Ann Med Res

Current issue list available at AnnMedRes

Annals of Medical Research

journal page: www.annalsmedres.org

Imumunohistochemical analysis of CD133 and nucleostemin in squamous cell carcinoma and adenocarcinoma

Semih Tan[a,*], Hulya Cetin[b], Ferda Bir[c], Sevin Baser Oncel[d], Gamze Gokoz Dogu[e]

[a] *Ordu University, Faculty of Medicine, Department of Histology and Embriology, Ordu, Türkiye*
[b] *Pamukkale University, Faculty of Medicine, Department of Histology and Embriology, Denizli, Türkiye*
[c] *Pamukkale University, Faculty of Medicine, Department of Pathology, Denizli, Türkiye*
[d] *Pamukkale University, Faculty of Medicine, Department of Pulmonary Diseases, Denizli, Türkiye*
[e] *Pamukkale University, Faculty of Medicine, Department of Medical Oncology, Denizli, Türkiye*

ARTICLE INFO

Keywords:
Adenocarcinoma
Squamous cell cancer
CD133
Nucleostemin
Lung cancer

Received: Nov 10, 2023
Accepted: Mar 27, 2024
Available Online: 26.04.2024

DOI:
10.5455/annalsmedres.2023.11.304

Abstract

Aim: Cancer stands as a significant health issue in our era, with lung cancer being among the most widespread types in both Turkey and worldwide. The diagnosis may be delayed due to the late detection of symptoms and signs. Cancer stem cells cause the initiation and progression of cancer. There is a risk of cancer recurrence due to cancer stem cells that cannot be destroyed. Therefore, it is important to detect cancer stem cells and prevent the proliferation of these stem cells.

Materials and Methods: In the Pathology Department of Pamukkale University Faculty of Medicine, 72 squamous cell carcinoma and 51 adenocarcinoma cases diagnosed with non-small cell lung cancer were retrospectively examined. Nucleostemin and CD133 expression levels were evaluated immunohistochemically in sections taken from cancerous tissue samples of the cases.

Results: Disease-free survival and tumor stages of the cases and immunohistochemical expression level were compared. Nucleostemin and CD133 expression was commonly observed in tumor tissue from both adenocarcinoma and squamous cell carcinoma cases.

Conclusion: Therefore, in line with the data we obtained, we think that both nucleostemin and CD133 cannot be used as prognostic markers in non-small cell lung cancer.

Introduction

Non-small cell lung cancer (NSCLC) is the most common type of lung cancer in our country as well as in the world. Despite advances in surgical techniques, new chemotherapeutic drugs, and adjustments in classification and staging, 5-year survival was approximately 12% in 1974-76, but today it has only reached 15%, and in advanced stage cases, this rate is below 10% [1, 2].

The most common histological subtypes among NSCLCs are squamous cell carcinoma (SCC) (38.2%) and adenocarcinoma (ADC) (47.1%). NSCLC accounts for 80% to 85% of lung cancer cases, and approximately 2/3 of the cases are advanced at diagnosis [3]. NSCLC is diagnosed late because symptoms and findings are detected late. 70% of cases do not have the opportunity for surgery at the time of diagnosis. In studies conducted worldwide, 30-40% of cases are diagnosed at a locally advanced stage [2].

Today, chemotherapy, radiotherapy and surgery are used in cancer treatment, and the aim of the treatment techniques is to shrink the tumor. There is a risk of cancer recurrence due to cancer stem cells that cannot be destroyed [4]. Conventional cancer treatments such as radiotherapy or chemotherapy can eliminate most tumors, but Cancer stem cells (CSCs) are thought to be resistant to treatment and responsible for tumor recurrence. To prevent or significantly delay relapse, these stem cells need to be targeted and eliminated [5].

If the cancer stem cells that maintain the tumor are not destroyed, the risk of cancer recurrence continues. However, cancer treatment techniques aim to shrink the tumor [4]. Since CSCs are responsible for the initiation, recurrence and metastatic growth of cancer, targeting these cells to destroy them provides successful treatment. Perfect identification of the type of CSC is the most important step for an appropriate treatment of CSCs. Targeting surface markers and activated signaling pathways is promising in creating specific treatments against CSCs [6-8].

