Review Article

Roles of nucleolin

Focus on cancer and anti-cancer therapy

Zhuo Chen, *MMed,* ***XinHua Xu,*** *MMed.*

ABSTRACT

يعد النوكليولين بروتين متعدد الوظائف منتشر في نوية، ويشارك في العديد من التحويرات بما في ذلك نسخ الحمض النووي والتمثيل الغذائي RNA وتجمع الريبوسوم. يظهر نوكليولين أيضاً في السيتوبلازم وعلى غشاء الخلية، ويمكن ربط سطح نوكليولين بروابط مختلفه تؤثر على العديد من الوظائف الفسيولوجية. التعبير وتوطين النوكليولين في كثير من الأحيان غير طبيعي في أمراض السرطان، والتوزيع المتفاوت للنوكليولين في السرطان يمكن أن تؤثر على انتشار السرطان وبقاءوه وورم الخلايا السرطانية الخبيث مؤدياً بذلك إلى تطور السرطان. وبالتالي، قد يكون نوكليولين هدف واعد وحديث لعلاج مضاد للسرطان. نستعرض هنا ونوصف كيفية عمل النوكليولين في تطور السرطان ووصف أدوية نوكليولين المضادة للسرطان.

Nucleolin, a multifunctional protein distributed in the nucleolus, participates in many modulations including rDNA transcription, RNA metabolism, and ribosome assembly. Nucleolin is also found in the cytoplasm and on the cell membrane, and surface nucleolin can bind to various ligands to affect many physiological functions. The expression and localization of nucleolin is often abnormal in cancers, as the differential distribution of nucleolin in cancer can influence the carcinogenesis, proliferation, survival, and metastasis of cancer cells, leading to the cancer progression. Thus, nucleolin may be a novel and promising target for anti-cancer treatment. Here, we describe how nucleolin act functions in cancer development and describe nucleolin-dependent anti-cancer therapies.

Saudi Med J 2016; Vol. 37 (12): 1312-1318
doi: 10.15537/smj.2016.12.15972

From the Department of Oncology, The First College of Clinical Medical Science, China Three Gorges University & Yichang Central People's Hospital, Yichang, China.

Address correspondence and reprint request to: Prof. XinHua Xu, Department of Oncology, The First College of Clinical Medical Science, China Three Gorges University & Yichang Central People's Hospital, Yichang, China. E-mail: xuxinhua@medmail.com.cn

Nucleolin, or called C23, is one of the most abundant proteins in the nucleolus, as it accounts for approximately 10% in the protein content in the nucleolus. It has been confirmed that nucleolin is involved in the remodeling of nucleolar chromatin, maturation of pre-RNA, rDNA transcription and ribosome assembly.[1] Nucleolin also plays significant roles in many physiological processes such as modulating the proliferation, survival and apoptosis of cells, especially in cancer cells.[2] Meanwhile, nucleolin on the cell surface has been found in various cancers and can specifically bind to ligands to regulate the progression of cancer. Thus, nucleolin may be a novel target in studying cancer progression and developing cancer diagnostics and therapies. Here, we briefly present the effects of nucleolin in cancer and in anti-cancer therapy.

The structure and function of nucleolin. Nucleolin has 3 main structural domains: the N-terminal domain (rich in acidic regions and containing multiple phosphorylation sites); the central domain (contains 4 RNA binding domains [RBDs]) and the C-terminal domain (rich in glycine, arginine and phenylalanine residues). The N-terminal domain participates in the rRNA transcription, and has interactions with components of the pre-rRNA processing complex. The central domain modulates the interactions with mRNAs and pre-rRNA. The C-terminal domain can interacts with target mRNAs/proteins.

Nucleolin is mainly distributed in the nucleus and is involved in many modulations of cellular progression. Some studies indicated that nucleolin might be necessary to controlling the transcriptional states of rDNA. Nucleolin also can affect the turnover and transcription of mRNA both positively and negatively

through binding to different components of mRNA. Evidences have suggested that the binding of nucleolin to the mRNA 5' UTR often suppresses translation, while binding to the 3' UTR enhances mRNA translation. It is well known that aberrant splicing of mRNA precursors results in the production of abnormal proteins. Das et al[3] showed that nucleolin interacted with some mRNAs or spliceosomes that could regulate the alternative splicing. Moreover, nucleolin has multiple roles in the process of ribosome biogenesis steps, including RNA polymerase (Pol) I transcription, processing of pre-rRNA and ribosome assembly.[4] Turner et al[5] demonstrated that nucleolin could facilitate the first processing step of pre-rRNA occurring at the 5' external transcribed spacer (5'-ETS), and lead to cleavage of the precursor transcript of rRNA.[5] Nucleolin RBDs were reported to bind to a stem-loop structure of RNA and worked as a chaperone to facilitate the proper folding of pre-rRNA.

