Oncogenic Role of Nucleophosmin/B23

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Nucleophosmin/B23 was first identified as a nucleolar protein expressed at higher levels in cancer cells compared to normal cells. Nucleophosmin/B23 has long been thus thought to have a role in tumor formation. With our efforts and others in the last 15 years, nucleophosmin/B23 has proven to have an oncogenic role. In this review, we provide evidence suggesting that nucleophosmin/B23 may be a crucial gene in regulation of cancer growth and discuss how nucleophosmin/B23 can contribute to tumorigenesis. (Chang Gung Med J 2007;30:285-93)

Key words: nucleolus, nucleophosmin/B23, tumorigenesis

Two important differences between cancer and normal cells are hyperactivity and pleomorphism of the nucleoli.\(^{(1)}\) The nucleoli in cancer cells have extreme variations in size, shape, fine structure and cytochemical composition.\(^{(2)}\) Uncontrolled cell proliferation is the hallmark of cancer, and tumor cells have typically acquired damage to genes that directly regulate their cell cycles and cell growth. Although rRNA transcription, processing and ribosome assembly have been established as major functions of the nucleolus, previous studies suggest that the nucleolus participates in many other aspects of gene expression as well.\(^{(3)}\) Additional results indicate that biosynthesis of signal recognition particle RNA and telomerase RNA involve a nucleolar stage, and the nucleolus is a site that is critical to cellular aging.\(^{(4)}\)

Nucleophosmin/B23 is a nucleolar phosphoprotein and is found to be mainly localized in the granular region of the nucleolus.\(^{(5,6)}\) Nucleophosmin/B23 rapidly increases in response to mitogenic stimuli, and is highly expressed in proliferating and malignant cells. Nucleophosmin/B23 may thus have a role in the regulation of cell growth, proliferation and transformation.\(^{(7,8)}\)

For the past decade, advancement in understanding nucleophosmin/B23 has revealed a much more complex scenario. Nucleophosmin/B23, being a multifunctional protein, is involved in many cellular activities and is related to proliferative roles in cancer.\(^{(9)}\) In this review, we will analyze and discuss the oncogenic role of nucleophosmin/B23.

Nucleophosmin/B23 is implicated in human tumorigenesis

Being over-expressed in tumors, nucleophosmin/B23 has been proposed as a marker for gastric,\(^{(10)}\) colon,\(^{(11)}\) ovarian\(^{(12)}\) and prostate\(^{(13)}\) carcinomas. In the analysis of clinical gastric cancer tissues, later stage cancers seem to have higher nucleophosmin/B23 mRNA levels relative to the matched adjacent “normal” gastric mucosa.\(^{(14)}\) Two-dimensional gel electrophoresis analyses identify nucleophosmin/B23...
as an estrogen-regulated protein associated with acquired estrogen independence in human breast cancer cells. Nucleophosmin/B23 expression can thus be correlated to specific pathophysiological features.\(^{(15)}\) Moreover, the analysis of nucleophosmin/B23 mRNA of clinical bladder tissues by reverse transcription polymerase chain reaction (RT-PCR) indicates that over-expression of nucleophosmin/B23 is an important prognosticator for bladder cancer recurrence. Patients with over-expression of nucleophosmin/B23 have the greater recurrence and disease progression rates.\(^{(16)}\) Treatment strategies could also be determined on the basis of the status of this molecular marker used as a part of evidence-based medical treatment. Nucleophosmin/B23 may play a role in transformation in these different cancers.

