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Review article

Nucleophosmin in leukemia: Consequences of anchor loss

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ABSTRACT

Nucleophosmin (NPM), one of the most abundant nucleolar proteins, has crucial functions in ribosome biogenesis, cell cycle control, and DNA-damage repair. In human cells, NPM occurs mainly in oligomers. It functions as a chaperone, undergoes numerous interactions and forms part of many protein complexes. Although NPM role in carcinogenesis is not fully elucidated, a variety of tumor suppressor as well as oncogenic activities were described. NPM is overexpressed, fused with other proteins, or mutated in various tumor types. In the acute myeloid leukemia (AML), characteristic mutations in *NPM1* gene, leading to modification of NPM C-terminus, are the most frequent genetic aberration. Although multiple mutation types of NPM are found in AML, they are all characterized by aberrant cytoplasmic localization of the mutated protein. In this review, current knowledge of the structure and function of NPM is presented in relation to its interaction network, in particular to the interaction with other nucleolar proteins and with proteins active in apoptosis. Possible molecular mechanisms of NPM mutation-driven leukemogenesis and NPM therapeutic targeting are discussed. Finally, recent findings concerning the immunogenicity of the mutated NPM and specific immunological features of AML patients with NPM mutation are summarized.

1. Introduction

Nucleophosmin (NPM, B23) was identified as one of the major nucleolar silver-stained proteins (Lischwe et al., 1979) with an important role in ribosome biogenesis (Yung et al., 1985; Li et al., 1996; Liu and Yung, 1999). It was found to be associated with the chromosomes during mitosis (Ochs et al., 1983; Zatsepina et al., 1999) and its role in the cell cycle progression was later confirmed (Amin et al., 2008b). Additionally, participation of NPM in DNA-damage repair processes (Poletto et al., 2014; Ziv et al., 2014), chromatin remodeling (Swaminathan et al., 2005; De Koning et al., 2007; Hisaoka et al., 2014), or apoptosis (Kerr et al., 2007; Li et al., 2007; Dhar and St Clair, 2009) was also reported.

Nucleophosmin structure and function was previously reviewed (Federici and Falini, 2013; Lindström, 2011), several papers compiled its particular role in centrosome duplication (Lim and Wang, 2006) or DNA repair function (Box et al., 2016; Scott and Oeffinger, 2016). Some excellent reviews recapitulated current knowledge of nucleophosmin aberrations in cancer (Grisendi et al., 2006; Meani and Alcalay, 2009), others focused on specific mutations of NPM occuring in the acute myeloid leukemia (AML) (Falini et al., 2011; Heath et al., 2017) or to novel possibilities for AML therapy (Di Matteo et al., 2016). This review aims to complete the previous articles with the summary of the known consequences of the AML-specific NPM mutations on the interaction network, and to outline the potential of immunotherapeutic strategies for treatment of AML with mutated NPM.

2. NPM structure

Nucleophosmin is a member of the nucleophosmin/nucleoplasmin family (Eirín-López et al., 2006), which encompasses four major protein types: NPM1 (Nucleophosmin), NPM2 (Nucleoplasmin-2, NP), NPM3 (Nucleoplasmin-3) and drosophila NP-like protein (dNPL). Gene expression, intracellular localization, and function of individual members

Abbreviations: AA, amino acids; AML, acute myeloid leukemia; APE1, apurinic/apyrimidinic endonuclease; APL, acute promyelocytic leukemia; AR, allele ratio; ATRA, all-trans retinoic acid; BER, base excision repair; del(5q), interstitial deletion of the chromosome 5; ELN, European LeukemiaNet; Flt3-ITD, fms-like tyrosine kinase 3 with internal tandem duplications; LSC, leukemia stem cells; MDS, myelodysplastic syndrome; MEF/ELF4, myeloid ELF1-like factor; MRD, minimal residual disease; MW, molecular weight; NCL, nucleolin; NES, nuclear export signal; NLS, nuclear localization signal; Nm/Np, nucleophosmin/nucleoplasmin; NoLS, nucleolar localization signal; NPM, nucleophosmin; NPMmut, nucleophosmin with C-terminal mutation; NPMwt, wild-type nucleophosmin; PD-L1, programmed-death ligand 1; pT199NPM, nucleophosmin with phosphorylated Threonine 199; SINE, selective inhibitor of nuclear export; T-ALL, T-cell acute lymphoblastic leukemia; TPA, 12-O-tetradecanoylphorbol 13-acetate; VAF, variant allele fraction; WT1, Wilms´ Tumor 1

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of the nucleophosmin/nucleoplasmin group vary substantially, but there is a high sequence similarity in their Nterminal regions (Frehlick et al., 2007). Five Nterminal domains associate to form a pentamer, and were found to assemble in a decamer structure using X-ray crystallography (Dutta et al., 2001; Lee et al., 2019; Namboodiri et al., 2003, 2004).

2.1. NPM protein variants

The human *NPM1* gene, located on the chromosome locus 5q35, is composed of 12 exons. Owing to alternative mRNA splicing, it encodes several protein isoforms, three of which being more closely described. The most common transcription variant 1 (NCBI Reference Sequence: NM_002520.6) lacks the exon 10 and corresponds to the longest mRNA transcript, coding for a 294-amino acid (AA) polypeptide. The calculated molecular weight (MW) of the resulting protein is 32.6 kDa, its specific band on SDS-PAGE is observed at 37 kDa (Chan et al., 1989; Frehlick et al., 2007; Lim and Wang, 2006). This isoform is usually denoted as NPM1 or NPM. The latter term will be used from now on in this review. The second protein isoform (product of the transcription variant 2, NCBI Reference Sequence NM_199185.3), has 265 A A, as a result of missing exon 8 (Lim and Wang, 2006). The function and expression of this isoform has not been evaluated so far. The third known variant (transcription variant 3, NCBI Reference Sequence: NM_1037738.2) is composed of 259 A A (Dalenc et al., 2002). Whereas NPM localizes mainly in the nucleoli (Cordell et al., 1999; Spector et al., 1984), the NPM isoform 3 has also been observed in the nucleoplasm (Okuwaki et al., 2002), likely due to a loss of 35 A A in the C-terminal region.

2.2. Primary and secondary structure

NPM consists of three regions with diverse properties regarding the structure, function and biochemical activities. An Nterminal core domain, which is involved in oligomerization and in chaperone activity, has high stability due to a belt of hydrophobic contacts between subunits (Herrera et al., 1996; Hingorani et al., 2000). The N-terminal domain of the folded NPM molecule is organized into eight antiparallel beta-strands. Five NPM molecules are associated in a complex with highly asymmetric distribution of charge, resulting in negative-charge accumulation on one side of the pentamer (Lee et al., 2019). The Cterminal domain is relatively rich in basic residues providing a positive charge to this region, which is implicated in binding to nucleic acids, ribonuclease activity and ATP binding (Chan et al., 1989; Chang et al., 1998; Hingorani et al., 2000). At the end of this domain, there is a stretch of aromatic AA including two conserved Tryptophan residues, W288 and W290, which are necessary for nucleolar localization of NPM (Grummitt et al., 2008). The DNA-binding domain including the Cterminus is formed by an intrinsically disordered part followed by three helical fragments (H1-H3). The two NPM (terminal) globular domains are separated by a central region (linker) harboring two acidic segments, which are required for NPM chaperone activity. The linker domain is crucial for histone binding (Hingorani et al., 2000; Okuwaki et al., 2001b) and mediates formation of nucleosome (Okuwaki et al., 2001b). The region between the two acidic patches contributes to ribonuclease activity of NPM (Hingorani et al., 2000). A binding site for G-quadruplex DNA-structures was mapped to 70 residues at C-terminal region, specifically to AA 225–242, with principal importance of Lysines K229 and K230 (Federici et al., 2010).

2.3. Oligomerization

The major native NPM form is probably pentameric, with individual molecules interacting through the Nterminal globular domains (Hingorani et al., 2000). Truncation of the first 24 A A induced loss of NPM ability to form oligomers (Enomoto et al., 2006), whereas a deletion mutant lacking 192 A A from C-terminus formed higher order multimers (Hingorani et al., 2000). Later on, the region with several extremely conserved residues, including a GSGP sequence (Glycine 105 – Proline 108), was identified to be necessary for the oligomerization. Mutations of AA L102, G105, S106 and G107 in this region were shown to destabilize NPM oligomers and to inhibit the nucleosome assembly (Enomoto et al., 2006). The nucleic acid-binding activity, which is mapped to the Cterminal 70 residues, is required for NPM nucleolar localization (Okuwaki et al., 2002). As NPM mutated in the GSGP sequence was localized in the nucleoplasm, its ability to bind RNA was also examined. However, the RNA-binding activity of these mutants was not altered (Lin et al., 2016).

Point mutations of Cysteine 21 (C21) were reported to impair pentamer formation detected by biochemical methods (Prinos et al., 2011). On the other hand, in vivo monitoring by fluorescence methods showed that C21 F mutant retained the ability to associate with NPMwt (Holoubek et al., 2018). The importance of Tyrosine 67 (Y67), in the oligomerization domain, for the pentamer stability was also documented (Duan-Porter et al., 2014).

Moreover, NPM oligomerization is tightly related to its conformational state, which is regulated by a high number of conserved phosphorylation sites within the oligomerization domain (Mitrea et al., 2014). Phosphorylation of these sites leads to the destabilization of NPM folding, whereas dephosphorylated NPM molecules fold into an ordered structure allowing for pentamer formation. The accessibility of various Serine and Threonine residues regulates the extent of their phosphorylation and thereby the oligomer/monomer ratio. The NPM oligomerization was also shown to depend on the cellular ionic strength: whereas NPM forms pentamers in the presence of mono- and divalent cations, it tends to dissociate in a „low-salt “buffer (Mitrea et al., 2014). Furthermore, the interaction of NPM with other proteins was proved to regulate NPM folding and assembly. Specifically, the presence of binding peptides derived from an NPM interaction partner, p14Arf, locked NPM in pentamers and inhibited its transition from the folded state to disordered monomers (Banerjee et al., 2016).

2.4. Localization

NPM is mainly localized in nucleoli, but it undergoes nucleocytoplasmic shuttling, which is mediated by motifs for nuclear localization and nuclear export. The Nterminus of NPM contains two predicted Leucine-rich nuclear export signals (NES) (Wang et al., 2005; Yu et al., 2006) putatively recognized by the nuclear export receptor CRM1. However, neither of them have been proven to be active as real NESs, possibly due to inconvenient structure of these motifs consisting in particular of β-strands (Arregi et al., 2015; Bolli et al., 2007; Lee et al., 2019). A bipartite nuclear localization signal (NLS) promoting nuclear localization has been discovered between the two acidic stretches of the NPM central region (Hingorani et al., 2000). The two Tryptophan residues located in the Cterminal domain have been long considered as the NPM nucleolus localization signal (NoLS), essential for nucleolar docking of NPM. As we will see later, its absence is critical for the aberrant localization of AML-associated NPM mutants (NPMmut) (Falini et al., 2005; Nishimura et al., 2002). However, the nucleolar attachment has recently been ascribed also to the affinity of the C-terminal region for G-rich nucleic acids (Mitrea et al., 2016). In addition, another putative, probably unfunctional, NES has recently been reported within the Cterminus (Arregi et al., 2015).

3. NPM function

NPM participates in numerous cellular processes. It controls rDNA transcription (Murano et al., 2008), serves as an endoribonuclease for the maturing rRNA transcript (Herrera et al., 1995; Savkur and Olson, 1998), and takes part in the export of pre-ribosomal particles (Borer et al., 1989). These findings pointed to an essential role of NPM in the

ribosome biogenesis. More detailed insight into this particular NPM function specified that NPM directs the nuclear export of both 40S and 60S ribosomal subunits, and that it serves as a rate limiting factor in protein synthesis (Maggi et al., 2008). NPM also contributes to the maintenance of the genomic stability. This function is mediated by participation in DNA-repair processes (Poletto et al., 2014; Wu et al., 2002), centrosome duplication (Okuda et al., 2000; Wang et al., 2005), DNA replication (Okuwaki et al., 2001b; Takemura et al., 1994, 1999), and RNA pol I and II transcription (Bergstralh et al., 2007; Gurumurthy et al., 2008; Lessard et al., 2010; Li et al., 2008; Liu et al., 2007a; Murano et al., 2008; Swaminathan et al., 2005). NPM ability to function as a molecular chaperone is related to the prevention of protein aggregation (Szebeni and Olson, 1999), to histone and nucleosome assembly (Okuwaki et al., 2001b), to chromatin condensation and decondensation events (Okuwaki et al., 2001b, a), and to promotion of acetylation-dependent chromatin transcription (Swaminathan et al., 2005). Furthermore, NPM is involved in the apoptotic response to a variety of stress stimuli, such as UV irradiation (Wu et al., 2002) and hypoxia (Li et al., 2004), and it can modulate p53 stability and activity (Colombo et al., 2002).