More than 90% of nucleolin is found in the nucleolus, nucleolin also has been found in the cytoplasm and on the cell surface. The shuttling of nucleolin between the nucleus, cytoplasm and plasmalemma is significant for normal nucleolin functions. Meng et al[6] indicated that a decrease of cell-surface nucleolin expression, or activity would inhibit the growth of cancer cells and trigger the apoptosis in endothelial cells. Surface-nucleolin participates in many pathways, or processes via binding to various ligands including DNA, RNA, and proteins.

Nucleolin in cancer. Some evidence has suggested that the expression and localization of nucleolin is abnormal in cancer. Dysregulated accumulation of nucleolin mRNA and protein is found in a diverse range of cancers, and the level of surface nucleolin in cancers is much higher than in normal cells.[7] The elevated expression of nucleolin is associated with a worse prognosis of cancer patients, and the presence of nucleolin on the cell surface increases the malignancy of cancer and modulates the metastasis. Thus, nucleolin is believed to facilitate the processes that affect the fate of cancer cells (the effect of nucleolin for cancer is summarized in **Table 1**).

Nucleolin in carcinogenesis. The dysregulation of cancer-related genes, or their pathways is an important factor for the transformation of normal cells to cancer cells; once a structural or regulatory abnormality occurs, the resulting products, or activity will accelerate the formation of cancer.

Most cancers have character with aberrant centrosome numbers, which can cause aneuploidy and result in the formation of cancer cells.[8] In interphase and during mitosis, nucleolin is found in the surrounding region including the vicinity of the outer kinetochore of chromosomes, as it is associated with spindle poles. Further studies showed that depletion of nucleolin could induce the amplification of immature centriole markers and a disorganization of the microtubule network. Nucleolin depletion also caused improper kinetochore attachments, and reduced tension and syntelic attachments.[1] There are a number of signaling pathways, such as the transforming growth factor β (TGF-ß) pathway and epidermal growth factor (EGF) pathway that are involved in oncogenesis. Lv et al[9] revealed that surface nucleolin could promote and regulate the TGF-ß pathway via the interaction with TGF-beta receptor I (TβR-I) in glioblastoma cells, and that nucleolin was required for the initiation and activation of the TGF-ß pathway.[9] Similarly, it has also been shown that nucleolin regulated the activation of epidermal growth factor (EGF)-induced ERK signaling and the PI3K-AKT pathway by interacting with EGFR, which could obviously affect the growth, viability, colony formation ability, and invasiveness of cancer cells.[10]

Moreover, there exist some high-risk factors/promoters for cancer initiation, and nucleolin is

Table 1 - The effects of nucleolin in cancers.

Stage	Effect	Mechanism
Carcinogenesis	Facilitate	Promote and regulate the oncogenesis-related TGF-β pathway and EGF pathway. Regulate high-risk promoters of cancer initiation.
Proliferation and survival	Facilitate	Interact with DNA repair proteins to maintain DNA stability. Regulate the stability of apoptosis-related mRNAs to enhance anti-apoptosis. Bind to apoptosis-related ligands to prevent apoptosis.
Infiltration and metastasis	Facilitate	Regulate the process of EMT and the expression of MMPs. Modulate the initiation and transduction of EGFR and CXCR4 signaling.
Angiogenesis	Facilitate	Up-regulate the level of VEGF and HIF1α.

TGF-ß - transforming growth factor ß, EGF - epidermal growth factor, EMT - epithelial mesenchymal transition, VEGF - vascular endothelial growth factor, MMPs - modulates matrix metalloproteinases, EGFR - epidermal growth factor receptor, CXCR4 - chemokine receptor type 4, HIF1α - Hypoxia-inducible factor 1alpha