Nucleophosmin/B23 is also involved in many chromosomal translocations that play a role in tumorigenesis. The N-terminal region of nucleophosmin/B23 is fused to ALK tyrosine kinase (NPM-ALK) in anaplastic lymphoma with t(2;5), retinoic acid (RA) receptor (NPM-RAR\(\alpha\)) in acute promyelocytic leukemia with t(5;17) and a novel gene called MLF1 (NPM-MLF1) in myelodysplastic syndrome (MDS) with t(3;5).\(^{(17-19)}\) Moreover, the region of chromosome 5 that nucleophosmin/B23 maps to, the 5q35 locus, is occasionally deleted in patients with de novo MDS, a genetically and clinically heterogeneous disease that often evolves into leukemia.\(^{(20)}\) Deletion of 5q35 is also observed in non-small cell lung carcinoma.\(^{(21)}\) Strikingly, nucleophosmin/B23 has also recently been found to be mutated and aberrantly localized in the cytoplasm of leukemic blasts in around 35% of patients with acute myeloid leukemia.\(^{(22)}\) The oncogenic function of the cytoplasmic localized mutant attracts intensive attention and further investigation.

**How nucleophosmin/B23 is upregulated in cancer**

Over the past two decades, many studies have been directed to the analysis of the genes in control of cell growth and their participation in the development of cancer. The steady-state level of nucleophosmin/B23 mRNA is found to be significantly higher in abnormal growth than in normal growth.\(^{(23)}\) There is a close correlation between DNA synthesis and induction of nucleophosmin/B23 in cell growth stimulated by serum.\(^{(24)}\) Previous studies have shown that expression of c-Myc correlates to nucleophosmin/B23 expression.\(^{(25-27)}\) Wild-type Rat1 fibroblasts maintain a nucleophosmin/B23 transcript level that is 3.5 times higher than fibroblasts bearing deletion of myc.\(^{(28)}\) In myc-overexpressing avian bursal neoplasia, the nucleophosmin/B23 transcript level is 3.5 times higher than in normal bursa.\(^{(29)}\) Adenoviral transfection of c-Myc in mice leads to a dramatic increase in liver nucleophosmin/B23 mRNA, which correlates to increasing Myc protein levels 3-5 days post-infection.\(^{(30)}\) These studies indicate that increased Myc expression results in an elevated nucleophosmin/B23 transcript level. Not only has a Myc binding site been identified but c-Myc has also been shown to be able to bind the nucleophosmin/B23 promoter in vivo and in vitro. c-Myc may play an active role in the transcription of the nucleophosmin/B23 promoter.\(^{(31)}\)

Elevated c-Myc expression is observed in a wide variety of human tumors.\(^{(27)}\) In many cases this is caused, at least in part, by gene amplification. In addition, point mutations are frequently found in the coding regions of c-Myc in some tumors. Hot spots for these mutations are found clustered around MAPK/ERK phosphorylation sites.\(^{(28,29)}\) Activation of the Ras pathway extends the half-life of c-Myc and thus the accumulation of c-Myc activity. Phosphorylations through the action of MAPK/ERK pathways are critical for controlling c-Myc protein accumulation.\(^{(28,29)}\) Our recent results showed a MEK inhibitor, PD98059, could depress the nucleophosmin/B23 transcription in minus serum conditions and during serum stimulation. Taken together, this shows that nucleophosmin/B23 is regulated in the signaling pathway involving MAPK/ERK and c-Myc.

In our analysis of the possible physiological role of Ras on c-Myc and nucleophosmin/B23, we have revealed that constitutive expression of dominant-negative Ras abrogates the serum-induced increase of nucleophosmin/B23 promoter activity as well as the binding of c-Myc to the promoter in U1 cells.\(^{(30)}\) The study has also linked Ras, c-Myc and nucleophosmin/B23 to cancer growth. Oncogenic Ras activates nucleophosmin/B23 expression by inducing c-Myc binding to the promoter. Our study has revealed an important molecular mechanism for Ras oncogenic function through which up-regulation of nucleolar protein nucleophosmin/B23 is achieved.\(^{(31)}\) Previous results have demonstrated that cells
with over-expressed nucleophosmin/B23 can reach a higher cell density, and exhibit anchorage-independent growth and tumorigenicity in nude mice.\(^{(8,31)}\) Nucleophosmin/B23 is notably correlated with growth capacity and malignancy of cancer cells. Our discovery thus strengthens the notion that nucleophosmin/B23, being a special target of the c-Myc-Ras activated pathway, plays a significant role in the neoplastic growth of human cancers. Considering that Ras and c-Myc are importantly associated with tumor progression in many types of malignant cells, nucleophosmin/B23, apart from Ras and c-Myc, could be a potential molecular target for therapeutic intervention of cancer development.

