containing the SUMO-1 conjugating enzyme Ubc9 and the SUMO-1 ligase protein inhibitor of activated STAT (PIAS)1.[51,144] However, addressing the function of p53 SUMOlation on p53 activity led to contradictory results, reaching from increased activity,[42,43] no effect[143] to decreased transcriptional activity of p53.[144] The reason for these discrepancies should be clarified in the future, although it is currently not clear whether SUMOlation of p53 might take place in PML-NBs. Interestingly, similar to SUMO-1, PIAS proteins also appear to localise to PML-NBs.[52] Recently, the deubiquitinating enzyme HAUSP, which colocalises with PML-NBs,[137] was found to stabilise p53 and to activate its proapoptotic activity.[138] Whether HAUSP can exert its function in PML-NBs remains to be studied.

Besides several p53-activating enzymes, factors implicated in negative regulation of p53 were also found in PML-NBs. For example, the evolutionarily conserved NAD-dependent histone deacetylase hSir2 localises to PML-NBs.[17] hSir2 inhibits p53 activity through deacetylation of Lys382 and antagonises genotoxic stress-induced apoptosis.[145,146] The localisation of CBP and hSir2 to PML-NBs, two factors that exert antagonistic functions on p53 activity, suggest a potential mechanism for the regulation of p53 function in PML-NBs the change of its acetylation status, depending on the molecular context and stimulus. In addition, a fraction of the p53 degrading E3 ubiquitin ligase Hdm2 (human Mdm2) was found associated with PML-NBs.[134] Although the functional meaning of this finding is currently not clear, this may indicate that PML-NBs can regulate p53 ubiquitination and proteasome-dependent degradation. If true, this would be another level of PML-NB-mediated regulation of apoptosis.

Taken together, there is accumulating evidence for the model that a fraction of p53 is post-translationally modified within the PML-NBs under specific cellular conditions (Figure 3). PML-NBs might function preferentially by serving as scaffolds to increase the local concentrations of factors implicated in p53 post-translational modification, including site-specific acetylation and phosphorylation. Subsequently, modified p53 may be released from PML-NBs and targeted to its site of action at its target gene promoters. Recent findings that SUMO-specific protease SuPr-1[54] and HIPK2 induce PML-NB disintegration[147] (Hofmann TG, Stollberg N, Schmitz ML, Will H, manuscript submitted), suggest an attractive molecular mechanism of how modified p53 and other factors can be released from PML-NBs. The specific molecular context of the multiple positive and negative regulators of p53 concentrated in PML-NBs may lead to stabilisation, activation, inactivation or even degradation of this cellular key protein. Answers to these many open questions are likely to contribute to a better understanding of the role of p53 and PML-NBs in human diseases and cancer.

Summary

Several independent lines of evidence point to an important function of PML-NBs and their components in regulating extrinsic and intrinsic apoptosis pathways, in particular those elicited by ligation of death-inducing cell surface receptors or those resulting in p53 activation. First, data from PML−/− cells show that these cells have multiple defects in different apoptosis pathways, including death receptor-mediated ones and p53-dependent pathways. Second, APL blasts that express the PML-RARα oncoprotein and have disrupted PML-NBs are resistant towards various apoptosis stimuli, and As_2O_3 treatment induces reformation of PML-NBs accompanied by apoptosis of the blasts. Third, there is evidence that signalling molecules participating in receptor-mediated apoptosis pathways (Daxx, TRADD) at a receptor proximal level localise to PML-NBs and also participate in apoptosis regulation employing nuclear pathways. This suggests a regulatory function of PML-NBs in these pathways by regulating the bioavailability of specific apoptotic signal transducers. Strikingly, a significant number of proteins that

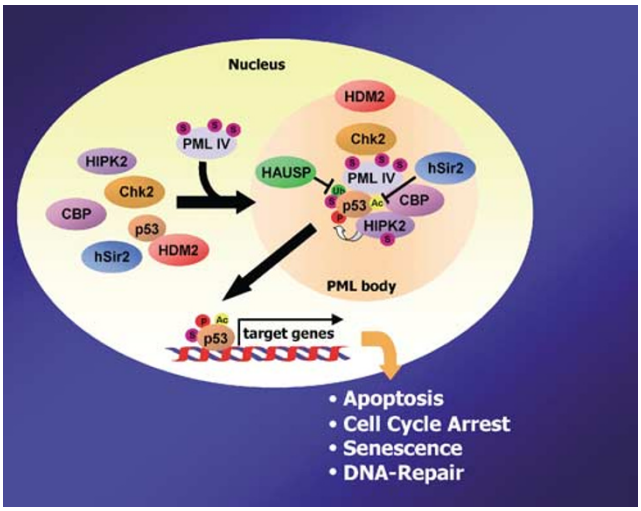

Figure 3 Speculative model of PML-NB components and their role in p53 regulation. Upon oncogenic transformation-induced cellular senescence, UV-induced stress and treatment with the chemotherapeutic drug arsenic trioxide (As_2O_3) PML mediates corecruitment of p53 and different p53 regulating factors to PML-NBs, where they colocalise with p53. p53 is subsequently differentially post-translationally modified and activated. Active, modified p53 leaves the PML-NBs and is targeted to the chromatin where it regulates the expression of its specific target genes, thereby triggering the different p53-effector pathways. The different post-translational modifications on p53 affected in these processes are indicated. S, SUMOlation, P, phosphorylation, Ac, acetylation, Ub, ubiquitination. For a detailed description, see text

fulfil an important function in regulating p53 activity (CBP, PML, HIPK2, HAUSP, hSir2, Chk2) are recruited along with p53 to PML-NBs in response to genotoxic insult or cellular senescence, and regulate p53 effector function in a PML-dependent manner. Even more striking, multiple important post-translational modifications associated with p53 stabilisation and activation, including Ser15, Ser20 and Ser46 phosphorylation as well as Lys382 acetylation, are dependent on the presence of PML, highlighting the emerging role of these nuclear domains in p53 regulation.

Concluding remarks and future perspectives

The mechanism by which PML-NBs and their components regulate the function of their components that participate in apoptosis signal transduction awaits further elucidation. Current knowledge about the function of PML and its NB components in apoptosis regulation suggests an intricate network between different apoptosis pathways and PML-NBs. As discussed, PML-NBs may modulate death receptor pathways by controlling the bioavailability and the quality of apoptogenic PML-NB components that operate in these pathways. Therefore, it will be important to know whether other factors involved in receptor-mediated apoptosis signalling or p53 regulation localise to PML-NBs, and if so, how their localisation affects the sensitivity towards different apoptosis pathways.

Although currently available evidence strongly suggests that PML-NBs represent nuclear post-translational modification platforms for p53, one important question to be answered in the future is whether the PML-NB-associated fractions of HIPK2, CBP, hSir2, HAUSP, Chk2 and/or the nucleoplasmic fractions thereof are catalytically active on p53. In order to catch a molecular glimpse of how PML-NBs are coupled to various apoptosis pathways, we still need the answer to a couple of open questions. At which level and how does PML act in death receptor and other apoptosis pathways? What are the functions of cytoplasmic and nuclear PML isoforms? Which are the players in the PML- and TRADD-induced caspase-independent apoptosis pathways? Does PML also regulate apoptosis pathways mediated by other death receptors? These and other related important questions will probably be answered in the near future and will help to shed light on the molecular function of the PML-NBs.

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