Together with other nucleolar proteins, in particular with Ki-67, nucleolin (NCL), and fibrillarin, NPM is found in the perichromosomal layer of mitotic cells, as a part of peripheral chromosome scaffold (Sheval and Polyakov, 2008). However, NPM depletion, in contrast to depletion of Ki-67 or of NCL, does not impair the mitotic processes (Booth et al., 2014; Booth and Earnshaw, 2017).

3.1. Posttranslational modifications

The structure and function of NPM are influenced by numerous posttranslational modifications, especially by phosphorylation. Various kinases have been shown to phosphorylate NPM at multiple sites. These modifications may induce conformational changes and modulate diverse NPM functions. The best explored phosphorylation, which occurs at Threonine 199 (pT199), is mediated by Cyclin E/Cdk2, promotes NPM dissociation from the centriole, and controls proper centrosome duplication (Tokuyama et al., 2001). Phospho-T95, which is located in one of the putative NES regions, also participates in centrosome duplication control: it is phosphorylated at the G2/M phase boundary and is rapidly dephosphorylated during mitosis (Zhao et al., 2015). A Cdk1-mediated NPM phosphorylation (T199, T219, T234 and T237) is required to inactivate its RNA-binding activity (Hisaoka et al., 2014). This correlates with the observed phosphorylation of Serine 4 (S4), T95, T199, and T234/T237 during G2/M transition (Zhao et al., 2015). A stepwise dephosphorylation of T199, T234/237 and S4 was also found to mediate the stress response to irradiation (Wiesmann et al., 2019). Phosphorylation of T199 by v-cyclin/Cdk6 regulates latency of Kaposi´s sarcoma herpesvirus (Sarek et al., 2010). Substitution of multiple NPM phosphorylation sites mimicking hypoxia stress conditions increased cell death mediated by association of monomeric NPM with BAX in mitochondria after ischemic injury (Wang et al., 2019). In metastatic hepatocellular carcinoma, decreased binding of NPM to ROCK2 was associated with a high level of T234/237-phosphorylation mediated by Cyclin B/Cdk1 (Ching et al., 2015). DNA damage-induced phosphorylation at Serine 48, mediated by Akt, disrupted NPM interaction with p14Arf and induced p53-dependent apoptosis (Hamilton et al., 2014).

Numerous Lysines within the whole NPM protein can be deacetylated by sirtuins (Sirt). The acetylation of NPM significantly affects its histone-chaperone and nucleosome assembly functions. Acetylated NPM shows an increased affinity towards acetylated histones and increases transcription activity (Swaminathan et al., 2005). Through the Sirt1-mediated deacetylation, NPM transcriptionally regulates genes involved in the cell survival and proliferation during carcinogenesis of oral tumors (Shandilya et al., 2009). Recently, Sirt6 and Sirt7 were confirmed to directly interact with NPM, and to decrease by deacetylation its transcription activity in senescent cells (Lee et al., 2014). The Lysines K230 and K263 can also be subjected to sumoylation. K263 sumoylation enhances NPM association with pRb during the cell cycle (Liu et al., 2007b). During oxidative stress, NPM undergoes S-glutathionylation on Cysteine 275, which triggers the dissociation of NPM from the nucleolar nucleic acids (Yang et al., 2016).

4. NPM interactions

Thanks to the variability of individual domain functions and probably under modulation by posttranslational modifications, NPM interacts with nucleic acids as well as with numerous proteins. Its RNA-binding and endonuclease activity helps rRNA processing (Dumbar et al., 1989), and the oligomeric structure allows for core histone binding (Dutta et al., 2001). The histone-binding domain regulates chromatin remodeling (Okuwaki et al., 2012), enhances the acetylation-dependent chromatin transcription (Swaminathan et al., 2005), and mediates nucleosome assembly (Okuwaki et al., 2001b) suggesting that NPM serves as a histone chaperone (Frehlick et al., 2007). However, NPM chaperone activity is not limited to histones. During ribosome biogenesis, NPM interacts with many ribosomal proteins (Lindström, 2011). Through direct interaction, it cooperates also with non-ribosomal nucleolar proteins, in particular with NCL (Liu and Yung, 1999), and likely also with fibrillarin, although the latter interaction is documented only on the basis of colocalization experiments (Amin et al., 2008a). Moreover, NPM Nterminal domain mediates nucleolar localization of human immunodeficiency virus proteins Tat and Rev (Fankhauser et al., 1991; Li, 1997; Nouri et al., 2015). In the mitotic phase, a phosphorylated form of NPM interacts with proteins responsible for the centrosome duplication, Aurora A and B (Reboutier et al., 2012; Shandilya et al., 2014).

The role of NPM in the DNA-damage repair is mediated mainly by its interaction with apurinic/apyrimidinic endonuclease (APE1), which is involved in the base excision repair (BER). As a consequence of APE1 interaction with the oligomerization domain of NPM, APE1 endonuclease activity on abasic double-stranded DNA is stimulated (Vascotto et al., 2009). Phosphorylated NPM (pT199-NPM) has a role in the formation of repair complexes after ionizing radiation-induced DNA double-strand breaks (Koike et al., 2010).

NPM was also reported to interact with many proteins operating in the apoptosis. A region near the NPM Cterminus was identified to account for its interaction with the tumor suppressor p53 (Colombo et al., 2002; Lambert and Buckle, 2006). Overexpression of NPM in hypoxia or UV-irradiated cells led to suppression of p53 activity through several mechanisms: inhibition of p53 phosphorylation on Ser15, direct interaction with the p53 Nterminus, and binding of the transcription factor HIF-1α to HIF-1-responsive element in the NPM promoter (Li et al., 2004; Maiguel et al., 2004). In epithelial cells, NPM overexpression enhanced the level of p53 in the nuclei, but it reduced p53 association with mitochondria and thereby blocked the apoptosis induced by 12-O-tetradecanoylphorbol 13-acetate (TPA) (Dhar and St Clair, 2009). Besides the direct interaction, the expression and stability of p53 is also regulated through NPM interaction with MDM2, an E3ubiquitin ligase promoting p53 degradation (Kurki et al., 2004), or with the tumor suppressor p14Arf (p19Arf in mouse (Bertwistle et al., 2004)), another player in this regulation loop. The pool of p14Arf is bound to NPM in nucleoli, where it is sequestered from interaction with nucleoplasmic proteins. When it is released from the complex, p14Arf interacts with MDM2 and regulates p53 degradation/activity (Lee et al., 2005). Detailed studies of NPM-p14Arf complex revealed the importance of the Nterminal NPM domain for this interaction (Itahana et al., 2003; Enomoto et al., 2006; Luchinat et al., 2018). NPM also directly interacts with several p53-target proteins, in particular with BAX (Kerr et al., 2007; Thompson et al., 2008) and p21WAF1 (Xiao et al., 2009).

Furthermore, association with NPM is documented for several transcription factors. In particular, through the interaction with NPM, the oncoprotein c-myc is directed into the nucleoli, where it stimulates

transcription of rDNA and other cmyc-target genes (Li et al., 2008; Li and Hann, 2013). Moreover, NPM regulates turnover of the c-myc by coupling with the F-box protein Fbw7γ, which is involved in the ubiquitination and proteasome degradation of cmyc (Bonetti et al., 2008). NPM interaction with another transcription factor, NFkB, was detected by affinity chromatography followed by mass spectrometry and further confirmed by immunoprecipitation. An assay analyzing the transcription of the NF-kB target gene, MnSOD, showed enhanced MnSOD mRNA expression after NPM overexpression, proving functionality of this interaction (Dhar et al., 2004). Recently, it was documented that NPM association with the DNA-binding domain of an NF-kB subunit, p65, enhances its DNA-binding activity and, subsequently, the transcription of its target genes (Lin et al., 2017). On the other hand, an inhibitory effect of interaction with the Nterminal domain of NPM was reported for the regulatory function of the myeloid ELF1-like factor (MEF/ELF4) on the MDM2 promoter (Ando et al., 2013). Direct interaction of NPM with two transcription factors of IFNγ signalling pathway, STAT1 and IRF1, enhancing their transcription activity, was also recently described (Abe et al., 2018). In this work, the NPM oligomerization domain was shown to be required for the interaction with IRF1. Interaction of NPM with the transcription factor PU.1, which regulates the terminal differentiation of myeloid cells to granulocytes and monocytes, was also described (Gu et al., 2018).

5. NPM in cancer

NPM is frequently overexpressed, fused or mutated in tumors (Grisendi et al., 2006). As it was recently reviewed in (Chen et al., 2018), overexpression of NPM in solid tumors usually correlates with poor prognosis. Lower NPM expression in comparison with the non-tumor tissue has been recently documented for gastric tumor, but the impact of NPM level on the prognosis was not investigated (Leal et al., 2014). Interestingly, inverse correlation between mRNA and protein expression and its dependence on histological subtype was shown in this study. Several fusion products with other genes resulting from chromosomal translocations were described, in particular in hematological malignancies. The fusion proteins contain the NPM N-terminal domain, which serves mostly as an oligomerization interface promoting the oncogenic potential of the fusion partner. Specifically, NPM-RARα, NPM-MLF1, or NPM-ALK fusions can be detected in the acute promyelocytic leukemia (APL, AML-M3), myelodysplastic syndrome (MDS), or nonHodgkin´s lymphoma, respectively (Morris et al., 1994; Redner et al., 1996; Yoneda-Kato et al., 1996). Interstitial deletion of the chromosome 5 (del(5q)) is widely detected in both *de novo* and therapy-related MDS (Tasaka et al., 2008)), in AML, and in T-cell acute lymphoblastic leukemia (T-ALL, (Ebert, 2010; La Starza et al., 2016)). Although the deletion is not exactly at 5q35 locus, it is associated with marked downregulation of NPM in advanced MDS (Pellagatti et al., 2011). Moreover, monosomy 5 leading to NPM haploinsufficiency occurs in about 38% of MDS cases (Tasaka et al., 2008).

6. AML-associated NPM mutation

A short (usually 4 base pairs) frameshift insertion in the exon 12 of *Npm1* gene is detected in about 30% of patients with AML. The protein NPMmut resulting from the altered gene, with the use of an alternative stop codon, is four aminoacids longer and has an altered Cterminus. In the mutated protein, the NoLS from NPM wild-type (NPMwt) sequence is lost and it is replaced with a newly acquired NES, which can be recognized by the exporting protein Crm1 (Bolli et al., 2007; Falini et al., 2006). The affinity of the acquired NES for the Crm1 depends on the type of NPM mutation (Arregi et al., 2015; Bolli et al., 2007). Basic characteristics of the AML-related mutations were described in 2005 (Falini et al., 2005; Grisendi and Pandolfi, 2005): besides the NPM cytoplasmic localization, which was detected immunohistochemically, the patients with NPMmut usually had normal karyotype, high white blood cell count, CD34 and CD133 negativity, and a good response to the induction therapy, in particular in the absence of internal tandem duplications in *Flt3* gene (Flt3-ITD). On the other hand, the incidence of Flt3-ITD, which is associated with worse prognosis in AML, was twice higher in NPMmut cases. A screen in neoplasms other than AML showed that the mutations were unique for AML. Subsequent analyses led to more precise specifications including CD33 positivity (De Propris et al., 2011), association with cup-like nuclear morphology (Chen et al., 2009), specificity for adult leukemia, and co-occurrence of other mutations, in particular in DNMT3A. Besides Flt3 and DNMT3A, higher frequency of mutations in IDH1, IDH2, and TET2 were also documented (Patel et al., 2017) in NPMmut AML.