Nucleophosmin/B23 is transcriptionally downregulated during RA-induced differentiation.\(^{(32)}\) The biochemical and molecular events involved in cellular response to RA include a modulation of nucleophosmin/B23 gene expression. The cellular protein levels of c-Myc and nucleophosmin/B23 decrease during RA-induced differentiation. Nucleophosmin/B23 promoter activity decreases upon RA treatment and upon antisense-mediated reduction of the intracellular amount of c-Myc. Binding of c-Myc to the nucleophosmin/B23 promoter decreases upon RA treatment. These results functionally implicate c-Myc in transcriptional regulation of nucleophosmin/B23 during RA-induced differentiation.\(^{(33)}\)

**Biological roles of nucleophosmin/B23 in cancer**

The nucleophosmin/B23 gene appears to be involved in control of cell growth, cell differentiation and programmed cell death.\(^{(14,32,34)}\) Nucleophosmin/B23 over-expression in RNA and protein levels may contribute to the onset of cancer.\(^{(7,8,31)}\) It can thus be speculated that an excess of nucleophosmin/B23 may be an important cause of cancer and not just a consequence.

Being involved in cellular susceptibility to UV-induced cell killing, nucleophosmin/B23 correlates to proliferating cellular nuclear antigen (PCNA) and the DNA repair capacity in cellular sensitivity to UV.\(^{(35)}\) Nucleophosmin/B23 over-expression in NIH-3T3 cells not only results in transformation but also in resistance to UV-induced apoptosis and sustained proliferation, by increasing DNA repair and upregulation of proliferating PCNA, which is an essential component of the DNA repair machinery.\(^{(35)}\) Treatment of cells with nucleophosmin/B23 antisense oligonucleotides decreases nucleophosmin/B23 and PCNA proteins, and potentiates UV-induced cell killing.\(^{(35)}\) The effect of PCNA upregulation may be one of the reasons that nucleophosmin/B23 over-expression makes cells resistant to UV-induced growth inhibition and cell killing. Since UV-induced nucleophosmin/B23 expression is super-induced by cycloheximide and cannot be blocked by UV-inducible pathway inhibitors, UV stimulation of nucleophosmin/B23 expression is mediated through a novel UV-inducible pathway and is an immediate-early gene response induced by damaged DNA.\(^{(36)}\)

Induction of immediate-early genes is an initial step in the regulation of cellular and genomic response to external stimuli. These results provide important evidence for the involvement of nucleophosmin/B23 in the acute response of mammalian cells to environmental stress.

In RA-induced differentiation of HL-60 cells, the involvement of nucleophosmin/B23 in oncogenic activity is that nucleophosmin/B23 may bind and inactivate the tumor suppressor in cancer cells.\(^{(32)}\) IRF-1 acts as a transcriptional activator in the interferon system and as a tumor suppressor.\(^{(37,38)}\) Nucleophosmin/B23 could bind to IRF-1, interfere with IRF-1 binding to IRF-1 response elements, inhibit IRF-1 transcriptional activity and manifest oncogenic potential.\(^{(31,32)}\) Interaction of nucleophosmin/B23 with some factor(s), such as the tumor suppressor, may be an important mechanism in the control of cellular response to induction of cellular differentiation and apoptosis. The availability of the binding site of nucleophosmin/B23 to interact with the tumor suppressor may be the basis for the cells being abnormal and resistant to induction of differentiation and apoptosis.

