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)*

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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 biosyntheses 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

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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-RARα) 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.[8] There is a close correlation between DNA synthesis and induction of nucleophosmin/B23 in cell growth stimulated by serum.[7] Previous studies have shown that expression of c-Myc correlates to nucleophosmin/B23 expression.[23-25] Wild-type Rat1 fibroblasts maintain a nucleophosmin/B23 transcript level that is 3.5 times higher than fibroblasts bearing deletion of myc.[23] In myc-overexpressing avian bursal neoplasia, the nucleophosmin/B23 transcript level is 3.5 times higher than in normal bursa.[24] 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.[25] 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.[26]

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.[30]

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 down-regulated 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.[42,43] 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.[44] 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.[33] 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 pre-mRNA 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 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

1. Busch H, Byvoet P, Smetana K. The nucleolus of the cancer cell: a review. Cancer Res 1963;23:313-39.
2. Bernhard W, Granboulan N. Electron microscopy of the nucleolus in vertebrate cells. In: Dalton AJ, Haguenau F, eds. Ultrastructure In Biological Systems. Vol. 3. The Nucleus. 3rd ed. New York: Academic Press, 1968:81-149.
3. Pederson T. The plurifunctional nucleolus. Nucleic Acids Res 1998;26:3871-6.
4. Johnson FB, Marciniak RA, Guarente L. Telomerase, the nucleolus and aging. Curr Opin Cell Biol 1998;10:332-8.
5. Kang YJ, Olson MO, Busch H. Phosphorylation of acid-soluble proteins in isolated nucleoli of Novikoff hepatoma ascites cells. Effects of divalent cations. J Biol Chem 1974;249:5580-5.
6. Kang YJ, Olson MO, Jones C, Busch H. Nucleolar phosphoproteins of normal rat liver and Novikoff hepatoma ascites cells. Cancer Res 1975;35:1470-5.
7. Feuerstein N, Spiegel S, Mond JJ. The nuclear matrix protein, numatrin (B23), is associated with growth factor-induced mitogenesis in Swiss 3T3 fibroblasts and with T lymphocyte proliferation stimulated by lectins and anti-T cell antigen receptor antibody. J Cell Biol 1988;107:1629-42.
8. Chan WY, Liu QR, Borjigin J, Busch H, Rennert OM, Tease LA, Chan PK. Characterization of the cDNA encoding human nucleophosmin and studies of its role in normal and abnormal growth. Biochemistry 1989;28:1033-9.
9. Grisendi S, Mecucci C, Falini B, Pandolfi PP. Nucleophosmin and cancer. Nat Rev Cancer 2006;6:493-505.
10. Tanaka M, Sasaki H, Kino I, Sugimura T, Terada M. Genes preferentially expressed in embryo stomach are predominantly expressed in gastric cancer. Cancer Res 1992;52:3372-7.
11. Nozawa Y, Van Belzen N, Van der Made AC, Dinjens WN, Bosman FT. Expression of nucleophosmin/B23 in normal and neoplastic colorectal mucosa. J Pathol 1996;178:48-52.
12. Shields LB, Gercel-Taylor C, Yashar CM, Wan TC, Katsanis WA, Spinnato JA, Taylor DD. Induction of immune responses to ovarian tumor antigens by multiparity. J Soc Gynecol Investig 1997;4:298-304.
13. Subong EN, Shue MJ, Epstein JI, Briggman JV, Chan PK, Partin AW. Monoclonal antibody to prostate cancer nuclear matrix protein (PRO:4-216) recognizes nucleophosmin/B23. Prostate 1999;39:298-304.
14. You BJ, Huang IJ, Liu WH, Hung YB, Chang JH, Yung BY. Decrease in nucleophosmin/B23 mRNA and telomerase activity during indomethacin-induced apoptosis of gastric KATO-III cancer cells. Naunyn Schmiedebergs Arch Pharmacol 1999;360:683-90.
15. Skaar TC, Prasad SC, Sharareh S, Lippman ME, Brunner N, Clarke R. Two-dimensional gel electrophoresis analyses identify nucleophosmin as an estrogen regulated protein associated with acquired estrogen-independence in human breast cancer cells. J Steroid Biochem Mol Biol 1998;67:391-402.
16. Tsui KH, Cheng AJ, Chang PL, Pan TL, Yung BY. Association of nucleophosmin/B23 mRNA expression with clinical outcome in patients with bladder carcinoma. Urology 2004;64:839-44.
17. Morris SW, Kirstein MN, Valentine MB, Dittmer KG, Shapiro DN, Saltman DL, Look AT. Fusion of a kinase gene, ALK, to a nucleolar protein gene, NPM, in non-Hodgkin's lymphoma. Science 1994;263:1281-4.
18. Redner RL, Rush EA, Faas S, Rudert WA, Corey SJ. The t(5;17) variant of acute promyelocytic leukemia expresses a nucleophosmin-retinoic acid receptor fusion. Blood 1996;87:882-6.
19. Yoneda-Kato N, Look AT, Kirstein MN, Valentine MB, Raimondi SC, Cohen KJ, Carroll AJ, Morris SW. The t(3;5)(q25.1;q34) of myelodysplastic syndrome and acute