The majority of patients harbor mutation type A. Type D mutation differs only in one base and is phenotypically identical with the type A. Type B has one likely nonessential substitution (L289 M) in comparison with the type A. Altogether, the mutation types A, D and B represent more than 90% of all NPMmut cases. Nevertheless, more than fifty mutation types in the most frequently affected specific locus of the exon 12 were found till now (Kawaguchi-Ihara et al., 2016). Although they generally consist of four base-pair insertions, several longer indel mutations were also reported (Jeziskova et al., 2017). Moreover, rare mutations in other exons (exons 5, 6, 9 or 11) also occur (Albiero et al., 2007; Mariano et al., 2006). Recently, internal tandem repetition in the exon 12 leading to the creation of a longer (by about 40 A A) protein was found in a patient with relapsed AML (Duployez et al., 2018). Despite this heterogeneity, all the mutations cause cytoplasmic localization of the resulting protein. Therefore, although routinely analyzed by PCR followed by sequencing to determine the mutation type, the presence of the mutation can be effectively detected also by immunofluorescence using combination of specific antibodies recognizing either NPMwt or NPMmut (Šašinková et al, 2018).

Specific characteristics associated with NPM mutation led to the definition of „AML with mutated *NPM1* “as a distinct entity in WHO classification of myeloid neoplasm and acute leukemia (WHO edition 2016, (Arber et al., 2016)). In the majority of cases, the mutation is recurrent and can be used to monitor the minimal residual disease (MRD). The persistence of the mutation was shown in about 15% of patients after second chemotherapy cycle (Ivey et al., 2016) and in 30% of samples after allogeneic stem cell transplantation (Delsing Malmberg et al., 2018). In both cases, MRD positivity significantly correlated with higher risk of relapse and with shorter overall survival. Hence, until recently, NPM mutation was supposed to be a founder genetic lesion (Di Matteo et al., 2016; Falini et al., 2011; Federici and Falini, 2013). However, several exceptions documenting the loss or a change of the mutation have been reported (Krönke et al, 2013; Ivey et al., 2016; Höllein et al, 2018). In a cohort of 104 AML patients with NPMmut, ten percent relapsed as NPMwt, concurrently retaining other mutations from diagnosis suggesting the existence of a premalignant leukemic clone without NPMmut (Höllein et al, 2018). Persistence of NPM mutation at relapse positively correlated with Flt3-ITD co-mutation, whereas loss of NPM mutation was frequently observed together with recurrent DNMT3A mutation (Höllein et al, 2018; Krönke et al, 2013). Importantly, a switch from TCGC insertion at diagnosis to mutation type A (i.e. TCTG insertion) at relapse has been documented pointing to the fact, that the loss of a mutation may be accompanied by manifestation of another mutation (Webersinke et al., 2014). Moreover, the screening of variant allele fractions (VAF) of coexisting mutations showed that median VAFs were higher for frequent co-mutations in genes regulating DNA methylation (DNMT3A, IDH1, IDH2 or TET2) than the VAFs for NPM mutations. This suggests that NPM mutations might be a secondary or later event in the pathogenesis of AML (Patel et al., 2017). On the other hand, Flt3 mutations had relatively low VAF and are thus assumed to occur later than NPM mutations (Metzeler et al., 2016), in agreement with the conclusions from the statistics of mutation recurrence reviewed above. In summary, mutations in epigenetic regulators occur early during clonal evolution, but they are

usually not sufficient to cause leukemia, which is manifested later due to additional subsequent mutations, typically that in NPM (Wang et al., 2017).

Several genetically engineered mouse models of the NPM mutation, including transgenic and knock-in alleles, showed that NPM mutation cooperated with other mutations in AML induction (Sportoletti et al., 2015). Recently, a mouse model of human AML was created using human hematopoietic stem/progenitor cells transduced with lentivirus expressing NPMmut. Immunodeficient mice engrafted with these cells rapidly developed myeloid leukemia of human origin. Interestingly, the untransduced engrafted cells gave rise to development of human-derived immune cells and the leukemia was thus targetable by immunotherapy (Kaur et al., 2019).

Although NPM mutations are usually reported as exclusive for AML, they can rarely be found also in other hematologic neoplasms. In MDS, occurrence of NPM mutation (4,4%) was restricted to intermediate- and high-risk disease (Bains et al., 2011). In the chronic myelomonocytic leukemia (CMML, < 5%), the mutation was associated with unfavorable prognosis with early blastic transformation (Vallapureddy et al., 2017). Moreover, NPM function is associated with BCR-ABL pathways and with ribosomal protein networks in the chronic myeloid leukemia (Chan et al., 2015).

The NPM nucleolar insufficiency leads to partial inhibition of ribosomal synthesis, which may cause slower cell proliferation. Concurrently, the nucleoli of NPMmut-containing cells are more fragile and these cells are thus more prone to p53-induced apoptosis. These facts may be the main reason for the better response of AML patients with NPM mutation to intensive chemotherapy (Derenzini et al., 2018). Interestingly, cells with NPMmut showed increased phosphorylation of T199 and, concurrently, delayed progression into mitosis. Therefore, a higher cytoplasmic level of NPM may be related to a better suppression of aberrant centrosome duplication (Chan and Lim, 2015). Recently, Brunetti et al (Brunetti et al., 2018) showed that clearance of NPMmut from the cytoplasm was associated with downregulation of homeobox (HOX) genes, induced differentiation of AML cells and prolonged survival of mice with NPMmut leukemia. Although CD34+ subpopulation can also be found in NPMmut patients, the frequency of these cells was significantly lower than that in NPMwt patients (Schneider et al., 2014). Moreover, several genes involved in Tcell immunity were found to be overexpressed in leukemia stem cell (LSC) subpopulation (CD34+CD38-) of AML patients with NPMmut compared to AML patients with NPMwt or to healthy donors. This analysis suggests an important role of the immune system in AML with NPMmut (Schneider et al., 2014). The known consequences of NPM mutation in leukemogenesis and therapy effectiveness are summarized in Fig. 1 and Table 1.

The latest European LeukemiaNet (ELN) Recommendations assign patients with NPM mutations and with positive-but-low allele ratio (low AR, < 0.5) Flt3-ITD into favorable risk group (Döhner et al, 2017). However, there are indications of unfavorable prognosis in NPMmut-positive AML with FLT3-ITD low AR when allogeneic hematopoietic stem cell transplantation was not carried out in the first complete remission (Sakaguchi et al., 2018).

7. NPMmut oligomerization and interactions

As homozygote NPM mutation is lethal (Grisendi et al., 2005), cells with NPM mutation always co-express the wild-type form. NPMmut retains the ability to form oligomers and the localization of NPMwt and NPMmut is mutually affected by heterodimer formation (Bolli et al., 2009; Brodska et al., 2017). Hence, a fraction of NPMwt is localized in the cytoplasm and, conversely, a part of NPMmut can be found in the nucleoli, despite the NoLS loss (Brodska et al., 2016a, b). The precise localization of the mutated NPM further depends on the mutation type (Brodska et al., 2017) and is influenced by drugs causing NPM delocalization (actinomycin D, (Brodska et al., 2016a, b)) or stabilization of NPM oligomers (all-trans retinoic acid (ATRA) (Šašinková et al, 2018)).

Besides the oligomerization, which is in fact a self-interaction, many NPM-interacting partners were found to interact also with NPMmut. Aberrant cytoplasmic localization, associated with a loss of function or with degradation of the partner proteins, was then observed as a consequence of NPM mutation (Colombo et al., 2006; Vascotto et al., 2014).

The presence of NPMmut perturbs the activity of the tumor suppressor p14Arf (den Besten et al., 2005). As p14Arf interacts with the NPM Nterminus, it is reasonable to conclude that the interaction is preserved in NPMmut and that delocalization of p14Arf into the cytoplasm accounts for the inhibition of p14Arf activity. Indeed, the p14Arf was detected in the cytoplasm of NPMmut expressing cells and its overall cellular level was lowered in comparison with NPMwt-only expressing cells, likely due to an enhanced degradation (Colombo et al., 2006). NPMmut-induced cytoplasmic localization of APE1 was associated with impaired BER activity and with higher cell sensitivity to DNA-damaging agents (Vascotto et al., 2014). Cytoplasmic delocalization of the transcription factor PU.1 and concurrent upregulation of HOX genes was observed in cells with NPMmut (Brunetti et al., 2018; Gu et al., 2018). It is broadly assumed, that Nterminal oligomerization domain is also the domain through which NPM interactions occur (Meani and Alcalay, 2009; Di Matteo et al., 2017) and that NPMmut thus generally interacts with NPMwt partners. However, numerous observations indicate that the interactome of NPMmut is not identical with that of NPMwt.

In our recent work, we have shown that NPM interaction with NCL is compromised by the Cterminal NPM mutation (Šašinková et al., 2018). Loss of interaction with G-quadruplex structures in ribosomal DNA due to the mutation was also reported (Chiarella et al., 2013). As the RNA-binding domain is located near the mutated part of NPMmut, the interaction between NPM and RNAs is likely to be influenced by the mutation, too. Indeed, RNA binding to NPM, detected by co-immunoprecipitation, was significantly reduced in cells with NPM mutation (Sakashita et al., 2018).

Interestingly, the presence of NPM mutation was associated with enhanced MEF/ELF4-mediated MDM2 transcription and with increased MDM2 mRNA level in malignant cells. However, although the MEF/ELF4 was proved to interact with the Nterminal domain of NPMwt, the interaction was lost for the NPMmut. In this case, the increased MEF/ELF4 activity is likely caused by lower amount of the inhibitory NPMwt in the nucleolus due to the mutation (Ando et al., 2013).

The NPM region within the residues 187–259 binds cmyc (Li et al., 2008). By similarity with NCL, one could expect that the association is disrupted by the NPM mutation. On the other hand, the tumor suppressor Fbw7γ, regulating c-myc, interacts with the NPM Nterminus and has been demonstrated to be displaced to the cytoplasm and degraded due to interaction with NPMmut (Bonetti et al., 2008; Di Matteo et al., 2017). As a consequence, stabilization and enhanced activity of cmyc occurred in cells harboring NPMmut (Bonetti et al., 2008).

8. Potential NPM targeting in AML therapy

AML patients with NPMmut without concomitant mutations belong to the category with good prognosis after intensive chemotherapy. This may be due to fragile nucleolar structures, which may be more efficiently targeted by cytotoxic drugs. However, as the risk of relapse is still considerable, targeting of NPM, either wt or mut, remains worth exploring. Various small molecules were tested for the ability to potentiate the apoptosis and dampen the leukemic burden. These include inhibitors of the oligomerization, causing depletion of NPMwt pool, or inhibitors of the nuclear export, targeting NPMmut back to the nucleus (Di Matteo et al., 2016).