Decrease of nucleophosmin/B23 has been observed in cells during induction of cellular differentiation and apoptosis.\(^{(32,34)}\) A more drastic decrease of nucleophosmin/B23 is detected in NIH-3T3 than in Ras-transformed cells during apoptosis induced by serum deprivation.\(^{(39)}\) Nucleophosmin/B23 in serum-deprived NIH-3T3 cells is found to be highly unstable, with a half-life of less than 4 h. Cell permeable caspase-3 inhibitor blocks the decrease of nucleophosmin/B23 induced by serum deprivation in NIH-3T3 cells.\(^{(39)}\) These studies indicate that increased stability of nucleophosmin/B23 is involved in anti-apoptosis. While no evidence has been found
of cleavage of nucleophosmin/B23 under apoptotic or necrotic conditions in HL-60 cells, the appearance of its degraded forms has been detected in serum-deprived NIH-3T3.(39) The signaling pathway and cell specificity involved in the cleavage of nucleophosmin/B23 in the induction of apoptosis and differentiation needs further investigation.

Nucleophosmin/B23 has been shown to be a natural repressor of p53 and may contribute to the dampening of p53 function during cellular growth or in the presence of low levels of DNA damage.(40) Previous data suggest that nucleophosmin/B23 could contribute to suppression of p53 activation until its functions are absolutely required. Over-expression of nucleophosmin/B23 in cancer cells could contribute to p53 inactivation and tumor progression. Additionally, p53 protein and its transcriptional activities are elevated in U1 bladder cancer cells treated with nucleophosmin/B23-siRNA.(41) On the other hand, nucleophosmin/B23 has been implicated in the stabilization of p53 and senescence in normal fibroblasts in two apparently contradictory reports. Nucleophosmin/B23 is required for the stability of tumor suppressor p19 ARF, and cells null for p53 and nucleophosmin/B23 are more susceptible to transformation by activated oncogene.(42,43) Such discrepancy may be due to the levels of nucleophosmin/B23 activity in the systems under study, as explained by Maiguel et al.(40) Given that nucleophosmin/B23 is functionally associated with p53 and p19 ARF, it is important, in the future, to determine how nucleophosmin/B23 differentially regulates p53 and p19 ARF in systems of different levels of nucleophosmin/B23 activity.

Nucleophosmin/B23 is a potential molecular target for cancer suppression

The import of proteins into the nucleus via the nuclear pore complex is directed by relatively short basic sequences called nuclear localization signals (NLSs) contained in these proteins.(45) Although the NLS sequences have been identified in a large number of proteins, only a few of the protein receptors to which they bind have been identified. One of these is nucleophosmin/B23, which has been shown to bind peptides containing NLS of the SV40 T-antigen.(46) Furthermore, nucleophosmin/B23 has also been shown to form a specific complex with human immunodeficiency virus-1 (HIV-1) Rev protein.(47,48) In a study to determine the relative affinities and stoichiometries of various NLS-containing peptides for nucleophosmin/B23, Szebeni et al. showed that a peptide (Rev 37-47) containing the sequence required for nucleolar localization of the HIV-1 Rev protein has an affinity for nucleophosmin/B23 ten times greater than that of SV40 T-NLS.(49)

A small molecule that binds to the cancer target would be a specific and powerful anti-cancer therapeutic agent. The Rev peptide that binds to nucleophosmin/B23 with the highest affinity exhibits the greatest cytotoxicity on Ras-3T3 cells and inhibited tumor growth most effectively in nude mice. The efficiency of colony formation in soft agar of Ras-3T3 cells is significantly inhibited by treatment with Rev peptide. In addition, Rev peptide can potentiate the doxorubicin-induced decrease of cellular viability in U1 bladder cancer cells and inhibit tumor growth in nude mice. Treatment with Rev peptide increases protein expression and transcriptional activity of p53, and inhibits nucleophosmin/B23-mediated PCNA promoter activation. Peptides having high affinity for binding to molecular targets, such as nucleophosmin/B23, represent a potentially useful approach to anti-cancer biotherapeutics. These results suggest that nucleophosmin/B23 is an important molecular target for cancer suppression.(41)