*Corresponding author:
Email address: tansemih@hotmail.com (Semih Tan)

Like CSCs, lung cancer stem cells (LCSCs) have spheroid and colony formation properties, with specific markers such as CD133, CD44, ABCG2 and ALDH1A1 [8]. Current information on the biology of LCSCs is limited. In addition, a number of CSC determinants have been identified and studied. These CSC markers are associated with resistance to cancer treatments. Some of those; include aldehyde dehydrogenase (ALDH1), CD133, side population (Hoechst-negative), CD44, CD87 and CD117 [8].

One of the most commonly used CSC markers is CD133. It is a cell surface glycoprotein with a molecular weight of 117 kDa, containing extracellular and five transmembrane regions [9]. It is a surface marker for hematopoietic stem cells. It ensures the preservation of stem cell properties in adult stem cells, and the expression of this gene is also associated with various cancers [8]. CD133 is a protein that forms membrane-membrane interactions and interacts with cholesterol [10]. CD133 (Prom1) was used to identify CSCs in lung cancer [11].

Nucleostemin, a nuclear protein, that plays a role in maintaining stem cell properties was first identified by Tsai and Mckay in 2002 [12]. Nucleostemin is encoded by GNL3 gene that located on chromosome 3p21.1. The nucleostemin gene is 8578 bases in size. Its structure includes an amino terminus, a coiled-coil region, 5 GTP binding motifs (G1-5) and an acidic carboxyl terminus. Nucleostemin is a protein that is found in low amounts in the nucleoplasm and can pass bidirectionally between the nucleolus and the nucleoplasm. It is thought that control of the transition between the nucleolus and nucleoplasm is provided through GTP binding. When GTP is not bound, nucleostemin localizes in the nucleolus. It binds to the p53 protein with the amino end of the nucleostemin, which binds with GTP and exits into the nucleoplasm, and causes active cell division by preventing the increase of p53 accumulation in the cell [13].

In case of nucleostemin deletion, the cell cycle is disrupted and the cell is eliminated by apoptosis. It is thought to play an important role in cell self-renewal, cell cycle regulation, apoptosis and cell proliferation, thanks to its interaction with molecules such as the tumor suppressor protein p53 [14]. p53 is a tumor suppressor protein. It regulates target genes that produce proteins that lead to DNA repair, cellular senescence, apoptosis and cell cycle arrest [13].

In a study, it was revealed that the decrease in nucleostemin caused cell cycle arrest at the G1 checkpoint by increasing the level of p53, and this effect was not observed in cells genetically deficient in p53 [15].

Dai et al. (2008) determined that nucleostemin inhibits MDM2-regulated p53 ubiquitylation and degradation by binding to the central acidic region of MDM2. In situations such as DNA damage, nucleolar and oncogenic stress, MDM2 is largely inhibited and p53 is activated. P53 is kept at low levels in normal unstressed cells by its inhibitor MDM2. They revealed that overexpression of ectopic nucleostemin induces G1 cell cycle arrest and suppresses cell proliferation by activating p53 by eliminating the repression of MDM2 on p53. They also reported that depletion of nucleostemin with siRNA induces G1 cell cycle arrest by activating p53 [16].

Hsu et al. In 2012, they demonstrated that nucleostemin has the ability to reduce telomere damage through TRF-1 modification and by enhancing promyelocytic leukemia protein isoform IV (PML-IV) in telomerase-negative human cancer cells [17]. Uema et al. (2013) state that nucleostem is a nuclear protein involved in ribosomal biogenesis and protection of telomeres [18].

Nucleostemin is expressed in embryonic stem cells, central nervous system stem cells, primitive cells in the bone marrow and germ cells in the testis [19]. Nucleostemin, which plays an important role in controlling the proliferation of stem cells, undergoes a significant loss of expression with the onset of cellular differentiation [14].