myeloid leukemia produces a novel fusion gene, NPM-MLF1. Oncogene 1996;12:265-75.
20. Olney HJ, Le Beau MM. The cytogenetics and molecular biology of the myelodysplastic syndromes. In: Bennett JM, ed. The Myelodysplastic Syndromes, Pathobiology and Clinical Management. New York: Marcel Dekker, 2002:89-120.
21. Mendes-da-Silva P, Moreira A, Duro-da-Costa J, Matias D, Monteiro C. Frequent loss of heterozygosity on chromosome 5 in non-small cell lung carcinoma. Mol Pathol 2000;53:184-7.
22. Falini B, Mecucci C, Tiacci E, Alcalay M, Rosati R, Pasqualucci L, La Starza R, Diverio D, Colombo E, Santucci A, Bigerna B, Pacini R, Pucciarini A, Liso A, Vignetti M, Fazi P, Meani N, Pettirossi V, Saglio G, Mandelli F, Lo-Coco F, Pelicci PG, Martelli MF; GIMEMA Acute Leukemia Working Party. Cytoplasmic nucleophosmin in acute myelogenous leukemia with a normal karyotype. N Eng J Med 2005;352:254-66.
23. Guo QM, Malek RL, Kim S, Chiao C, He M, Ruffy M, Sanka K, Lee NH, Dang CV, Liu ET. Identification of c-myc responsive genes using rat cDNA microarray. Cancer Res 2000;60:5922-8.
24. Neiman PE, Ruddell A, Jasoni C, Loring G, Thomas SJ, Brandvold KA, Lee RM, Burnside J, Delrow J. Analysis of gene expression during myc oncogene-induced lymphomagenesis in the bursa of Fabricius. Proc Natl Acad Sci USA 2001;98:6378-83.
25. Kim S, Li Q, Dang CV, Lee LA. Induction of ribosomal genes and hepatocyte hypertrophy by adenovirus-mediated expression of c-Myc in vivo. Proc Natl Acad Sci USA 2000;97:11198-202.
26. Zeller KI, Haggerty TJ, Barrett JF, Guo Q, Wonsey DR, Dang CV. Characterization of nucleophosmin (B23) as a Myc target by scanning chromatin immunoprecipitation. J Biol Chem 2001;276:48285-91.
27. Nesbit CE, Tersak JM, Prochownik EV. MYC oncogenes and human neoplastic disease. Oncogene 1999;18:3004-16.
28. Sears R, Leone G, DeGregori J, Nevins JR. Ras enhances Myc protein stability. Mol Cell 1999;3:169-79.
29. Sears R, Nuckolls F, Haura E, Taya Y, Tamai K, Nevins JR. Multiple Ras-dependent phosphorylation pathways regulate Myc protein stability. Genes Dev 2000;14:2501-14.
30. Yeh CW, Huang SS, Lee RP, Yung BY. Ras-dependent recruitment of c-Myc for transcriptional activation of nucleophosmin/B23 in highly malignant U1 bladder cancer cells. Mol Pharmacol 2006;70:1443-53.
31. Kondo T, Minamino N, Nagamura-Inoue T, Matsumoto M, Taniguchi T, Tanaka N. Identification and characterization of nucleophosmin/B23/numatrin which binds the anti-oncogenic transcription factor IRF-1 and manifests oncogenic activity. Oncogene 1997;15:1275-81.