One of the first putative oligomer inhibitors, NSC348884, exhibited promising activity in NPMmut-harboring cells, sensitizing them to ATRA- or cytarabine-induced apoptosis (Qi et al., 2008; Balusu et al., 2011). However, the effect on NPM oligomerization was hard to detect

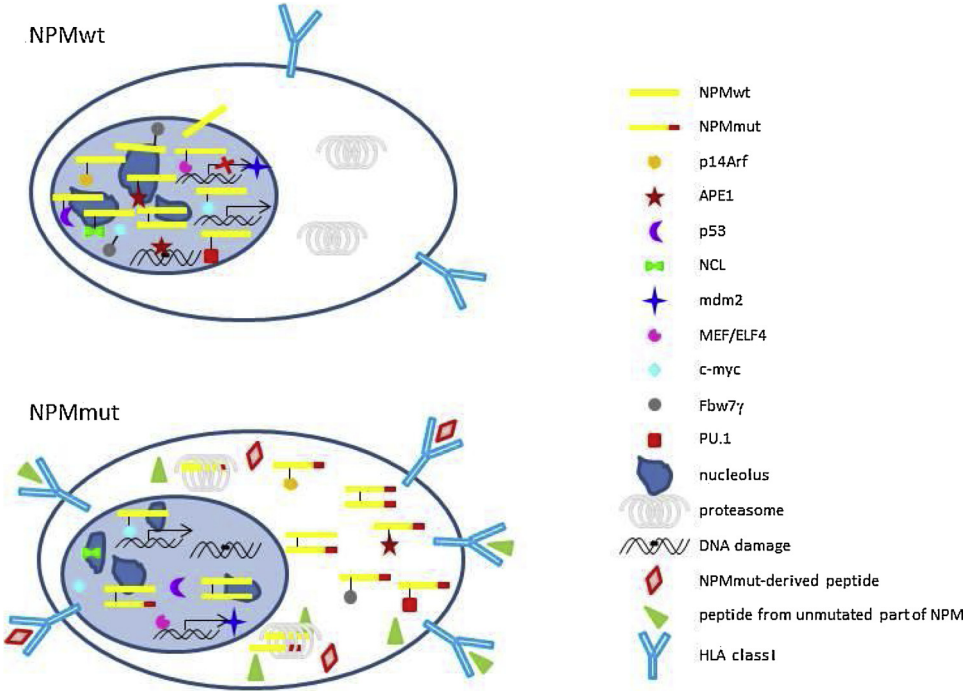

Fig. 1. Schematic illustration of possible consequences of NPM mutation in AML.

due to massive p53-dependent apoptosis induced in cells irrespectively of their NPM status (Holoubek et al., 2018). An alternative inhibitory compound, the small synthetic peptide N6L, was originally designed to target cellsurface NCL (Destouches et al., 2011). Later on, it was found to interact also with the Nterminal domain of NPM and to interfere with NPM association with partner proteins (De Cola et al., 2018). Therefore, it was presumed to initiate apoptosis preferentially in cells containing NPMmut, owing to the expected nuclear relocalization of the released pro-apoptotic proteins. However, the inversed effect was observed: N6L launched p53-dependent apoptosis in NPMwt, but not in NPMmut cells. We speculate that in this case, N6L induced disruption of NPMwt oligomers rather than NPMmut uncoupling from its interacting partners. Importantly, N6L sensitized NPMmut-harboring cells to doxorubicin and/or cytarabine treatment in the above-mentioned study. Another approach using RNA aptamers to target the NPM oligomerization also showed promising results (Jian et al., 2009).

The nuclear exporter Crm1 (also denoted as XPO1) is known to export most of the crucial tumor suppressors, including p53, p21, p73, and also NPM (Turner et al., 2012). Enhanced Crm1 expression was associated with worse prognosis in AML patients (Kojima et al., 2013). Therefore, numerous selective inhibitors of nuclear export (SINE) were analysed for their ability to reduce leukemic burden (Talati and Sweet, 2018). The most promising Crm1 inhibitor, selinexor (KPT-330), induced p53-dependent apoptosis and differentiation in leukemic cell lines (Ranganathan et al., 2012) and prolonged the survival in leukemia mouse model (Ranganathan et al., 2016). A Phase I clinical trial showed its safety and effectivity as monotherapy for relapsed or refractory AML (Garzon et al., 2017). Synergistic effect in AML therapy was observed when selinexor was combined with Topoisomerase II inhibitors (Ranganathan et al., 2016) or other DNA-damaging agents (Kashyap et al., 2016), fludarabine and cytarabine (Alexander et al., 2016), or HiDAC/Mito regimen (Amy et al., 2018). Recently, Gu et al (Gu et al., 2018) demonstrated, that selinexor treatment of patient-derived xenotransplant model of AML with NPMmut caused nuclear retention of

Table 1
Possible consequences of NPM mutation.

manifestation of the mutation	consequence in cellular processes	reference(s)
insufficient NPM level in nucleoli	lower ribogenesis – slower proliferation	(Derenzini et al., 2018)
	fragile nucleoli – p53-mediated apoptosis	(Derenzini et al., 2018)
NPM in cytoplasm	delocalization of APE1 – reduced DDR	(Vascotto et al., 2014)
	delocalization of p14Arf – dysregulation of p53/mdm2/Arf apoptotic pathway	(Colombo et al., 2006)
	delocalization of Fbw7g – enhanced activity of c-MYC	(Bonetti et al., 2008)
	delocalization of PU.1 – upregulation of HOX genes	(Brunetti et al., 2018; Gu et al., 2018)
	lower aberrant centrosome duplication	(Chan and Lim, 2015)
	higher immunogenicity due to better NPM accessibility for proteasome	(Kuzelova et al., 2015; Kuželová et al., 2018b)
altered AA sequence at the C-terminus	generation of a novel NES	(Bolli et al., 2007; Falini et al., 2006)
	loss of interaction with NCL	(Šašinková et al, 2018)
	loss of association with rRNA	(Sakashita et al., 2018)
	loss of interaction with MEF/ELF4 – enhanced MDM2	(Ando et al., 2013)
	loss of interaction with p53?	
	loss of interaction with c-myc?	
	misfolding of the C-terminus	(Di Natale et al., 2015)
	neoantigen production	(Forghieri et al., 2018; Greiner et al., 2012; van der Lee et al., 2019)

NPM and of the transcription factor PU.1. In parallel, it reverted monocyte and granulocyte terminal differentiation, which had been originally disrupted due to NPMmut/PU.1 complex delocalization.

Other approaches employed higher susceptibility of cells with decreased nuclear NPM content to undergo apoptosis induced by DNA-damaging agents (Falini et al., 2011; Federici and Falini, 2013, 2013). For example, dactinomycin was successfully used to treat one NPMmut-patient, ineligible for intensive chemotherapy due to cardiologic reasons (Falini et al., 2015). On the other hand, DNA damage was shown to induce NPMmut relocalization to the nucleoli (Bailey et al., 2019), and the effects of NPM-targeting strategies are thus not easily predictable. Inhibitors of APE1/NPM interaction suppressed cell proliferation and synergized with therapeutically relevant DNA damaging agents (Poletto et al., 2016). The three-helical conformation of the NPM C-terminal domain also undergoes several changes following the mutation. Significant propensity to the aggregation due to aberrant folding was observed for the isolated Cterminal domain of the mutated NPM, inducing potential structure-related toxic properties of NPMmut (Di Natale et al., 2015). Compounds stabilizing the C-terminal domain also displayed moderate effect in relocalizing NPMmut into nucleoli (Urbaneja et al., 2017). Moreover, demethylating drugs or histonedeacetylase inhibitors are currently tested in various clinical trials and their effectivity should be investigated also in correlation with NPM mutation.

9. NPM targeting for immunotherapy

From both clinical and molecular point of view, AML is a very heterogenous disease. It is thus practically impossible to find unique genetic markers for diagnostics, and patient-specific sets of common mutations should be used to monitor the minimal residual disease (MRD). Similarly, specific target antigens for potential immunotherapeutic intervention of AML are lacking. Several general tumor-associated antigens, in particular Wilms´ Tumor 1 (WT1), were more or less successfully tested for vaccination in clinical trials with various modifications of vaccine length or adjuvans composition (Hofmann et al., 2015). With regard to the frequency of NPM mutations, the search for immunogenic peptides derived from NPM sequence is appealing. Indeed, several proofs of immunogenic potential of NPM were reported, considering either the neoantigens produced from the mutated NPM part or enhanced processing of both NPM forms due to their cytoplasmic localization. Two HLA-A*02-binding 9-mer peptides derived from the border between the unmutated and mutated regions of NPMmut were shown to induce specific response of CD8+ lymphocytes in AML NPMmut patients and in healthy donors (Greiner et al., 2012). Longer peptides from the same region also activated CD4+ lymphocytes from healthy donors. Recently, a naturally occurring T-cell receptor specific for a peptide from the mutated NPM C-terminal domain in the context of HLA-A*02 was found and cloned into donor CD4+ or CD8+ lymphocytes. These engineered immune cells were reactive against HLA-A*02-positive primary cells from AML patients with NPMmut and markedly reduced the tumor growth in the mouse model of NPMmut AML (van der Lee et al., 2019). The existence of T-cells specifically recognizing the mutated NPM Cterminal domain was confirmed by another study showing, in addition, that the appearance and persistence of these immune cells inversely correlate with the course of the minimal residual disease in NPMmut AML (Forghieri et al., 2018).

As the mutated sequence of NPM is rather short, the repertoire of HLA alleles with affinity to NPMmut-derived neoantigens is restricted. However, the increased amount of the mutated protein in the cytoplasm could facilitate NPM processing and enhance the presentation of peptides from the unmutated parts as well. In a similar way, immunopeptides from NPM interaction partners that are delocalized along with NPMmut might also be more efficiently processed and presented on HLA molecules. A statistical evaluation of HLA class I distribution revealed several HLA allelic groups with lower frequency in patients with NPMmut compared to NPMwt patients or to healthy individuals. Screening of NPM-derived immunopeptides with high potency to bind the selected underrepresented HLA alleles (B*40, B*07) uncovered several candidate peptides from the unmutated part of NPM (Kuzelova et al., 2015). An effect of patient HLA class I type on the overall survival was later demonstrated in NPMmut AML (Kuželová et al., 2018b).

Importantly, the anti-cancer immune responses are regulated at many levels. The inhibitory ligand PD-L1 (Programmed-death ligand of checkpoint inhibitor PD-1) is one of the most frequent immunotherapeutic targets in tumors, including hematologic malignancies. In a small cohort of 30 patients, surface expression of PD-L1 on leukemia stem cells was reported to be significantly higher in NPMmut group in comparison with the NPMwt (Greiner et al., 2017). Interestingly, differences observed using flow-cytometry measurements were not found on the mRNA level. This is in accordance with our previous findings that in AML patients, the surface expression of PD-L1 does not simply correlate with its mRNA level but depends on the ratio of individual PD-L1 transcript variants (Brodska et al., 2016a, b). Our results also confirm the existence of specific immunological features of NPMmut AML patients compared to the NPMwt group (Kuželová et al., 2018a). Thus, combination of chemotherapy with an immune system stimulation with the aim to eradicate the residual disease is a promising treatment strategy for this type of leukemia.

10. Conclusions

Since the discovery of Falini et al that NPM cytoplasmic localization and specific mutation correlated with distinct features of AML patients, hundreds of studies have been published about the consequences of this mutation. AML with NPM mutation received its own category in WHO molecular classification of AML and new therapy recommendations were included in ELN risk stratification (Döhner et al, 2017). Many studies are currently ongoing with the aim to elucidate how NPM mutation contributes to leukemogenesis and how to take advantage of specific features of this AML subgroup to improve the therapy. We provide a summary of the current knowledge in this field, with an emphasis on the interaction network of the mutated protein and its possible therapeutic targeting.

In general, two scenarios may occur as a consequence of the mutation. First, the most important characteristic of the mutated NPM is the displacement from nucleoli to the cytoplasm. Persisting interaction of other proteins with the unmutated part of NPM may induce their delocalization. This is usually associated with a loss of function of the interaction partner, which is physically separated from its site of action. This mechanism was documented for p14Arf, APE1 or Fbw7γ, for example. The second possibility involves reduced capacity of the mutated NPM to form complex with an interaction partner and thereby induced deregulation of the expression and/or activity of the interacting protein, such as NCL or MEF/ELF4. Moreover, the ability of both wild-type and mutated NPM to form homo- and heterooligomers results in the presence of a small fraction of NPMmut in the nucleoli as well as of NPMwt in the cytoplasm, which further enhances the intricacy of NPM function and interaction network.

Therapies based on the drugs interfering with NPM oligomerization, interaction capacity, or with its aberrant localization appear promising. However, it is necessary to be aware of possible side effects associated with simultaneous targeting of NPMwt, such as abrogation of the ribogenesis in healthy cells.

The immunogenic potential of neoantigens derived from the mutated C-terminus of NPM, as well as an enhanced processing of immunopeptides from the unmutated part of NPM due to its cytoplasmic localization offer the opportunity for immunotherapeutic intervention in AML with NPMmut. This approach should likely be beneficial especially in combination of chemotherapy with immunotherapeutic methods, e.g. with vaccination or immune checkpoint inhibition.