This study aimed to detect the immunohistochemical expression of nucleostemin and CD133 in lung cancer, to investigate the correlations between nucleostemin and CD133 expression levels and clinicopathological parameters, and to evaluate the effects of expressions on prognosis.

Materials and Methods

A total of 123 lung carcinoma cases, including NSCLC, 72 SCC and 51 ADC, diagnosed in the Pathology Department of Pamukkale University Faculty of Medicine between 2007 and 2018, ranging in stages from I to IV, without receiving preoperative anticancer treatment, were retrospectively examined. Ethics committee approval for our study was received from Pamukkale University Non-Interventional Clinical Research Ethics Committee with the decision numbered 16 dated 07.08.2018 and supported by Pamukkale University Scientific Research Projects Coordination Unit (project number: 2018SABE041).

Of the cases included in the study, 74 (60.2%) had lobectomy, 25 (20.3%) had wedge resection, 16 (13%) had pneumonectomy, and 8 (6.5%) had excisional biopsy. Information regarding standard demographic information of the cases, preoperative stage of the tumor, type of treatment, follow-up periods and disease outcomes (disease-free survival data, relapse, cancer-related death data) were obtained from the records. Diagnosis of lung carcinoma, differentiation of the tumor in the histological sections of the resection materials of each patient; The presence of metastatic lymph nodes in the lymph node dissection materials was reviewed and evaluated.

Tumor staging was performed according to the 2015 "International Association for the Study of Lung Cancer" (8th Edition of the TNM Classification for Lung Cancer) criteria.

A sample that best reflected the tumor tissue was determined. For each case, two H&E sections were taken from the selected paraffin blocks onto positively charged slides, and three 3-micron sections were taken to study nucleostemin and CD133 antibodies. The sections were taken from the bain-marie onto slides and placed in the slide carrying basket. The slide carrying basket was kept in the oven at 60 °C for 1 night. It was kept in xylene for 1 hour for deparaffinization. The tissues were removed from xylene, dried in air, and marked with a PAP pen. The sections were kept in ethyl alcohol series of 100%, 96%, 80%, 70%, 50%, respectively, for 2 minutes each. The alcohol

preparations were washed with distilled water 3 times for 5 minutes. For the antigen retrieval process, the tissues were kept in a microwave oven in citrate buffer until they boiled, and after the boiling started, they were kept for 20 minutes and then allowed to cool to room temperature. It was kept in Phosphate Buffered Saline (PBS) for 10 minutes. Endogenous peroxidase activity in tissues was eliminated by a 10-minute application with a 30% H_2O_2: Methanol (1: 9) mixture. The sections were washed with PBS and kept at room temperature for 10 minutes with serum blocking solution added to them. Nucleostemin (FineTest, Wuhan Biotech Co, Ltd. FNab01510) and CD133 (Bioassay Tech. Lab. BT-AP02809) primary antibodies were added to the sections and kept overnight. After washing with PBS, the sections were treated with secondary antibodies with biotinylated affinity that reacted with the primary antibodies for 20 minutes. A broad-spectrum universal secondary antibody kit (Thermo Scientific) was used for this procedure. The sections were washed again with PBS and treated with horseradish peroxidase conjugate streptavidin (HRP-SA), which can easily bind to biotinylated secondary antibodies, for 10 minutes. After the sections were washed with PBS for the last time, they were treated with chromogen dye DAB (Thermo Scientific) for 3-10 minutes. To better observe the localization of the antigen, the sections were counterstained with hematoxylin (Merk Harris' hematoxylin). The sections were washed in running water and kept in ethyl alcohol series of 50%, 70%, 80%, 96%, 100%, respectively, for 2 minutes each. The tissues obtained from the alcohol series were kept in xylene I and xylene II for 2 minutes each. The tissues taken from xylene were covered with entellan without waiting for them to dry. It was evaluated with a light microscope.