involved with these regulators. For instance, gastrin is highly expressed in malignancies such as pancreatic cancer and colorectal cancer. Nucleolin is required to maintain the stabilization of gastrin related mRNA. Human papilloma virus (HPV) is associated with a high frequency of cervical carcinogenesis, and Sato indicated that nucleolin could bind to plasmids containing the tHPV16 genomic region in cancer cells, which is related to the stable maintenance of the HPV16 genome.[11] Tumor necrosis factor (TNF)-α-inducing protein (Tipα), release from *Helicobacter pylori (H. pylori)*, can strongly induce the expression of TNF-α and chemokine genes by mediating NF-κB activation in stomach cancer cells, leading to the development of cancer in human stomachs infected with *H. pylori*.[12] Watanabe et al[13] revealed that surface nucleolin acted as a receptor for Tipα through binding of HB-19 to the RGG domain at the C-terminal region and shuttled Tipα from the cell surface to the cytosol and nuclei. It has been found that overexpression of the interleukin-9 (IL-9) receptor occurs in several types of human leukemias. Shang et al[14] confirmed the increased localization of nucleolin in the nuclei of TL cell lines (which have elevated expression of the IL-9R gene (Il9r), which suggests that nucleolin might favor IL-9R transcription during leukemogenesis.

Nucleolin in cancer proliferation and survival. Cancer cells often have characteristically increased proliferation, often to the point without limits. During this event, nucleolin can safeguard the stability of genome and limit DNA damage accumulation due to rapid proliferation, leading to high levels of protein synthesis that can benefit the elevated proliferation rate of cancer.

Under stress conditions (namely, heat shock or radiation), nucleolin can redistribute from the nucleolus to the nucleoplasm, and the relocalization will increase the formation of nucleolin-replication protein A (RPA) complex. Because RPA is an important ssDNA-binding protein during the initiation and elongation stages of DNA replication, this complex would sequester RPA and block the function of RPA during DNA replication. With the sequestration of nucleolin and other factors, the conditions allow for the maintenance of genome stability via transiently delaying cell proliferation to support the activation of DNA repair machinery. Nucleolin also interacts with DNA repair proteins such as PCNA and gH2AX[15] to promote nucleolin-related DNA repair. De et al[16] indicated that nucleolin and Rad51 were involved in the pathway of homologous recombination repair and that nucleolin might regulate the DNA repair activity of Rad51.[16] Nucleolin is also involved in the regulation of telomerase maintenance. It was confirmed that nucleolin interacts with telomeric repeats (TTAGGG) and the human telomerase reverse transcriptase subunit (hTERT). The binding of nucleolin to the active telomerase complex via protein-protein and protein-RNA interactions may regulate the function of telomerase.[2]

Meanwhile, cancer cells have a low apoptosis rate. Nucleolin can regulate the stability of apoptosis-related mRNAs by the binding of nucleolin RBD to the 5' and 3'UTR of mRNAs and enhance the anti-apoptosis. It has been reported that increased nucleolin expression could elevate the levels of BCL-2 in cancer cells by the specific binding of nucleolin to AU-rich elements (AREs) in the 3'UTR of BCL-2 mRNA, which protects the mRNA from degradation.[17] Nucleolin also interacts with 15a and 16 miRNAs, which are negative regulators of BCL-2 expression, to control their maturation process.[18] Moreover, nucleolin can reduce the translation of p53 by associating with the 5' UTR of TP53 mRNA and enhance the translation of AKT1 and cyclin I (pro-survival proteins) via binding to their mRNA.[19]

Surface nucleolin has gained increasing attention due to its roles in many physiological modulations. Wise et al[20] showed that cell-surface nucleolin could bind to Fas and block the interaction of Fas/FasL, which prevents cells from entering Fas-induced apoptosis.[20] The interaction of surface nucleolin with ErbB1 and Ras[21] also favor cell proliferation. Therefore, nucleolin can facilitate an anti-apoptotic phenotype and induce the initiation and survival of cancer.

Nucleolin in cancer infiltration and metastasis. During the progression of cancer, cancer cells will break away from cancer tissue, and intrude into and drift in the circulation before implanting in novel regions. It is well known that cancer cells can undergo epithelial mesenchymal transition (EMT) to enhance their metastatic potential. Some studies implied that the disturbance of nucleolin could inhibit the process of EMT. For instance, transfecting cells with nucleolin-targeted small interfering RNA could result in the inhibition of the EMT phenotypes.[22] Yang et al[23] showed that si-nucleolin treatment attenuated the BMP2-induced expression of p-Erk1/2, p-Akt, vimentin, N-cadherin, and MMP2, leading to decreased migration and invasion of gastric cancer cells. Nucleolin also modulates matrix metalloproteinases (MMPs). Hsu et al[24] found the nucleolin was observably cleaved to form C-terminal truncated nucleolin (TNCL) in lung cancer, TNCL could increase the expression of MMP9, anaplastic lymphoma kinase (ALK), and HIF1a as well

as decrease the expression of cancer suppressors by regulating mRNA stability via binding to the 3′ UTR.[24]