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References

Abe, M., Lin, J., Nagata, K., Okuwaki, M., 2018. Selective regulation of type II interferon-inducible genes by NPM1/nucleophosmin. FEBS Lett. 592, 244–255.
Albiero, E., Madeo, D., Bolli, N., Giaretta, I., Bona, E.D., Martelli, M.F., et al., 2007. Identification and functional characterization of a cytoplasmic nucleophosmin leukaemic mutant generated by a novel exon-11 NPM1 mutation. Leukemia 21, 1099–1103.
Alexander, T.B., Lacayo, N.J., Choi, J.K., Ribeiro, R.C., Pui, C., Rubnitz, J.E., 2016. Phase I study of selinexor, a selective inhibitor of nuclear export, in combination with fludarabine and cytarabine, in pediatric relapsed or refractory acute leukemia. J. Clin. Oncol. 34, 4094–4101.
Amin, M.A., Matsunaga, S., Uchiyama, S., Fukui, K., 2008a. Depletion of nucleophosmin leads to distortion of nucleolar and nuclear structures in HeLa cells. Biochem. J. 415, 345–351.
Amin, M.A., Matsunaga, S., Uchiyama, S., Fukui, K., 2008b. Nucleophosmin is required for chromosome congression, proper mitotic spindle formation, and kinetochore-microtubule attachment in HeLa cells. FEBS Lett. 582, 3839–3844.
Amy, Y.W., Weiner, H., Green, M., Chang, H., Fulton, N., Richard, A.L., et al., 2018. A phase I study of selinexor in combination with high-dose cytarabine and mitoxantrone for remission induction in patients with acute myeloid leukemia. J. Hematol. Oncol. 11, 4.
Ando, K., Tsushima, H., Matsuo, E., Horio, K., Tominaga-Sato, S., Imanishi, D., et al., 2013. Mutations in the nucleolar phosphoprotein, nucleophosmin, promote the expression of the oncogenic transcription factor MEF/ELF4 in leukemia cells and potentiates transformation. J. Biol. Chem. 288, 9457.
Arber, D.A., Orazi, A., Hasserjian, R., Thiele, J., Borowitz, M.J., Le Beau, M.M., et al., 2016. The 2016 revision to the world health organization classification of myeloid neoplasms and acute leukemia. Blood 127, 2391–2405.
Arregi, I., Falces, J., Olazabal-Herrero, A., Alonso-Mariño, M., Taneva, S.G., Rodríguez, J.A., et al., 2015. Leukemia-associated mutations in nucleophosmin alter recognition by CRM1: molecular basis of aberrant transport. PLoS One 10, e0130610.
Bailey, G.D., Qutob, H.M.H., Akhtar, A., Russell, N.H., Seedhouse, C.H., 2019. DNA damage corrects the aberrant cytoplasmic localisation of nucleophosmin in NPM1 mutated acute myeloid leukaemia. Br. J. Haematol.
Bains, A., Luthra, R., Medeiros, L.J., Zuo, Z., 2011. FLT3 and NPM1 mutations in myelodysplastic syndromes: frequency and potential value for predicting progression to acute myeloid leukemia. Am. J. Clin. Pathol. 135, 62–69.
Balusu, R., Fiskus, W., Rao, R., Chong, D.G., Nalluri, S., Mudunuru, U., et al., 2011. Targeting levels or oligomerization of nucleophosmin 1 induces differentiation and loss of survival of human AML cells with mutant NPM1. Blood 118, 3096–3106.
Banerjee, P.R., Mitrea, D.M., Kriwacki, R.W., Deniz, A.A., 2016. Asymmetric modulation of protein order-disorder transitions by phosphorylation and partner binding. Angew. Chem. Int. Ed. Engl. 55, 1675–1679.
Bergstralh, D.T., Conti, B.J., Moore, C.B., Brickey, W.J., Taxman, D.J., Ting, J.P.-, 2007. Global functional analysis of nucleophosmin in taxol response, cancer, chromatin regulation, and ribosomal DNA transcription. Exp. Cell Res. 313, 65–76.
Bertwistle, D., Sugimoto, M., Sherr, C.J., 2004. Physical and functional interactions of the arf tumor suppressor protein with nucleophosmin/B23. Mol. Cell. Biol. 24, 985–996.
Bolli, N., Nicoletti, I., De Marco, M.F., Bigerna, B., Pucciarini, A., Mannucci, R., et al., 2007. Born to be exported: COOH-terminal nuclear export signals of different strength ensure cytoplasmic accumulation of nucleophosmin leukemic mutants. Cancer Res. 67, 6230–6237.
Bolli, N., De Marco, M.F., Martelli, M.P., Bigerna, B., Pucciarini, A., Rossi, R., et al., 2009. A dose-dependent tug of war involving the NPM1 leukaemic mutant, nucleophosmin, and ARF. Leukemia 23, 501–509.
Bonetti, P., Davoli, T., Sironi, C., Amati, B., Pelicci, P.G., Colombo, E., 2008. Nucleophosmin and its AML-associated mutant regulate c-myc turnover through Fbw7 gamma. J. Cell Biol. 182, 19–26.
Booth, D.G., Earnshaw, W.C., 2017. Ki-67 and the chromosome periphery compartment in mitosis. Trends Cell Biol. 27, 906–916.
Booth, D.G., Takagi, M., Sanchez-Pulido, L., Petfalski, E., Vargiu, G., Samejima, K., et al., 2014. Ki-67 is a PP1-interacting protein that organises the mitotic chromosome periphery. Elife 3, e01641.
Borer, R.A., Lehner, C.F., Eppenberger, H.M., Nigg, E.A., 1989. Major nucleolar proteins shuttle between nucleus and cytoplasm. Cell 56, 379–390.
Box, J.K., Paquet, N., Adams, M.N., Boucher, D., Bolderson, E., O'Byrne, K.J., et al., 2016. Nucleophosmin: from structure and function to disease development. BMC Mol. Biol. 17, 19.
Brodska, B., Holoubek, A., Otevrelova, P., Kuzelova, K., 2016a. Low-dose actinomycin-D induces redistribution of wild-type and mutated nucleophosmin followed by cell death in leukemic cells. J. Cell. Biochem. 117, 1319–1329.
Brodska, B., Otevrelova, P., Kuzelova, K., 2016b. Correlation of PD-L1 surface expression on leukemia cells with the ratio of PD-L1 mRNA variants and with electrophoretic mobility. Cancer Immunol. Res. 4, 815–819.
Brodska, B., Kracmarova, M., Holoubek, A., Kuzelova, K., 2017. Localization of AML-related nucleophosmin mutant depends on its subtype and is highly affected by its interaction with wild-type NPM. PLoS One 12, e0175175.
Brunetti, L., Gundry, M.C., Sorcini, D., Guzman, A.G., Huang, Y., Ramabadran, R., et al., 2018. Mutant NPM1 maintains the leukemic state through HOX expression. Cancer Cell 34, 512 e9.
Chan, N., Lim, T.M., 2015. Cytoplasmic nucleophosmin has elevated T199 phosphorylation upon which G2/M phase progression is dependent. Sci. Rep. 5, 11777.
Chan, W.Y., Liu, Q.R., Borjigin, J., Busch, H., Rennert, O.M., Tease, L.A., et al., 1989. Characterization of the cDNA encoding human nucleophosmin and studies of its role in normal and abnormal growth. Biochemistry 28, 1033–1039.
Chan, L.W.C., Lin, X., Yung, G., Lui, T., Chiu, Y.M., Wang, F., et al., 2015. Novel structural co-expression analysis linking the NPM1-associated ribosomal biogenesis network to chronic myelogenous leukemia. Sci. Rep. 5, 10973.
Chang, J.H., Lin, J.Y., Wu, M.H., Yung, B.Y., 1998. Evidence for the ability of nucleophosmin/B23 to bind ATP. Biochem. J. 329 (Pt 3), 539–544.
Chen, W., Konoplev, S., Medeiros, L.J., Koeppen, H., Leventaki, V., Vadhan-Raj, S., et al., 2009. Cuplike nuclei (prominent nuclear invaginations) in acute myeloid leukemia are highly associated with FLT3 internal tandem duplication and NPM1 mutation. Cancer 115, 5481–5489.
Chen, S., He, H., Wang, Y., Liu, L., Liu, Y., You, H., et al., 2018. Poor prognosis of nucleophosmin overexpression in solid tumors: a meta-analysis. BMC Cancer 18, 838.
Chiarella, S., De Cola, A., Scaglione, G.L., Carletti, E., Graziano, V., Barcaroli, D., et al., 2013. Nucleophosmin mutations alter its nucleolar localization by impairing G-quadruplex binding at ribosomal DNA. Nucleic Acids Res. 41, 3228–3239.
Ching, R.H.H., Lau, E.Y.T., Ling, P.M.T., Lee, J.M.F., Ma, M.K.F., Cheng, B.Y.L., et al., 2015. Phosphorylation of nucleophosmin at threonine 234/237 is associated with HCC metastasis. Oncotarget 6, 43483–43495.
Colombo, E., Marine, J.C., Danovi, D., Falini, B., Pelicci, P.G., 2002. Nucleophosmin regulates the stability and transcriptional activity of p53. Nat. Cell Biol. 4, 529–533.
Colombo, E., Martinelli, P., Zamponi, R., Shing, D.C., Bonetti, P., Luzi, L., et al., 2006. Delocalization and destabilization of the arf tumor suppressor by the leukemia-associated NPM mutant. Cancer Res. 66, 3044–3050.
Cordell, J.L., Pulford, K.A., Bigerna, B., Roncador, G., Banham, A., Colombo, E., et al., 1999. Detection of normal and chimeric nucleophosmin in human cells. Blood 93, 632–642.
Dalenc, F., Drouet, J., Ader, I., Delmas, C., Rochaix, P., Favre, G., et al., 2002. Increased expression of a COOH-truncated nucleophosmin resulting from alternative splicing is associated with cellular resistance to ionizing radiation in HeLa cells. Int. J. Cancer 100, 662–668.
De Cola, A., Franceschini, M., Di Matteo, A., Colotti, G., Celani, R., Clemente, E., et al., 2018. N6L pseudopeptide interferes with nucleophosmin protein-protein interactions and sensitizes leukemic cells to chemotherapy. Cancer Lett. 412, 272–282.
De Koning, L., Corpet, A., Haber, J.E., Almouzni, G., 2007. Histone chaperones: an escort network regulating histone traffic. Nat. Struct. Mol. Biol. 14, 997–1007.
De Propris, M.S., Raponi, S., Diverio, D., Milani, M.L., Meloni, G., Falini, B., et al., 2011. High CD33 expression levels in acute myeloid leukemia cells carrying the nucleophosmin (NPM1) mutation. Haematologica 96, 1548–1551.
Delsing Malmberg, E., Johansson Alm, S., Nicklasson, M., Lazarevic, V., Ståhlman, S., Samuelsson, T., et al., 2018. Minimal residual disease assessed with deep sequencing of NPM1 mutations predicts relapse after allogeneic stem cell transplant in AML. Leuk. Lymphoma 0, 1–9.
den Besten, W., Kuo, M.L., Williams, R.T., Sherr, C.J., 2005. Myeloid leukemia-associated nucleophosmin mutants perturb p53-dependent and independent activities of the arf tumor suppressor protein. Cell Cycle 4, 1593–1598.
Derenzini, E., Rossi, A., Treré, D., 2018. Treating hematological malignancies with drugs inhibiting ribosome biogenesis: when and why. J. Hematol. Oncol. 11, 75.
Destouches, D., Page, N., Hamma-Kourbali, Y., Machi, V., Chaloin, O., Frechault, S., et al., 2011. A simple approach to cancer therapy afforded by multivalent pseudopeptides that target cell-surface nucleoproteins. Cancer Res. 71, 3296–3305.
Dhar, S.K., St Clair, D.K., 2009. Nucleophosmin blocks mitochondrial localization of p53 and apoptosis. J. Biol. Chem. 284, 16409–16418.
Dhar, S.K., Lynn, B.C., Daosukho, C., St Clair, D.K., 2004. Identification of nucleophosmin as an NF-kappaB co-activator for the induction of the human SOD2 gene. J. Biol. Chem. 279, 28209–28219.
Di Matteo, A., Franceschini, M., Chiarella, S., Rocchio, S., Travaglini-Allocatelli, C., Federici, L., 2016. Molecules that target nucleophosmin for cancer treatment: an update. Oncotarget 7, 44821–44840.
Di Matteo, A., Franceschini, M., Paiardini, A., Grottesi, A., Chiarella, S., Rocchio, S., et al., 2017. Structural investigation of nucleophosmin interaction with the tumor suppressor Fbw7γ. Oncogenesis 6, e379.
Di Natale, C., Scognamiglio, P.L., Cascella, R., Cecchi, C., Russo, A., Leone, M., et al., 2015. Nucleophosmin contains amyloidogenic regions that are able to form toxic aggregates under physiological conditions. FASEB J. 29, 3689–3701.
Döhner, H., Estey, E., Grimwade, D., Amadori, S., Appelbaum, F.R., Büchner, T., et al., 2017. Diagnosis and management of AML in adults: 2017 ELN recommendations from an international expert panel. Blood 129, 424–447.
Duan-Porter, W.D., Woods Jr, V.L., Maurer, K.D., Li, S., Rosen, A., 2014. Dynamic conformations of nucleophosmin (NPM1) at a key monomer-monomer interface affect oligomer stability and interactions with granzyme B. PLoS One 9, e115062.
Dumbar, T.S., Gentry, G.A., Olson, M.O., 1989. Interaction of nucleolar phosphoprotein B23 with nucleic acids. Biochemistry 28, 9495–9501.
Duployez, N., Chebrek, L., Helevaut, N., Fournier, E., Bemba, M., Caillault, A., et al., 2018. A novel type of NPM1 mutation characterized by multiple internal tandem repeats in a case of cytogenetically normal acute myeloid leukemia. Haematologica 103, e577.
Dutta, S., Akey, I.V., Dingwall, C., Hartman, K.L., Laue, T., Nolte, R.T., et al., 2001. The