32. Hsu CY, Yung BY. Down-regulation of nucleophosmin/B23 during retinoic acid-induced differentiation of human promyelocytic leukemia HL-60 cells. Oncogene 1998;16:915-23.
33. Yung BY. c-Myc-mediated expression of nucleophosmin/B23 decreases during retinoic acid-induced differentiation of human leukemia HL-60 cells. FEBS Lett 2004;578:211-6.
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.
35. Wu MH, Chang JH, Yung BY. 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 2002;23:93-100.
36. Wu MH, Yung BY. UV stimulation of nucleophosmin/B23 expression is an immediate-early gene response induced by damaged DNA. J Biol Chem 2002;277:48234-40.
37. Harada H, Fujita T, Miyamoto M, Kimura Y, Maruyama M, Furia A, Miyata T, Taniguchi T. Structurally similar but functionally distinct factors, IRF-1 and IRF-2, bind to the same regulatory elements of IFN and IFN-inducible genes. Cell 1989;58:729-39.
38. Harada H, Kitagawa M, Tanaka N, Yamamoto H, Harada K, Ishihara M, Taniguchi T. Anti-oncogenic and oncogenic potentials of interferon regulatory factor-1 and -2. Science 1993;259:971-4.
39. Chou CC, Yung BY. Increased stability of nucleophosmin/B23 in anti-apoptotic effect of ras during serum deprivation. Mol Pharmacol 2001;59:38-45.
40. Maiguel DA, Jones L, Chakravarty D, Yang C, Carrier F. Nucleophosmin sets a threshold for p53 response to UV radiation. Mol Cell Biol 2004;24:3703-11.
41. Chan HJ, Weng JJ, Yung BY. Nucleophosmin/B23-binding peptide inhibits tumor growth and up-regulates transcriptional activity of p53. Biochem Biophys Res Commun 2005;333:396-403.
42. Colombo E, Marine JC, Danovi D, Falini B, Pelicci PG. Nucleophosmin regulates the stability and transcriptional activity of p53. Nat Cell Biol 2002;4:529-33.
43. Kurki S, Peltonen K, Latonen L, Kiviharju TM, Ojala PM, Meek D, Laiho M. Nucleolar protein NPM interacts with HDM2 and protects tumor suppressor protein p53 from HDM2-mediated degradation. Cancer Cell 2004;5:465-75.
44. Colombo E, Bonetti P, Lazzerini Denchi D, Martinelli P, Zamponi R, Marine JC, Helin K, Falini B and Pelicci PG. Nucleophosmin is required for DNA integrity and p19Arf protein stability. Mol Cell Biol 2005;25:8874-86.
45. Boulikas T. Putative nuclear localization signals (NLS) in protein transcription factors. J Cell Biochem 1994;55:32-58.