Qi et al[25] indicated that nucleolin was extensively located in the nucleus, cytoplasm and cell membrane in esophageal squamous cell carcinoma (ESCC) tissues with metastasis, while nucleolin was merely confined to the nucleus in tissues without metastasis. Nucleolin was implicated in the migration and invasion of ESCC cells via modulation of the initiation and transduction of EGFR and CXCR4 signaling. Similarly, Dai et al[26] showed that nucleolin was crucial in the activation of CXCR4 signaling, which affected the growth, migration, and invasiveness of cancer cells. Studies also found that nucleolin participated in the initiation of the CCR6 pathway to modulate the adhesion, migration, and invasive of hepatocellular carcinoma cells that the expression of nucleolin, and CCR6 in cancer patients was associated with advanced stage, lymph node metastasis, and a poor 5-year prognosis.[27]

Nucleolin in cancer angiogenesis. Angiogenesis is an essential factor for cancer progression, as it not only provides the blood supply to the cancer locus, but also offers more opportunities for metastasis. Recent findings showed nucleolin played a significant role in angiogenesis. Nucleolin can affect cancer angiogenesis by modulating the levels of blood vessel-related factors. It has been indicated that over-expression of nucleolin up-regulated the expression of vascular endothelial growth factor (VEGF) via interacting with the G- and C-rich sequences of the VEGF promoter.[28] Nucleolin also could bind to the G-quadruplex structure in the 5' UTR of HIF1-a mRNA, and the inhibition of nucleolin led to decreased HIF1a protein, and mRNA levels.[29] However, Zhuo et al[30] demonstrated that surface nucleolin on cancer cells, and angiogenesis-related endothelial cells had a high affinity to endostatin, thus, nucleolin inhibition might result in the anti-angiogenesis caused by endostatin.

Surface nucleolin can be used as a transport protein to transfer regulatory factors from the cell surface to the nuclei, or nucleoli. Acharan sulfate (AS), an anti-tumor and anti-angiogenesis glycosaminoglycan, has a strong affinity specifically to surface nucleolin in lung adenocarcinoma cells; after binding to nucleolin, AS can be absorbed into the cytoplasm via nucleolin.[31] Interestingly, VEGF can regulate the relocation of nucleolin. Wu et al[32] indicated that VEGF expression was correlated with nucleolin distribution in colorectal carcinoma clinical samples that VEGF could promote the phosphorylation and relocation of nucleolin through the PI3K/Akt pathway in cancer cell lines.

Nucleolin in anti-cancer therapy. Nucleolin is a remarkable target for cancer therapy given its higher abundance, selective presence on plasma membrane, and multifaceted influence on initiation, and progression of cancer. A number of studies have indicated that the proliferation and progression of cancer cells would be inhibited by suppressing or blocking nucleolin. Meanwhile, owing to its affinity and specific binding to extracellular ligands, cell-surface nucleolin may act as a novel delivery system in cancer therapies.

Nucleolin-based siRNA and microRNA treatment. siRNA and miRNA can modulate the expression of proteins by silencing specific genes, and binding to target mRNAs. Thus, the abnormal expression levels of nucleolin might be decreased via siRNA, or miRNA. Many efforts have been made to develop an siRNA-based therapy; for example, Xu et al[33] found that the decrease of nucleolin expression via siRNA-mediated knockdown resulted in an obvious reduction in the proliferation of glioblastoma cells and induced cell cycle arrest in vitro. Decreased nucleolin expression also caused a dramatic decrease of tumor size in an intracranial xenograft model. Wu et al[34] showed that antisense phosphorothioate-modified oligodeoxynucleotides (S-ODNs) directed at nucleolin mRNA could trigger the apoptosis of nasopharyngeal carcinoma (NPC) cells and that S-ODN treatment would result in the suppression of NPC growth in tumor xenografts. It was also reported that upon binding of miRNA-494 to nucleolin, nucleolin expression was inhibited and led to an obvious reduction of cancer cell survival.[35] Upon treating lung cancer cells with 2 nucleolin aptamer siRNA chimeras (aptNCL-SLUGsiR and aptNCL-NRP1siR), Lai et al[36] found the aptNCL-siRNA could specifically and significantly knock down the expression of SLUG and NRP1 by nucleolin-mediated endocytosis; furthermore, this combination treatment also suppressed the growth, invasiveness and angiogenesis of cancer in a xenograft mouse model without affecting the functions of the liver, or kidney.