crystal structure of nucleoplasmin-core: implications for histone binding and nucleosome assembly. Mol. Cell 8, 841–853.
Ebert, B.L., 2010. Genetic deletions in AML and MDS. Best Pract. Res. Clin. Haematol. 23, 457–461.
Eirín-López, J.M., Frehlick, L.J., Ausió, J., 2006. Long-term evolution and functional diversification in the members of the nucleophosmin/nucleoplasmin family of nuclear chaperones. Genetics 173, 1835–1850.
Enomoto, T., Lindstrom, M.S., Jin, A., Ke, H., Zhang, Y., 2006. Essential role of the B23/NPM core domain in regulating ARF binding and B23 stability. J. Biol. Chem. 281, 18463–18472.
Falini, B., Mecucci, C., Tiacci, E., Alcalay, M., Rosati, R., Pasqualucci, L., et al., 2005. Cytoplasmic nucleophosmin in acute myelogenous leukemia with a normal karyotype. N. Engl. J. Med. 352, 254–266.
Falini, B., Bolli, N., Shan, J., Martelli, M.P., Liso, A., Pucciarini, A., et al., 2006. Both carboxy-terminus NES motif and mutated tryptophan(s) are crucial for aberrant nuclear export of nucleophosmin leukemic mutants in NPMc+ AML. Blood 107, 4514–4523.
Falini, B., Gionfriddo, I., Cecchetti, F., Ballanti, S., Pettirossi, V., Martelli, M.P., 2011. Acute myeloid leukemia with mutated nucleophosmin (NPM1): Any hope for a targeted therapy? Blood Rev. 25, 247–254.
Falini, B., Brunetti, L., Martelli, M.P., 2015. Dactinomycin in NPM1-mutated acute myeloid leukemia. N. Engl. J. Med. 373, 1180–1182.
Fankhauser, C., Izaurralde, E., Adachi, Y., Wingfield, P., Laemmli, U.K., 1991. Specific complex of human immunodeficiency virus type 1 rev and nucleolar B23 proteins: dissociation by the rev response element. Mol. Cell. Biol. 11, 2567–2575.
Federici, L., Falini, B., 2013. Nucleophosmin mutations in acute myeloid leukemia: a tale of protein unfolding and mislocalization. Protein Sci. 22, 545–556.
Federici, L., Arcovito, A., Scaglione, G.L., Scaloni, F., Lo Sterzo, C., Di Matteo, A., et al., 2010. Nucleophosmin C-terminal leukemia-associated domain interacts with G-rich quadruplex forming DNA. J. Biol. Chem. 285, 37138–37149.
Forghieri, F., Comoli, P., Marasca, R., Potenza, L., Luppi, M., 2018. Minimal/measurable residual disease monitoring in NPM1-mutated acute myeloid leukemia: a clinical viewpoint and perspectives. Int. J. Mol. Sci. 19.
Frehlick, L.J., Eirín-López, J.M., Ausió, J., 2007. New insights into the nucleophosmin/nucleoplasmin family of nuclear chaperones. Bioessays 29, 49–59.
Garzon, R., Savona, M., Baz, R., Andreeff, M., Gabrail, N., Gutierrez, M., et al., 2017. A phase 1 clinical trial of single-agent selinexor in acute myeloid leukemia. Blood 129, 3165–3174.
Greiner, J., Ono, Y., Hofmann, S., Schmitt, A., Mehring, E., Götz, M., et al., 2012. Mutated regions of nucleophosmin 1 elicit both CD4(+) and CD8(+) T-cell responses in patients with acute myeloid leukemia. Blood 120, 1282–1289.
Greiner, J., Hofmann, S., Schmitt, M., Götz, M., Wiesneth, M., Schrezenmeier, H., et al., 2017. Acute myeloid leukemia with mutated nucleophosmin 1: an immunogenic acute myeloid leukemia subtype and potential candidate for immune checkpoint inhibition. Haematologica 102, e501.
Grisendi, S., Pandolfi, P.P., 2005. NPM mutations in acute myelogenous leukemia. N. Engl. J. Med. 352, 291–292.
Grisendi, S., Bernardi, R., Rossi, M., Cheng, K., Khandker, L., Manova, K., et al., 2005. Role of nucleophosmin in embryonic development and tumorigenesis. Nature 437, 147–153.
Grisendi, S., Mecucci, C., Falini, B., Pandolfi, P.P., 2006. Nucleophosmin and cancer. Nat. Rev. Cancer 6, 493–505.
Grummitt, C.G., Townsley, F.M., Johnson, C.M., Warren, A.J., Bycroft, M., 2008. Structural consequences of nucleophosmin mutations in acute myeloid leukemia. J. Biol. Chem. 283, 23326–23332.
Gu, X., Ebrahem, Q., Mahfouz, R.Z., Hasipek, M., Enane, F., Radivoyevitch, T., et al., 2018. Leukemogenic nucleophosmin mutation disrupts the transcription factor hub that regulates granulomonocytic fates. J. Clin. Invest. 128, 4260–4279.
Gurumurthy, M., Tan, C.H., Ng, R., Zeiger, L., Lau, J., Lee, J., et al., 2008. Nucleophosmin interacts with HEXIM1 and regulates RNA polymerase II transcription. J. Mol. Biol. 378, 302–317.
Hamilton, G., Abraham, A.G., Morton, J., Sampson, O., Pefani, D.E., Khoronenkova, S., et al., 2014. AKT regulates NPM dependent ARF localization and p53mut stability in tumors. Oncotarget 5, 6142–6167.
Heath, E.M., Chan, S.M., Minden, M.D., Murphy, T., Shlush, L.I., Schimmer, A.D., 2017. Biological and clinical consequences of NPM1 mutations in AML. Leukemia 31, 798–807.
Herrera, J.E., Savkur, R., Olson, M.O., 1995. The ribonuclease activity of nucleolar protein B23. Nucleic Acids Res. 23, 3974–3979.
Herrera, J.E., Correia, J.J., Jones, A.E., Olson, M.O., 1996. Sedimentation analyses of the salt- and divalent metal ion-induced oligomerization of nucleolar protein B23. Biochemistry 35, 2668–2673.
Hingorani, K., Szebeni, A., Olson, M.O., 2000. Mapping the functional domains of nucleolar protein B23. J. Biol. Chem. 275, 24451–24457.
Hisaoka, M., Nagata, K., Okuwaki, M., 2014. Intrinsically disordered regions of nucleophosmin/B23 regulate its RNA binding activity through their inter- and intra-molecular association. Nucleic Acids Res. 42, 1180–1195.
Hofmann, S., Mead, A., Malinovskis, A., Hardwick, N.R., Guinn, B., 2015. Analogue peptides for the immunotherapy of human acute myeloid leukemia. Cancer Immunol. Immunother. 64, 1357–1367.
Höllein, A., Meggendorfer, M., Dicker, F., Jeromin, S., Nadarajah, N., Kern, W., et al., 2018. NPM1 mutated AML can relapse with wild-type NPM1: persistent clonal hematopoiesis can drive relapse. Blood Adv. 2, 3118–3125.
Holoubek, A., Herman, P., Sýkora, J., Brodská, B., Humpolíčková, J., Kráčmarová, M., et al., 2018. Monitoring of nucleophosmin oligomerization in live cells. Methods Appl. Fluoresc. 6, 035016.
Itahana, K., Bhat, K.P., Jin, A., Itahana, Y., Hawke, D., Kobayashi, R., et al., 2003. Tumor suppressor ARF degrades B23, a nucleolar protein involved in ribosome biogenesis and cell proliferation. Mol. Cell 12, 1151–1164.
Ivey, A., Hills, R.K., Simpson, M.A., Jovanovic, J.V., Gilkes, A., Grech, A., et al., 2016. Assessment of minimal residual disease in standard-risk AML. N. Engl. J. Med. 374, 422–433.
Jeziskova, I., Semerad, L., Dvorakova, D., Janeckova, V., Culen, M., Kunetkova, T., et al., 2017. Novel complex mutation in NPM1 gene in patient with acute myeloid leukemia. Leuk. Lymphoma 58, 746–748.
Jian, Y., Gao, Z., Sun, J., Shen, Q., Feng, F., Jing, Y., et al., 2009. RNA aptamers interfering with nucleophosmin oligomerization induce apoptosis of cancer cells. Oncogene 28, 4201–4211.
Kashyap, T., Argueta, C., Aboukameel, A., Unger, T.J., Klebanov, B., Mohammad, R.M., et al., 2016. Selinexor, a selective inhibitor of nuclear export (SINE) compound, acts through NF-κB deactivation and combines with proteasome inhibitors to synergistically induce tumor cell death. Oncotarget 7, 78883–78895.
Kaur, M., Drake, A.C., Hu, G., Rudnick, S., Chen, Q., Phennicie, R., et al., 2019. Induction and therapeutic targeting of human NPM1c+ myeloid leukemia in the presence of autologous immune system in mice. J. Immunol.
Kawaguchi-Ihara, N., Itoh, M., Murohashi, I., Tohda, S., 2016. Establishment of a quenching probe method for detection of NPM1 mutations in acute myeloid leukemia cells. Oncol. Lett. 11, 2429–2432.
Kerr, L.E., Birse-Archbold, J.L., Short, D.M., McGregor, A.L., Heron, I., Macdonald, D.C., et al., 2007. Nucleophosmin is a novel bax chaperone that regulates apoptotic cell death. Oncogene 26, 2554–2562.
Koike, A., Nishikawa, H., Wu, W., Okada, Y., Venkitaraman, A.R., Ohta, T., 2010. Recruitment of phosphorylated NPM1 to sites of DNA damage through RNF8-dependent ubiquitin conjugates. Cancer Res. 70, 6746–6756.
Kojima, K., Kornblau, S.M., Ruvolo, V., Dilip, A., Duvvuri, S., Davis, R.E., et al., 2013. Prognostic impact and targeting of CRM1 in acute myeloid leukemia. Blood 121, 4166–4174.
Krönke, J., Bullinger, L., Teleanu, V., Tschürtz, F., Gaidzik, V.I., Kühn, M.W.M., et al., 2013. Clonal evolution in relapsed NPM1-mutated acute myeloid leukemia. Blood 122, 100–108.
Kurki, S., Peltonen, K., Latonen, L., Kiviharju, T.M., Ojala, P.M., Meek, D., et al., 2004. Nucleolar protein NPM interacts with HDM2 and protects tumor suppressor protein p53 from HDM2-mediated degradation. Cancer Cell 5, 465–475.
Kuzelova, K., Brodska, B., Fuchs, O., Dobrovolna, M., Soukup, P., Cetkovsky, P., 2015. Altered HLA class I profile associated with type A/D nucleophosmin mutation points to possible anti-nucleophosmin immune response in acute myeloid leukemia. PLoS One 10, e0127637.
Kuželová, K., Brodská, B., Otevřelová, P., Šálek, C., 2018a. 90PImmunosuppressive mechanisms in acute myeloid leukemia with mutated nucleophosmin. Ann. Oncol. 29.
Kuželová, K., Brodská, B., Schetelig, J., Röllig, C., Ráčil, Z., Walz, J.S., et al., 2018b. Association of HLA class I type with prevalence and outcome of patients with acute myeloid leukemia and mutated nucleophosmin. PLoS One 13, e0204290.
La Starza, R., Barba, G., Demeyer, S., Pierini, V., Di Giacomo, D., Gianfelici, V., et al., 2016. Deletions of the long arm of chromosome 5 define subgroups of T-cell acute lymphoblastic leukemia. Haematologica 101, 951–958.
Lambert, B., Buckle, M., 2006. Characterisation of the interface between nucleophosmin (NPM) and p53: potential role in p53 stabilisation. FEBS Lett. 580, 345–350.
Leal, M.F., Mazzotti, T.K.F., Calcagno, D.Q., Cirilo, P.D.R., Martinez, M.C., Demachki, S., et al., 2014. Deregulated expression of nucleophosmin 1 in gastric cancer and its clinicopathological implications. BMC Gastroenterol. 14, 9.
Lee, C., Smith, B.A., Bandyopadhyay, K., Gjerset, R.A., 2005. DNA damage disrupts the p14ARF-B23(nucleophosmin) interaction and triggers a transient subnuclear redistribution of p14ARF. Cancer Res. 65, 9834–9842.
Lee, N., Kim, D., Kim, E., Park, S.J., Kwon, J., Shin, J., et al., 2014. Comparative interactomes of SIRT6 and SIRT7: implication of functional links to aging. Proteomics 14, 1610–1622.
Lee, H.H., Kim, H.S., Kang, J.Y., Lee, B.I., Ha, J.Y., Yoon, H.J., et al., 2007. Crystal structure of human nucleophosmin-core reveals plasticity of the pentamer-pentamer interface. Proteins 69, 672–678.
Lessard, F., Morin, F., Ivanchuk, S., Langlois, F., Stefanovsky, V., Rutka, J., et al., 2010. The ARF tumor suppressor controls ribosome biogenesis by regulating the RNA polymerase I transcription factor TTF-I. Mol. Cell 38, 539–550.
Li, Y.P., 1997. Protein B23 is an important human factor for the nucleolar localization of the human immunodeficiency virus protein tat. J. Virol. 71, 4098–4102.
Li, Z., Hann, S.R., 2013. Nucleophosmin is essential for c-myc nucleolar localization and c-myc-mediated rDNA transcription. Oncogene 32, 1988–1994.
Li, Y.P., Busch, R.K., Valdez, B.C., Busch, H., 1996. C23 interacts with B23, a putative nucleolar-localization-signal-binding protein. Eur. J. Biochem. 237, 153–158.
Li, J., Zhang, X., Sejas, D.P., Bagby, G.C., Pang, Q., 2004. Hypoxia-induced nucleophosmin protects cell death through inhibition of p53. J. Biol. Chem. 279, 41275–41279.
Li, J., Sejas, D.P., Burma, S., Chen, D.J., Pang, Q., 2007. Nucleophosmin suppresses oncogene-induced apoptosis and senescence and enhances oncogenic cooperation in cells with genomic instability. Carcinogenesis 28, 1163–1170.
Li, Z., Boone, D., Hann, S.R., 2008. Nucleophosmin interacts directly with c-myc and controls c-myc-induced hyperproliferation and transformation. Proc. Natl. Acad. Sci. U. S. A. 105, 18794–18799.
Lim, M.J., Wang, X.W., 2006. Nucleophosmin and human cancer. Cancer Detect. Prev. 30, 481–490.
Lin, J., Hisaoka, M., Nagata, K., Okuwaki, M., 2016. Functional characterization and efficient detection of nucleophosmin/NPM1 oligomers. Biochem. Biophys. Res. Commun.