46. Goldfarb DS. Karyophilic peptides: applications to the study of nuclear transport. Cell Biol Int Rep 1988;12:809-32.
47. Fankhauser C, Izaurralde E, Adachi Y, Wingfield P, Laemmli UK. Specific complex of human immunodeficiency virus type 1 rev and nucleolar B23 proteins: dissociation by the Rev response element. Mol Cell Biol 1991;11:2567-75.
48. Umekawa H, Chang JH, Correia J, Wang D, Wingfield PT, Olson MO. Nucleolar protein B23: bacterial expression, purification, oligomerization and secondary structures of two isoforms. Cell Mol Biol Res 1993;39:635-45.
49. Szebeni A, Herrera JE, Olson MO. Interaction of nucleolar protein B23 with peptides related to nuclear localization signals. Biochemistry 1995;34:8037-42.
50. Gao H, Jin S, Song Y, Fu M, Wang M, Liu Z, Wu M, Zhan Q. B23 regulates GADD45a nuclear translocation and contributes to GADD45a-induced cell cycle G2-M arrest. J Biol Chem 2005;280:10988-96.
51. Grisendi S, Bernardi R, Rossi M, Cheng K, Khandker L, Manova K, Pandolfi PP. Role of nucleophosmin in embryonic development and tumorigenesis. Nature 2005;437:147-53.
52. Herrera JE, Savkur R, Olson MO. The ribonuclease activity of nucleolar protein B23. Nucleic Acids Res 1995;23:3974-9.
53. Okuda M, Horn HF, Tarapore P, Tokuyama Y, Smulian AG, Chan PK, Knudsen ES, Hofmann IA, Snyder JD, Bove KE, Fukasawa K. Nucleophosmin/B23 is a target of CDK2/cyclin E in centrosome duplication. Cell 2000;103:127-40.
54. Okuwaki M, Matsumoto K, Tsujimoto M, Nagata K. Function of nucleophosmin/B23, a nucleolar acidic protein, as a histone chaperone. FEBS Lett 2001;506:272-6.
55. Yun JP, Chew EC, Liew CT, Chan JY, Jin ML, Ding MX, Fai YH, Li HK, Liang XM, Wu QL. Nucleophosmin/B23 is a proliferate shuttle protein associated with nuclear matrix. J Cell Biochem 2003;90:1140-8.
56. Dhar SK, Lynn BC, Daosukho C, St Clair DK. Identification of nucleophosmin as an NF-kappaB co-activator for the induction of the human SOD2 gene. J Biol Chem 2004;279:28209-19.
57. Inouye CJ, Seto E. Relief of YY1-induced transcriptional repression by protein-protein interaction with the nucleolar phosphoprotein B23. J Biol Chem 1994;269:6506-10.
58. Korgaonkar C, Hagen J, Tompkins V, Frazier AA, Allamargot C, Quelle FW, Quelle DE. Nucleophosmin (B23) targets ARF to nucleoli and inhibits its function. Mol Cell Biol 2005;25:1258-71.
59. Lin CY, Liang YC, Yung BY. Nucleophosmin/B23 regulates transcriptional activation of E2F1 via modulating the promoter binding of NF-κB, E2F1 and pRB. Cell Signal 2006;18:2041-8.
60. Tokuyama Y, Horn HF, Kawamura K, Tarapore P, Fukasawa K. Specific phosphorylation of nucleophosmin on Thr(199) by cyclin-dependent kinase 2-cyclin E and its role in centrosome duplication. J Biol Chem 2001;276:21529-37.
61. Okuwaki M, Tsujimoto M, Nagata K. 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 2002;13:2016-30.
62. Peter M, Nakagawa J, Doree M, Labbe JC, Nigg EA. Identification of major nucleolar proteins as candidate mitotic substrates of cdc2 kinase. Cell 1990;60:791-801.
63. Chan PK, Liu QR, Durban E. The major phosphorylation site of nucleophosmin (B23) is phosphorylated by a nuclear kinase II. Biochem J 1990;270:549-52.
64. Jiang PS, Chang JH, Yung BY. Different kinases phosphorylate nucleophosmin/B23 at different sites during G(2) and M phases of cell cycle. Cancer Lett 2000;153:151-60.
65. Szebeni A, Hingorani K, Negi S, Olson MO. Role of protein kinase CK2 phosphorylation in the molecular chaperone activity of nucleolar protein b23. J Biol Chem 2003;278:9107-15.
66. Zhang H, Shi X, Paddon H, Hampong M, Dai W, Pelech S. B23/nucleophosmin serine 4 phosphorylation mediates mitotic functions of polo-like kinase 1. J Biol Chem 2004;279:35726-34.
67. Szebeni A, Mehrotra B, Baumann A, Adam SA, Wingfield PT, Olson MO. Nucleolar protein B23 stimulates nuclear import of the HIV-1 Rev protein and NLS-conjugated albumin. Biochemistry 1997;36:3941-9.
68. Tarapore P, Shinmura K, Suzuki H, Tokuyama Y, Kim SH, Mayeda A, Fukasawa K. Thr199 phosphorylation targets nucleophosmin to nuclear speckles and represses pre-mRNA processing. FEBS Lett 2006;580:399-409.

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

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