Nucleolin-based anti-cancer aptamers. An aptamer is single and short nucleic acid sequence, either DNA or RNA that can specifically target cellular and extracellular targets with high affinity. The aptamer AS1411, an unmodified guanosine (G)-rich oligonucleotide (5'-d GGT GGT GGT GGT TGT GGT GGT GGT GG-3'), has a high affinity for nucleolin and can bind to cell-surface nucleolin, then be internalized. The binding of AS1411 to nucleolin will disturb nucleolin-related modulations. In a phase II single-arm study, 35 metastatic renal cell carcinoma (RCC) patients were administered AS1411, but only one patient (2.9%)

had a response to treatment; however, the response was dramatic (84% reduction in cancer burden by RECIST 1.0 criteria) and durable (patient remains free of progression 2 years after completing therapy). Approximately 34% patients experienced AS1411-related side effects, but they were mild or moderate.[37] Although AS1411 can be uptaken directly, the efficiency is not very high. Malik et al[38] showed that AS1411-linked gold nanospheres (AS1411-GNS) were superior with regard to cell uptake and markedly showed increased anti-proliferative/cytotoxic effects compared to AS1411. An AS1411-GNS also completely inhibited cancer growth without signs of toxicity.[38]

Owing to the specific binding of AS1411 to nucleolin, AS1411 may work as promising delivery system in anti-cancer treatments. Li et al[39] used an AS1411-PEG-liposome/siRNA complex in a melanoma cancer xenograft mice and discovered remarkable silencing activity and inhibition of growth in cancer cells. Alibolandi et al[40] showed that AS1411-GEM-NPs could enhance the inhibitory effect on proliferation in lung cancer cells overexpressing nucleolin. Liao et al[41] demonstrated that AS1411/doxorubicin (DOX)/liposomes could obviously increase the intercellular accumulation of DOX compared to treatment with either free DOX or liposomes in a DOX-resistant breast cancer xenograft mouse model, resulting in an inhibition of cancer growth and a reduction of side effects. The combination of AS1411-functionalized composite micelles increased DOX accumulation in breast cancer cells and decreased cardiotoxicity.[42] Similarly, the AS1411-related polymeric nanosystem also can function as a potential drug delivery mechanism against various cancers such as ovarian, pancreatic, and lung cancer.[43]

Nucleolin-based anti-cancer peptides. Except for nucleic acid-dependent therapy, peptides also can be significant anti-nucleolin drugs in cancer therapy. HB-19 is a synthetic multimeric pseudopeptide that can bind to surface nucleolin. Once bound with HB-19, the organization of the existing 500 kDa complex in surface nucleolin can be changed and interfere with the native functions of surface nucleolin. Some findings showed that HB-19 could inhibit adhesion or spreading in epithelial tumor cells.[44] In a xenograft mouse model, Destouches et al[45] found that HB-19 treatment could markedly suppress the progression of established breast cancer cells; in some cases, it even eliminated measurable cancers while displaying no toxicity to normal tissue. Krust et al[44] indicated that HB-19 restored the contacted inhibition and impaired the growth of rhabdoid tumor-derived G401 cells, while the restoration of contact inhibition in HB-19-treated cells is related to an obvious decrease of transcripts coding the Wilms' tumor 1 gene, MMP-2, the epithelial isoform of CD44, and VEGF.[46] Similarly, N6L, another synthetic peptide targeting surface nucleolin, also displayed anti-proliferative activities, enhanced apoptosis, and decreased angiogenesis in cancers.[47]

In conclusion, the level and localization of nucleolin is aberrant and contributes to the progression of cancer, including carcinogenesis, proliferation, angiogenesis, and metastasis. Thus, nucleolin is a promising target for anti-cancer therapy. Although some achievements have been gained, there are many challenges. First, due to the mechanisms of controlling nucleolin abundance and relocation, the interactions of surface nucleolin with ligands are poorly understood, and nucleolin-related drugs are very restricted. A better understanding of those mechanisms is needed. Secondly, cell-surface nucleolin in cancers may be a specific marker for drug delivery, but whether cell-surface nucleolin in cancer cells has an obvious distinction from normal cells remains unclear. Thus, analyzing the molecular activities of cell-surface nucleolin and the distribution of nucleolin in cells is requisite. Most of the studies of nucleolin-targeted treatments are still at the cellular and animal stages, and more clinical trials are required to verify the safety and effectivity of these therapies. In summary, nucleolin is very promising target for anti-cancer therapy and is worth intensive further study.