Lin, J., Kato, M., Nagata, K., Okuwaki, M., 2017. Efficient DNA binding of NF-κB requires the chaperone-like function of NPM1. Nucleic Acids Res. 45, 3707–3723.
Lindström, M.S., 2011. NPM1/B23: a multifunctional chaperone in ribosome biogenesis and chromatin remodeling. Biochem. Res. Int. 2011, 195209.
Lischwe, M.A., Smetana, K., Olson, M.O.J., Busch, H., 1979. Proteins C23 and B23 are the major nucleolar silver staining proteins. Life Sci. 25, 701–708.
Liu, H.T., Yung, B.Y., 1999. In vivo interaction of nucleophosmin/B23 and protein C23 during cell cycle progression in HeLa cells. Cancer Lett. 144, 45–54.
Liu, H., Tan, B.C., Tseng, K.H., Chuang, C.P., Yeh, C., Chen, K., et al., 2007a. Nucleophosmin acts as a novel AP2alpha-binding transcriptional corepressor during cell differentiation. EMBO Rep. 8, 394–400.
Liu, X., Liu, Z., Jang, S., Ma, Z., Shinmura, K., Kang, S., et al., 2007b. Sumoylation of nucleophosmin/B23 regulates its subcellular localization, mediating cell proliferation and survival. Proc. Natl. Acad. Sci. U. S. A. 104, 9679–9684.
Luchinat, E., Chiarella, S., Franceschini, M., Di Matteo, A., Brunori, M., Banci, L., et al., 2018. Identification of a novel nucleophosmin-interaction motif in the tumor suppressor p14arf. FEBS J. 285, 832–847.
Maggi, L.B., Kuchenruether, M., Dadey, D.Y.A., Schwope, R.M., Grisendi, S., Townsend, R.R., et al., 2008. Nucleophosmin serves as a rate-limiting nuclear export chaperone for the mammalian ribosome. Mol. Cell. Biol. 28, 7050–7065.
Maiguel, D.A., Jones, L., Chakravarty, D., Yang, C., Carrier, F., 2004. Nucleophosmin sets a threshold for p53 response to UV radiation. Mol. Cell. Biol. 24, 3703–3711.
Mariano, A.R., Colombo, E., Luzi, L., Martinelli, P., Volorio, S., Bernard, L., et al., 2006. Cytoplasmic localization of NPM in myeloid leukemias is dictated by gain-of-function mutations that create a functional nuclear export signal. Oncogene 25, 4376–4380.
Meani, N., Alcalay, M., 2009. Role of nucleophosmin in acute myeloid leukemia. Expert Rev. Anticancer Ther. 9, 1283–1294.
Metzeler, K.H., Herold, T., Rothenberg-Thurley, M., Amler, S., Sauerland, M.C., Görlich, D., et al., 2016. Spectrum and prognostic relevance of driver gene mutations in acute myeloid leukemia. Blood 128, 686–698.
Mitrea, D.M., Grace, C.R., Buljan, M., Yun, M.K., Pytel, N.J., Satumba, J., et al., 2014. Structural polymorphism in the N-terminal oligomerization domain of NPM1. Proc. Natl. Acad. Sci. U. S. A. 111, 4466–4471.
Mitrea, D.M., Cika, J.A., Guy, C.S., Ban, D., Banerjee, P.R., Stanley, C.B., et al., 2016. Nucleophosmin integrates within the nucleolus via multi-modal interactions with proteins displaying R-rich linear motifs and rRNA. Elife 5 10.7554/eLife.13571.
Morris, S.W., Kirstein, M.N., Valentine, M.B., Dittmer, K.G., Shapiro, D.N., Saltman, D.L., et al., 1994. Fusion of a kinase gene, ALK, to a nucleolar protein gene, NPM, in non-Hodgkin's lymphoma. Science 263, 1281–1284.
Murano, K., Okuwaki, M., Hisaoka, M., Nagata, K., 2008. Transcription regulation of the rRNA gene by a multifunctional nucleolar protein, B23/nucleophosmin, through its histone chaperone activity. Mol. Cell. Biol. 28, 3114–3126.
Namboodiri, V.M.H., Dutta, S., Akey, I.V., Head, J.F., Akey, C.W., 2003. The crystal structure of drosophila NLP-core provides insight into pentamer formation and histone binding. Structure 11, 175–186.
Namboodiri, V.M.H., Akey, I.V., Schmidt-Zachmann, M.S., Head, J.F., Akey, C.W., 2004. The structure and function of xenopus NO38-core, a histone chaperone in the nucleolus. Structure 12, 2149–2160.
Nishimura, Y., Ohkubo, T., Furuichi, Y., Umekawa, H., 2002. Tryptophans 286 and 288 in the C-terminal region of protein B23.1 are important for its nucleolar localization. Biosci. Biotechnol. Biochem. 66, 2239–2242.
Nouri, K., Moll, J.M., Milroy, L.G., Hain, A., Dvorsky, R., Amin, E., et al., 2015. Biophysical characterization of nucleophosmin interactions with human immunodeficiency virus rev and herpes simplex virus US11. PLoS One 10, e0143634.
Ochs, R., Lischwe, M., O'Leary, P., Busch, H., 1983. Localization of nucleolar phosphoproteins B23 and C23 during mitosis. Exp. Cell Res. 146, 139–149.
Okuda, M., Horn, H.F., Tarapore, P., Tokuyama, Y., Smulian, A.G., Chan, P.K., et al., 2000. Nucleophosmin/B23 is a target of CDK2/cyclin E in centrosome duplication. Cell 103, 127–140.
Okuwaki, M., Iwamatsu, A., Tsujimoto, M., Nagata, K., 2001a. Identification of nucleophosmin/B23, an acidic nucleolar protein, as a stimulatory factor for in vitro replication of adenovirus DNA complexed with viral basic core proteins. J. Mol. Biol. 311, 41–55.
Okuwaki, M., Matsumoto, K., Tsujimoto, M., Nagata, K., 2001b. Function of nucleophosmin/B23, a nucleolar acidic protein, as a histone chaperone. FEBS Lett. 506, 272–276.
Okuwaki, M., Tsujimoto, M., Nagata, K., 2002. The RNA binding activity of a ribosome biogenesis factor, nucleophosmin/B23, is modulated by phosphorylation with a cell cycle-dependent kinase and by association with its subtype. Mol. Biol. Cell 13, 2016–2030.
Okuwaki, M., Sumi, A., Hisaoka, M., Saotome-Nakamura, A., Akashi, S., Nishimura, Y., et al., 2012. Function of homo- and hetero-oligomers of human nucleoplasmin/nucleophosmin family proteins NPM1, NPM2 and NPM3 during sperm chromatin remodeling. Nucleic Acids Res. 40, 4861–4878.
Patel, J.L., Schumacher, J.A., Frizzell, K., Sorrells, S., Shen, W., Clayton, A., et al., 2017. Coexisting and cooperating mutations in NPM1-mutated acute myeloid leukemia. Leuk. Res. 56, 7–12.
Pellagatti, A., Cazzola, M., Giagounidis, A., Perry, J., Malcovati, L., Della Porta, M.G., et al., 2011. Marked down-regulation of nucleophosmin-1 is associated with advanced del(5q) myelodysplastic syndrome. Br. J. Haematol. 155, 272–274.
Poletto, M., Lirussi, L., Wilson, D.M., Tell, G., 2014. Nucleophosmin modulates stability, activity, and nucleolar accumulation of base excision repair proteins. Mol. Biol. Cell 25, 1641–1652.
Poletto, M., Malfatti, M.C., Dorjsuren, D., Scognamiglio, P.L., Marasco, D., Vascotto, C., et al., 2016. Inhibitors of the apurinic/apyrimidinic endonuclease 1 (APE1)/nucleophosmin (NPM1) interaction that display anti-tumor properties. Mol. Carcinog. 55, 688–704.
Prinos, P., Lacoste, M.C., Wong, J., Bonneau, A.M., Georges, E., 2011. Mutation of cysteine 21 inhibits nucleophosmin/B23 oligomerization and chaperone activity. Int. J. Biochem. Mol. Biol. 2, 24–30.
Qi, W., Shakalya, K., Stejskal, A., Goldman, A., Beeck, S., Cooke, L., et al., 2008. NSC348884, a nucleophosmin inhibitor disrupts oligomer formation and induces apoptosis in human cancer cells. Oncogene 27, 4210–4220.
Ranganathan, P., Yu, X., Na, C., Santhanam, R., Shacham, S., Kauffman, M., et al., 2012. Preclinical activity of a novel CRM1 inhibitor in acute myeloid leukemia. Blood 120, 1765–1773.
Ranganathan, P., Kashyap, T., Yu, X., Meng, X., Lai, T., McNeil, B., et al., 2016. XPO1 inhibition using selinexor synergizes with chemotherapy in acute myeloid leukemia by targeting DNA repair and restoring topoisomerase IIα to the nucleus. Clin. Cancer Res. 22, 6142–6152.
Reboutier, D., Troadec, M.B., Cremet, J.Y., Fukasawa, K., Prigent, C., 2012. Nucleophosmin/B23 activates aurora A at the centrosome through phosphorylation of serine 89. J. Cell Biol. 197, 19–26.
Redner, R.L., Rush, E.A., Faas, S., Rudert, W.A., Corey, S.J., 1996. The t(5;17) variant of acute promyelocytic leukemia expresses a nucleophosmin-retinoic acid receptor fusion. Blood 87, 882–886.
Sakaguchi, M., Yamaguchi, H., Najima, Y., Usuki, K., Ueki, T., Oh, I., et al., 2018. Prognostic impact of low allelic ratio FLT3-ITD and NPM1 mutation in acute myeloid leukemia. Blood Adv. 2, 2744–2754.
Sakashita, G., Kiyoi, H., Naoe, T., Urano, T., 2018. Analysis of the oligomeric states of nucleophosmin using size exclusion chromatography. Sci. Rep. 8, 4008.
Sarek, G., Järviluoma, A., Moore, H.M., Tojkander, S., Vartia, S., Biberfeld, P., et al., 2010. Nucleophosmin phosphorylation by v-cyclin-CDK6 controls KSHV latency. PLoS Pathog. 6, e1000818.
Šašinková, M., Holoubek, A., Otevřelová, P., Kuželová, K., Brodská, B., 2018. AML-associated mutation of nucleophosmin compromises its interaction with nucleolin. Int. J. Biochem. Cell Biol. 103, 65–73.
Savkur, R.S., Olson, M.O., 1998. Preferential cleavage in pre-ribosomal RNA byprotein B23 endoribonuclease. Nucleic Acids Res. 26, 4508–4515.
Schneider, V., Zhang, L., Bullinger, L., Rojewski, M., Hofmann, S., Wiesneth, M., et al., 2014. Leukemic stem cells of acute myeloid leukemia patients carrying NPM1 mutation are candidates for targeted immunotherapy. Leukemia 28, 1759–1762.
Scott, D.D., Oeffinger, M., 2016. Nucleolin and nucleophosmin: nucleolar proteins with multiple functions in DNA repair. Biochem. Cell Biol. 94, 419–432.
Shandilya, J., Swaminathan, V., Gadad, S.S., Choudhari, R., Kodaganur, G.S., Kundu, T.K., 2009. Acetylated NPM1 localizes in the nucleoplasm and regulates transcriptional activation of genes implicated in oral cancer manifestation. Mol. Cell. Biol. 29, 5115–5127.
Shandilya, J., Senapati, P., Dhanasekaran, K., Bangalore, S.S., Kumar, M., Kishore, A.H., et al., 2014. Phosphorylation of multifunctional nucleolar protein nucleophosmin (NPM1) by aurora kinase B is critical for mitotic progression. FEBS Lett. 588, 2198–2205.
Sheval, E.V., Polyakov, V.Y., 2008. The peripheral chromosome scaffold, a novel structural component of mitotic chromosomes. Cell Biol. Int. 32, 708–712.
Spector, D.L., Ochs, R.L., Busch, H., 1984. Silver staining, immunofluorescence, and immunoelectron microscopic localization of nucleolar phosphoproteins B23 and C23. Chromosoma 90, 139–148.
Sportoletti, P., Varasano, E., Rossi, R., Mupo, A., Tiacci, E., Vassiliou, G., et al., 2015. Mouse models of NPM1-mutated acute myeloid leukemia: biological and clinical implications. Leukemia 29, 269–278.
Swaminathan, V., Kishore, A.H., Febitha, K.K., Kundu, T.K., 2005. Human histone chaperone nucleophosmin enhances acetylation-dependent chromatin transcription. Mol. Cell. Biol. 25, 7534–7545.
Szebeni, A., Olson, M.O., 1999. Nucleolar protein B23 has molecular chaperone activities. Protein Sci. 8, 905–912.
Takemura, M., Ohta, N., Furuichi, Y., Takahashi, T., Yoshida, S., Olson, M.O., et al., 1994. Stimulation of calf thymus DNA polymerase alpha activity by nucleolar protein B23. Biochem. Biophys. Res. Commun. 199, 46–51.
Takemura, M., Sato, K., Nishio, M., Akiyama, T., Umekawa, H., Yoshida, S., 1999. Nucleolar protein B23.1 binds to retinoblastoma protein and synergistically stimulates DNA polymerase alpha activity. J. Biochem. 125, 904–909.
Talati, C., Sweet, K.L., 2018. Nuclear transport inhibition in acute myeloid leukemia: recent advances and future perspectives. Int. J. Hematol. Oncol. 7, IJH04.
Tasaka, T., Tohyama, K., Kishimoto, M., Ohyashiki, K., Mitani, K., Hotta, T., et al., 2008. Myelodysplastic syndrome with chromosome 5 abnormalities: a nationwide survey in japan. Leukemia 22, 1874–1881.
Thompson, J., Finlayson, K., Salvo-Chirnside, E., MacDonald, D., McCulloch, J., Kerr, L., et al., 2008. Characterisation of the bax-nucleophosmin interaction: the importance of the bax C-terminus. Apoptosis 13, 394–403.
Tokuyama, Y., Horn, H.F., Kawamura, K., Tarapore, P., Fukasawa, K., 2001. Specific phosphorylation of nucleophosmin on thr(199) by cyclin-dependent kinase 2-cyclin E and its role in centrosome duplication. J. Biol. Chem. 276, 21529–21537.
Turner, J.G., Dawson, J., Sullivan, D.M., 2012. Nuclear export of proteins and drug resistance in cancer. Biochem. Pharmacol. 83, 1021–1032.
Urbaneja, M.A., Skjærven, L., Aubi, O., Underhaug, J., López, D.J., Arregi, I., et al., 2017. Conformational stabilization as a strategy to prevent nucleophosmin mislocalization in leukemia. Sci. Rep. 7, 13959.
Vallapureddy, R., Lasho, T.L., Hoversten, K., Finke, C.M., Ketterling, R., Hanson, C., et al., 2017. Nucleophosmin 1 (NPM1) mutations in chronic myelomonocytic leukemia and their prognostic relevance. Am. J. Hematol. 92, E618.
van der Lee, Dyantha I., Reijmers, R.M., Honders, M.W., Hagedoorn, R.S., de Jong, R.C., Kester, M.G., et al., 2019. Mutated nucleophosmin 1 as immunotherapy target in