Semi-quantitative evaluation

Immunostained sections were examined under a light microscope (40x) and lesion regions (ROI) were selected using Visiopharm's new-CAST™ software. Among the selected areas, the area where the first count would be made was randomly selected and the cells were counted in every 3 areas using an unbiased counting frame at 400x magnification. The number of positively stained cells was scored as 0-10% (1), 11-50% (2), 51-80% (3), and more than 80% (4). Staining intensity, 0=negative; 1=weak; It was rated from 0 to 3, with 2=moderate and 3=strong. Theoretically, scoring could range from 0 to 12. An immunohistochemistry score of 9 to 12 was considered strong immunoreactivity, 5 to 8 as moderate, 1 to 4 as weak, and 0 as negative [20]. P values were evaluated by one-way analysis of variance.

Statistical analysis

Data were analyzed with SPSS 25.0 (IBM SPSS Statistics 25 software (Armonk, NY: IBM Corp.)). Continuous variables are expressed as mean ± standard deviation, median (minimum-maximum values). The suitability of the data for normal distribution was examined with the Shapiro Wilk test. One-Way Analysis of Variance in comparing independent group differences when parametric test assumptions are met; When parametric test assumptions were not met, Bonferroni multiple comparison test was used to compare independent group differences. In all analyses, $p<0.05$ was considered statistically significant.

Results

Clinical findings

Among the 123 patients included in our study, the number of patients with ADC was 72 and the number of patients with SCC was 51. The distribution of the patients included in our study in terms of age, gender, location of the carcinoma, presence or absence of distant metastasis, local recurrence and patient survival is shown in Table 1.

Histopathological features

The patients included in our study were evaluated according to tumor diameter, lymph node metastasis, angiolymphatic invasion and pleural Patient distribution according to invasion criteria is shown in Table 2. When we look at the distribution of the cases according to the stages according to the AJCSS lung cancer staging system, it is seen that a large proportion of the patients included in the study are in the stage IIA-IIIA range (Table 3).

In our study, patient groups diagnosed with ADC (Figure 1) and SCC (Figure 2) were evaluated in terms of nucleostemin and CD133 expression. In microscopic examination of immunohistochemical staining, patient results within groups vary. In this context, our analysis results

Table 1. Clinicopathological characteristics of the patients.

Clinicopathological Features	Number of SCC Patients (72)	SCC Percentage (%)	Number of ADC Patients (51)	ADC Percentage (%)
Age				
<40	0	0.00%	0	0%
40-65	42	58.33%	20	39%
>65	30	41.67%	31	61%
Gender				
Male	72	100.00%	39	76%
Woman	0	0.00%	12	24%
Localization				
Right	34	47.22%	34	67%
Left	38	52.78%	17	33%
Distant Metastasis				
There is	6	8.33%	8	16%
None	66	91.67%	43	84%
Local Relapse				
There is	8	11.11%	7	14%
None	64	88.89%	44	86%
Patient Survival				
Alive	30	41.67%	22	43%
Ex	42	58.33%	29	57%

Table 2. Distribution of patients according to histopathological features in the patient groups included in the study.

Clinicopathological Features	Number of SCC Patients	SCC %(Percent)	Number of ADC Patients	ADC %(Percent)
Tumor Diameter				
<3	23	32%	19	37%
>3	49	68%	32	63%
Lymph Node Metastasis				
There is	27	38%	20	39%
None	45	63%	31	61%
Angiolymphatic Invasion				
There is	28	39%	20	39%
None	44	61%	31	61%
Patient Survival				
There is	25	35%	22	43%
None	47	65%	29	57%

Table 3. Distribution of cases according to stages according to the AJCSS lung cancer staging system.

AJCSS Stage	Number of SCC Patients	SCC %(Percent)	Number of ADC Patients	ADC %(Percent)
Stage IA1	0	0%	1	2%
Stage IA2	0	0%	0	0%
Stage IA3	9	13%	4	8%
Stage IB	6	8%	3	6%
Stage IIA	21	29%	12	24%
Stage IIB	20	28%	18	35%
Stage IIIA	14	19%	11	22%
Stage IIIB	2	3%	2	4%

did not show a statistically significant difference between the groups in terms of nucleostemin and CD133 expression. Since no significant change could be detected in nucleostemin and CD133 expression, no comparison was made with patient demographic data.