References

1. Li N, Yuan K, Yan F, Huo Y, Zhu T, Liu X, et al. PinX1 is recruited to the mitotic chromosome periphery by Nucleolin and facilitates chromosome congression. ***Biochem Biophys Res Commun*** 2009; 384: 76-81.
2. Lee JH, Lee YS, Jeong SA, Khadka P, Roth J, Chung IK. Catalytically active telomerase holoenzyme is assembled in the dense fibrillar component of the nucleolus during S phase. ***Histochem Cell Biol*** 2014; 141: 137-152.
3. Das S, Cong R, Shandilya J, Senapati P, Moindrot B, Monier K, et al. Characterization of nucleolin K88 acetylation defines a new pool of nucleolin colocalizing with pre-mRNA splicing factors. ***FEBS Lett*** 2013; 587: 417-424.
4. Salvetti A, Couté Y, Epstein A, Arata L, Kraut A, Navratil V, et al. Nuclear Functions of Nucleolin through Global Proteomics and Interactomic Approaches. ***J Proteome Res*** 2016; 15: 1659-1669.
5. Turner AJ, Knox AA, Prieto JL, McStay B, Watkins NJ. A novel small-subunit processome assembly intermediate that contains the U3 snoRNP, nucleolin, RRP5, and DBP4. ***Mol Cell Biol*** 2009; 29: 3007-3017.
6. Meng GZ, Xiao SJ, Zeng SE, Li YQ. [Down regulation of cell surface expressed nucleolin inhibits the growth of hepatocellular carcinoma cells in vitro]. ***Zhonghua Zhong Liu Za Zhi*** 2011; 33: 23-27. Chinese