acute myeloid leukemia. J. Clin. Invest.
Vascotto, C., Fantini, D., Romanello, M., Cesaratto, L., Deganuto, M., Leonardi, A., et al., 2009. APE1/ref-1 interacts with NPM1 within nucleoli and plays a role in the rRNA quality control process. Mol. Cell. Biol. 29, 1834–1854.
Vascotto, C., Lirussi, L., Poletto, M., Tiribelli, M., Damiani, D., Fabbro, D., et al., 2014. Functional regulation of the apurinic/apyrimidinic endonuclease 1 by nucleophosmin: impact on tumor biology. Oncogene 33, 2876–2887.
Wang, W., Budhu, A., Forgues, M., Wang, X.W., 2005. Temporal and spatial control of nucleophosmin by the ran-Crm1 complex in centrosome duplication. Nat. Cell Biol. 7, 823–830.
Wang, M., Yang, C., Zhang, L., Schaar, D.G., 2017. Molecular mutations and their cooccurrences in cytogenetically normal acute myeloid leukemia. Stem Cells Int. 2017, 6962379.
Wang, Z., Salih, E., Igwebuike, C., Mulhern, R., Bonegio, R.G., Havasi, A., et al., 2019. Nucleophosmin phosphorylation as a diagnostic and therapeutic target for ischemic AKI. J. Am. Soc. Nephrol. 30, 50–62.
Webersinke, G., Kranewitter, W., Deutschbauer, S., Zach, O., Hasenschwandtner, S., Wiesinger, K., et al., 2014. Switch of the mutation type of the NPM1 gene in acute myeloid leukemia (AML): relapse or secondary AML? Blood Cancer J. 4, e221.
Wiesmann, N., Gieringer, R., Grus, F., Brieger, J., 2019. Phosphoproteome profiling reveals multifunctional protein NPM1 as part of the irradiation response of tumor cells. Transl. Oncol. 12, 308–319.
Wu, M.H., Chang, J.H., Yung, B.Y.M., 2002. Resistance to UV-induced cell-killing in nucleophosmin/B23 over-expressed NIH 3T3 fibroblasts: enhancement of DNA repair and up-regulation of PCNA in association with nucleophosmin/B23 over-expression. Carcinogenesis 23, 93–100.
Xiao, J., Zhang, Z., Chen, G.G., Zhang, M., Ding, Y., Fu, J., et al., 2009. Nucleophosmin/B23 interacts with p21WAF1/CIP1 and contributes to its stability. Cell Cycle 8, 889–895.
Yang, K., Wang, M., Zhao, Y., Sun, X., Yang, Y., Li, X., et al., 2016. A redox mechanism underlying nucleolar stress sensing by nucleophosmin. Nat. Commun. 7, 13599.
Yoneda-Kato, N., Look, A.T., Kirstein, M.N., Valentine, M.B., Raimondi, S.C., Cohen, K.J., et al., 1996. The t(3;5)(q25.1;q34) of myelodysplastic syndrome and acute myeloid leukemia produces a novel fusion gene, NPM-MLF1. Oncogene 12, 265–275.
Yu, Y., Maggi Jr, L.B., Brady, S.N., Apicelli, A.J., Dai, M.S., Lu, H., et al., 2006. Nucleophosmin is essential for ribosomal protein L5 nuclear export. Mol. Cell. Biol. 26, 3798–3809.
Yung, B.Y., Busch, H., Chan, P., 1985. Translocation of nucleolar phosphoprotein B23 (37kDapI 5.1) induced by selective inhibitors of ribosome synthesis. Biochim. Et Biophys. Acta (BBA) - Gene Struct. Express. 826, 167–173.
Zatsepina, O.V., Rousselet, A., Chan, P.K., Olson, M.O., Jordan, E.G., Bornens, M., 1999. The nucleolar phosphoprotein B23 redistributes in part to the spindle poles during mitosis. J. Cell. Sci. 112 (Pt 4), 455–466.
Zhao, X., Ji, J., Yu, L., Veenstra, T., Wang, X.W., 2015. Cell cycle-dependent phosphorylation of nucleophosmin and its potential regulation by peptidyl-prolyl cis/trans isomerase. J. Mol. Biochem. 4, 95–103.
Ziv, O., Zeisel, A., Mirlas-Neisberg, N., Swain, U., Nevo, R., Ben-Chetrit, N., et al., 2014. Identification of novel DNA-damage tolerance genes reveals regulation of translesion DNA synthesis by nucleophosmin. Nat. Commun. 5, 5437.