Nucleophosmin/B23 is involved in transcriptional regulation

Nucleophosmin/B23 plays critical roles in the control of different aspects of cell growth and homeostasis, such as ribosome biogenesis, centrosome duplication, cell cycle progression, apoptosis, cell differentiation and transcriptional regulation.(50-55) Cumulative evidence indicates that one aspect of cell growth control in which nucleophosmin/B23 may be involved is transcriptional regulation. Its role in this regard is partly mediated by interaction with a number of transcription factors, including YY1, NF-κB, ARF and IRF-1.(31,42,56-58) Previously, it has been shown that nucleophosmin/B23 can form a stable complex with YY1 and, consistent with the ability of these two proteins to interact, nucleophosmin/B23 can reverse the transcription repression function of YY1.(57) Nucleophosmin/B23 has also been implicated in the regulation of the transcriptional activity of IRF-1 and p53.(31,42) Interestingly, a recent study has demonstrated its role as an NF-κB co-activator in...
regulating the expression of MnSOD.\(^{(56)}\)

In identifying physiologically important factors that may occupy the E2F1 promoter and regulate its activity in vivo, it has been found that the pattern of NF-κB, E2F1 and pRB recruitment to the E2F1 promoter changes in a strikingly dynamic fashion as cells progress from quiescence into serum-stimulated growth. E2F1 promoter activity in quiescent cells is associated with the recruitment of NF-κB. NF-κB is largely replaced by E2F1 in concert with gene activation during an early stage (12 h) of serum stimulation. At a late stage (24 h) of serum stimulation, pRB is then recruited to the E2F1-promoter complex to counterbalance its activity. Upon siRNA-mediated reduction of intracellular nucleophosmin/B23, E2F1 and pRB are recruited to the promoter with dissociation of NF-κB concomitant with gene inactivation. Based on immunoprecipitation experiments, nucleophosmin/B23 is associated with NF-κB in cells grown in serum-supplemented but not in serum-deprived medium. Furthermore, nucleophosmin/B23 can also be co-immunoprecipitated with pRB at an early stage (12 h) but not at a late stage (24 h) of serum stimulation. These results demonstrate a novel mechanism for transcriptional regulation of E2F1 and identify the functional role of nucleophosmin/B23 in modulating the binding of NF-κB, E2F1 and pRB to activate the E2F1 promoter.\(^{(59)}\)

In our current preliminary study, a transcription factor-transcription factor (TF-TF) binding array analysis of nuclear lysates has been carried out to first identify possible nucleophosmin/B23-binding partners. Results from this assay confirm previously identified interactions (YY1 and NF-κB) and also uncover novel associated factors of AP-2α and EGR-1. Among these proteins, AP-2α has been chosen for further study based on the observed interaction with nucleophosmin/B23 as well as its potential link to the RA-mediated gene expression, under which nucleophosmin/B23 is regulated.\(^{(53)}\) Using chromatin immunoprecipitation (ChIP) assay, we have clarified the transcriptional mechanism underlying the down-regulation of the nucleophosmin/B23 gene, which involves dynamic changes in the promoter occupancy of various transcriptional regulators including AP-2α and nucleophosmin/B23. Furthermore, AP-2α could recruit nucleophosmin/B23 to the promoters of certain RA-responsive genes, such as p120, Hsp60 and b-Myb, and such binding is concomitant with their transcriptional repression. For the first time nucleophosmin/B23, other than being a target of RA-induced gene repression, may also be directly involved in mediating the RA signaling-induced gene expression, acting as a negative co-regulator. Such research will underscore the functional significance of nucleophosmin/B23 in controlling cell growth and differentiation, and delineate a novel mechanism by which nucleophosmin/B23 may exert such a regulatory role.

Collectively, all of these studies signify the potentially important function of nucleophosmin/B23 in regulating gene expression at the transcriptional level. Nucleophosmin/B23 may play a role in regulating genes involved in tumorigenesis. The exact role and underlying mechanism remains largely undefined, and needs further investigation.