Histopathological features

The patients included in our study were evaluated according to tumor diameter, lymph node metastasis, angiolymphatic invasion and pleural Patient distribution according to invasion criteria is shown in Table 2.

When we look at the distribution of the cases according to the stages according to the AJCSS lung cancer staging system, it is seen that a large proportion of the patients included in the study are in the stage IIA-IIIA range (Table 3).

In our study, patient groups diagnosed with ADC (Figure 1) and SCC (Figure 2) were evaluated in terms of nucleostemin and CD133 expression. In microscopic examination of immunohistochemical staining, patient results within groups vary. In this context, our analysis results did not show a statistically significant difference between the groups in terms of nucleostemin and CD133 expres-

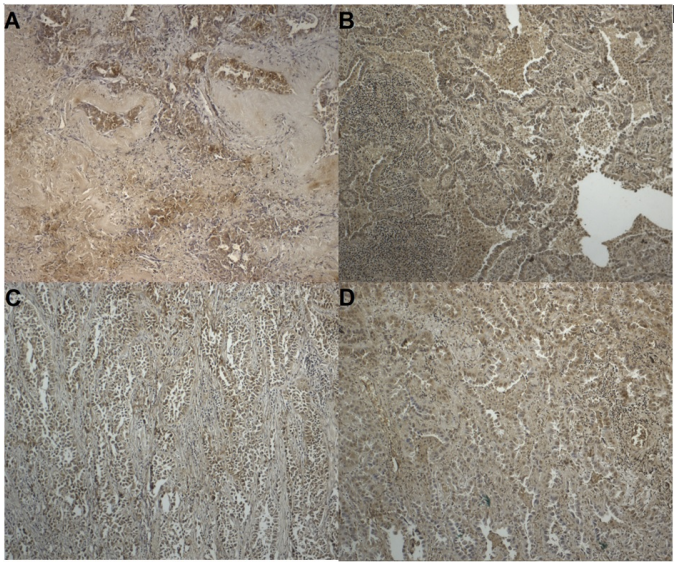

Figure 1. Immunohistochemical analysis of tissue sections obtained from patients with ADC using CD133 and nucleostemin primary antibodies. A-B Sections obtained from two different ADC patients stained with DAP (Brown) show areas expressing CD133. C-D Sections obtained from two different ADC patients stained with DAP (Brown) show areas expressing nucleostemin. Magnification: (200X).

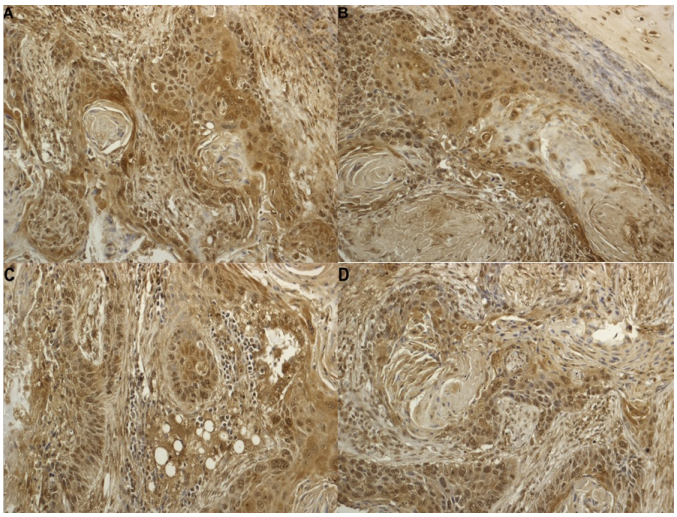

Figure 2. Immunohistochemical analysis of tissue sections obtained from patients with SCC using CD133 and nucleostemin primary antibodies. A-B Sections obtained from two different SCC patients stained with DAP (Brown) show areas expressing CD133. C-D Sections obtained from two different SCC patients stained with DAP (Brown) show areas expressing nucleostemin. Magnification: (200X).

sion. Since no significant change could be detected in nucleostemin and CD133 expression, no comparison was made with patient demographic data.