7. Qiu W, Wang G, Sun X, Ye J, Wei F, Shi X, et al. The involvement of cell surface nucleolin in the initiation of CCR6 signaling in human hepatocellular carcinoma. ***Med Oncol*** 2015; 32: 75.
8. Nigg EA, Cajanek L, Arquint C. The centrosome duplication cycle in health and disease. ***FEBS Lett*** 2014; 588: 2366-2372.
9. Lv S, Zhang J, Han M, Wang W, Zhang Y, Zhuang D et al. Nucleolin promotes TGF-ß signaling initiation via TGF-ß receptor I in glioblastoma. ***J Mol Neurosci*** 2015; 55: 1-6.
10. Lv S, Dai C, Liu Y, Sun B, Shi R, Han M, Bian R, et al. Cell surface protein C23 affects EGF-EGFR induced activation of ERK and PI3K-AKT pathways. ***J Mol Neurosci*** 2015; 55: 519-524.
11. Sato H, Kusumoto-Matsuo R, Ishii Y, Mori S, Nakahara T, Shinkai-Ouchi F, et al. Identification of nucleolin as a protein that binds to human papillomavirus type 16 DNA. ***Biochem Biophys Res Commun*** 2009; 387: 525-530.
12. Suganuma M, Watanabe T, Yamaguchi K, Takahashi A, Fujiki H. Human gastric cancer development with TNF-ß-inducing protein secreted from Helicobacter pylori. ***Cancer Lett*** 2012; 322: 133-138.
13. Watanabe T, Hirano K, Takahashi A, Yamaguchi K, Beppu M, Fujiki H, et al. Nucleolin on the cell surface as a new molecular target for gastric cancer treatment. ***Biol Pharm Bull*** 2010; 33: 796-803.
14. Shang Y, Kakinuma S, Nishimura M, Kobayashi Y, Nagata K, Shimada Y. Interleukin-9 receptor gene is transcriptionally regulated by nucleolin in T-cell lymphoma cells. ***Mol Carcinog*** 2012; 51: 619-627.
15. Kobayashi J, Fujimoto H, Sato J, Hayashi I, Burma S, Matsuura S, et al. Nucleolin participates in DNA double-strand break-induced damage response through MDC1-dependent pathway. ***PLoS One*** 2012; 7: e49245.
16. De A, Donahue SL, Tabah A, Castro NE, Mraz N, Cruise JL, et al. A novel interaction [corrected] of nucleolin with Rad51. ***Biochem Biophys Res Commun*** 2006; 344: 206-213.
17. Ishimaru D, Zuraw L, Ramalingam S, Sengupta TK, Bandyopadhyay S, Reuben A, et al. Mechanism of regulation of bcl-2 mRNA by nucleolin and AþU-rich element-binding factor 1 (AUF1). ***J Biol Chem*** 2010; 285: 27182-27191.
18. Pickering BF, Yu D, Van Dyke MW. Nucleolin protein interacts with microprocessor complex to affect biogenesis of microRNAs 15a and 16. ***J Biol Chem*** 2011; 286: 44095-44103.
19. Chen J, Guo K, Kastan MB. Interactions of nucleolin and ribosomal protein L26 (RPL26) in translational control of human p53 mRNA. ***J Biol Chem*** 2012; 287: 16467-16476.
20. Wise JF, Berkova Z, Mathur R, Zhu H, Braun FK, Tao RH, et al. Nucleolin inhibits Fas ligand binding and suppresses Fas-mediated apoptosis in vivo via a surface nucleolin-Fas complex. ***Blood*** 2013; 121: 4729-4739.
21. Farin K, Schokoroy S, Haklai R, Cohen-Or I, Elad-Sfadia G, Reyes-Reyes ME,et al. Oncogenic synergism between ErbB1, nucleolin, and mutant Ras. ***Cancer Res*** 2011; 71: 2140-2151.
22. Watanabe T, Takahashi A, Suzuki K, Kurusu-Kanno M, Yamaguchi K, Fujiki H, et al. Epithelial-mesenchymal transition in human gastric cancer cell lines induced by TNF-ß-inducing protein of Helicobacter pylori. ***Int J Cancer*** 2014; 134: 2373-2382.
23. Yang Y, Yang C, Zhang J. C23 protein meditates bone morphogenetic protein 2-mediated EMT via up-regulation of Erk1/2 and Akt in gastriccancer. ***Med Oncol*** 2015; 32: 76.
24. Hsu TI, Lin SC, Lu PS, Chang WC, Hung CY, Yeh YM, et al. MMP7-mediated cleavage of nucleolin at Asp255 induces MMP9 expression to promote tumor malignancy. ***Oncogene*** 2015; 34: 826-837.
25. Qi J, Li H, Liu N, Xing Y, Zhou G, et al. The implications and mechanisms of the extra-nuclear nucleolin in the esophageal squamous cell carcinomas. ***Med Oncol*** 2015; 32: 45.
26. Dai C, Lv S, Shi R, Ding J, Zhong X, et al. Nuclear Protein C23 on the Cell Surface Plays an Important Role in Activation of CXCR4 Signaling in Glioblastoma. ***Mol Neurobiol*** 2015; 52: 1521-1526.
27. Qiu W, Wang G, Sun X, Ye J, Wei F, Shi X, et al. The involvement of cell surface nucleolin in the initiation of CCR6 signaling in human hepatocellular carcinoma. ***Med Oncol*** 2015; 32: 75.
28. Liang P, Jiang B, Lv C, Huang X, Sun L, Zhang P, et al. The expression and proangiogenic effect of nucleolin during the recovery of heat-denatured HUVECs. ***Biochim Biophys Acta*** 2013; 1830: 4500-4512.
29. Cheng DD, Zhao HG, Yang YS, Hu T, Yang QC. GSK3 negatively regulates HIF1a mRNA stability via nucleolin in the MG63 osteosarcoma cell line. ***Biochem Biophys Res Commun*** 2014; 443: 598-603.
30. Zhuo W, Luo C, Wang X, Song X, Fu Y, Luo Y, et al. Endostatin inhibits tumour lymphangiogenesis and lymphatic metastasis via cell surface nucleolin on lymphangiogenic endothelial cells. ***J Pathol*** 2010; 222: 249-260.
31. Joo EJ, Yang H, Park Y, Park NY, Toida T, Linhardt RJ, et al. Induction of nucleolin translocation by acharan sulfate in A549 human lung adenocarcinoma. ***J Cell Biochem*** 2010; 110: 1272-1278.
32. Wu DM, Zhang P, Liu RY, Sang YX, Zhou C, Xu GC, et al. Phosphorylation and changes in the distribution of nucleolin promote tumor metastasis via the PI3K/Akt pathwayin colorectal carcinoma. ***FEBS Lett*** 2014; 588: 1921-1929.
33. Xu Z, Joshi N, Agarwal A, Dahiya S, Bittner P, Smith E, et al. Knocking down nucleolin expression in gliomas inhibits tumor growth and induces cell cycle arrest. ***J Neurooncol*** 2012; 108: 59-67.
34. Wu CD, Chou HW, Kuo YS, Lu RM, Hwang YC, Wu HC, et al. Nucleolin antisense oligodeoxynucleotides induce apoptosis and may be used as a potential drug for nasopharyngeal carcinoma therapy. ***Oncol Rep*** 2012; 27: 94-100.
35. Tominaga K, Srikantan S, Lee EK, Subaran SS, Martindale JL, Abdelmohsen K, et al. Competitive regulation of nucleolin expression by HuR and miR-494. ***Mol Cell Biol*** 2011; 31: 4219-4231.
36. Lai WY, Wang WY, Chang YC, Chang CJ, Yang PC, Peck K. et al. Synergistic inhibition of lung cancer cell invasion, tumor growth and angiogenesis using aptamer-siRNA chimeras. ***Biomaterials*** 2014; 35: 2905-2914.
37. Rosenberg JE1, Bambury RM, Van Allen EM, Drabkin HA, Lara PN Jr, Harzstark AL, et al. A phase II trial of AS1411 (a novel nucleolin-targeted DNA aptamer) in metastatic renal cell carcinoma. ***Invest New Drugs*** 2014; 32: 178-187.
38. Malik MT, O'Toole MG, Casson LK, Thomas SD, Bardi GT, Reyes-Reyes EM, et al. AS1411 conjugated gold nanospheres and their potential for breast cancer therapy. ***Oncotarget*** 2015; 6: 22270-22281.
39. Li L, Hou J, Liu X, Guo Y, Wu Y, Zhang L, et al. Nucleolin-targeting liposomes guided by aptamer AS1411 for the delivery of siRNA for the treatment of malignant melanomas. ***Biomaterials*** 2014; 35: 3840-3850.