**Post-translational modification of nucleophosmin/B23**

Nucleophosmin/B23 is phosphorylated by different kinases, including casein kinase 2 (CK2), nuclear kinase II, Polo-like kinase (PLK1) and cyclin-dependent kinases (CDK1/cyclin B, CDK2/cyclin E, and CDK2/cyclin A).\(^{(53,60-66)}\)

Phosphorylation by CK2 increases nucleophosmin/B23’s affinity to the NLS sequences derived from the SV40 large T antigen and the HIV Rev protein\(^{(49,67)}\) as well as modulating its molecular chaperoning activity, especially for its interaction with target proteins.\(^{(65)}\) Phosphorylation of nucleophosmin/B23 on Ser-4 by PLK1 has been shown to play a role in numeral homeostasis of centrosomes as well as cytokineses.\(^{(66)}\) Phosphorylation by CDK2/cyclin E and A on Thr199 of nucleophosmin/B23 is critical for the regulation of centrosome duplication by affecting its binding affinity to centrosomes.\(^{(61)}\) Moreover, the RNA-binding activity of nucleophosmin/B23 is controlled by phosphorylation. CDK1/cyclin B phosphorylates nucleophosmin/B23 on several residues, which results in a decrease in the RNA-binding affinity of nucleophosmin/B23.\(^{(61)}\) Phosphorylation of nucleophosmin/B23 on Thr199 by CDK2/cyclin E and A targets nucleophosmin/B23 to nuclear speckles, enhances the RNA-binding activity of nucleophosmin/B23 and represses premRNA processing.\(^{(68)}\) Taken together, this suggests that it is thus important in the future to determine how phosphorylations of nucleophosmin/B23 differ-
entially regulate its target genes in tumorigenesis.

**Future directions**

Nucleophosmin/B23 is an important nucleolar phosphoprotein with pleiotropic functions in various cellular processes, such as ribosome biogenesis, centrosome duplication, cell cycle progression, apoptosis and cell differentiation. The exact mechanism underlying nucleophosmin/B23-mediated transcriptional control remains largely elusive. In the future work, we will explore this function by undertaking an integrated and systematic approach entailing methods such as ChIP-on-ChIP, microarray and TF-TF array analyses. Examination of nucleophosmin/B23-associated transcriptional co-regulators as well as epigenetic regulation at nucleophosmin/B23-targeted loci will contribute to delineate the molecular mechanism underlying nucleophosmin/B23-orchestrated transcriptional regulation. The physiological significance of the nucleophosmin/B23 transcriptional network can be illustrated by assessing potential alteration of such a network during tumorigenesis and the tumorigenic association of nucleophosmin/B23’s targets. The future study will advance our functional and mechanistic understanding of nucleophosmin/B23’s transcriptional roles, and further expand our view on the link between gene expression regulation (or misregulation) and tumorigenesis.

**REFERENCES**

Nucleophosmin/B23’s oncogenic role


34. Liu WH, Yung BY. Mortalization of human promyelocytic leukemia HL-60 cells to be more susceptible to sodium butyrate-induced apoptosis and inhibition of telomerase activity by down-regulation of nucleophosmin/B23. Oncogene 1998;17:3055-64.


核仁磷酸蛋白 B23 的致癌角色

翁一鳴

核仁磷酸蛋白 (B23) 最初是在細胞核仁裡面被發現，且在癌細胞裡面的表現量比正常細胞高。在腫瘤形成過程中，核仁磷酸蛋白被認爲參與其中。在我們過去 15 年的研究與其他相關文獻，證明了 B23 扮演致癌角色。在這篇回顧文獻，我們說明了 B23 是調控癌症的重要基因，並探討 B23 如何影響腫瘤的形成。(長庚醫誌 2007;30:285-93)

關鍵詞：核仁、核仁磷酸蛋白 B23、腫瘤形成