40. Alibolandi M, Ramezani M, Abnous K, Hadizadeh F. AS1411 Aptamer-Decorated Biodegradable Polyethylene Glycol-Poly(lactic-co-glycolic acid) Nanopolymersomes for the Targeted Delivery of Gemcitabine to Non-Small Cell Lung Cancer In Vitro. ***J Pharm Sci*** 2016; 105: 1741-1750.
41. Liao ZX, Chuang EY, Lin CC, Ho YC, Lin KJ, Cheng PY, et al. An AS1411 aptamer-conjugated liposomal system containing a bubble-generating agent for tumor-specific chemotherapy that overcomes multidrug resistance. ***J Control Release*** 2015; 208: 42-51.
42. Li X, Yu Y, Ji Q, Qiu L. Targeted delivery of anticancer drugs by aptamer AS1411 mediated Pluronic F127/cyclodextrin-linked polymer composite micelles. ***Nanomedicine*** 2015; 11: 175-184.
43. Lale SV, Aravind A, Kumar DS, Koul V. AS1411 aptamer and folic acid functionalized pH-responsive ATRP fabricated pPEGMA-PCL-pPEGMA polymeric nanoparticles for targeted drug delivery in cancer therapy. ***Biomacromolecules*** 2014; 15:1737-1752.
44. Krust B, El Khoury D, Nondier I, Soundaramourty C, Hovanessian AG. Targeting surface nucleolin with multivalent HB-19 and related Nucant pseudopeptides results in distinctinhibitory mechanisms depending on the malignant tumor cell type. ***BMC Cancer*** 2011; 11: 333.
45. Destouches D, E Khoury D, Hamma-Kourbali Y, Krust B, Albanese P, et al. Suppression of tumor growth and angiogenesis by a specific antagonist of the cell-surface expressed nucleolin. ***PLoS One*** 2008; 3: e2518.
46. Krust B, E Khoury D, Soundaramourty C, Nondier I, Hovanessian AG. Suppression of tumorigenicity of rhabdoid tumor derived G401 cells by the multivalent HB-19 pseudopeptide that targets surface nucleolin. ***Biochimie*** 2011; 93: 26-33.
47. Benedetti E, Antonosante A, d'Angelo M, Cristiano L, Galzio R, Destouches D, et al. Nucleolin antagonist triggers autophagic cell death in human glioblastoma primary cells and decreased in vivo tumor growth in orthotopic brain tumor model. ***Oncotarget*** 2015; 6: 42091-42104.