Discussion

Cancer stem cells enable the initiation and progression of cancer because they form tumor cells and have the capacity to renew themselves. One of the most commonly used CSC markers is CD133 cell surface glycoprotein. As a result of the immunohistochemical evaluation of ADC and SCC cases in our study, widespread staining was observed in the tumor tissues of both groups (Figure 1,2). No statistical difference could be detected between both groups.

A study in lung cancer cell lines showed that $CD133^+$ cancer cells proliferated by forming tumor spheres, that $CD133^+$ cells were resistant to chemotherapy , and expressed high levels of the ATP-binding cassette G2. It has also been shown that the same cell group is associated with poor survival in NSCLC patients treated with chemotherapy [21]. It has been stated that CD133+ cells have tumor initiation, self-renewal and drug resistance [22].

High levels of CD133 expression were detected in 26% of osteosarcoma patient tissues. It was stated that no significant correlation was observed between CD133 expression and clinicopathological factors [23]. In ADC, CD133 expression, low pathological stage and lymphovascular It has been associated with the absence of invasion [24]. It has been stated that both MFG-E8 and CD133 expression levels in the tumor are strong predictors of poor clinical outcome in epithelial ovarian cancer patients [25].

Aggressive in breast cancer Nucleostemin expression is emphasized as a marker of phenotype and poor prognosis [26]. Nucleostemin is located in the cervix in humans. It has been reported to be expressed in SCCs, esophageal cancers, renal cell cancers and prostate cancers [27-30]. Kobayashi et al. (2014) nucleostema in invasive breast cancers They conducted a study investigating its clinicopathological and prognostic effects. In the study, nucleostem positivity was found to be 64.5% and was reported to correlate with estrogen receptor (ER), HER-2 and p53 positivity. Additionally , significantly shorter disease-free survival was observed in nucleostemin- positive cases compared to nucleostemin- negative cases. Additionally, it has been revealed that p53 positive, nucleostemin negative patients have a worse prognosis than other patient groups. As a result, it has been suggested that nucleostem status may be a useful prognostic marker in luminal and HER-2 type breast cancers [19]. Nucleostemin transcription levels in acute myeloid leukemias (AML), the highest nucleostemin level was found in the AML M1 subtype and the lowest level was found in the AML M3 subtype. It has also been stated that nucleostemin transcription levels correlate with blast rate, CD34 and CD117 expression. Based on these results, it has been suggested that nucleostemin expression may be useful in monitoring minimal residual disease in AML patients [31]. It has been stated that there is evidence that nucleostemin can accelerate proliferation in gliomas through the Wnt /β- Catenin pathway [32].

Li X et al. (2015) investigated the expression of nucleostemin in NSCLC. They detected higher nucleostemin expression levels in poorly differentiated tumors than in well- differentiated ones. In the same study, while more intense nucleostemin expression was detected in SCC than ADC, nucleostemin expression was not observed in normal lung tissue [25].

There is a correlation between lymph node metastasis, distant metastasis and TNM stage and nucleostemin expression in colorectal carcinomas. It has also been shown that cases with low nucleostemin expression levels have longer survival times than those with high expression [33].

In our study, we observed that the nuclear protein nucleostemin and the cell surface glycoprotein CD133 were widely expressed in the tumor tissue immunohistochemically (Figures 1,2). We could not detect any statistical difference in nucleostemin and CD133 expression in both tumor types. We observed that the patient survival rate was close to each other in both tumor types. CSCs are an important step in appropriate cancer treatment. We believe that studies on the detection and treatment of CSC have an important place in the treatment of cancer and that studies on this subject are needed.

Ethical approval

Ethics committee approval for the study was received from Pamukkale University Non-Interventional Clinical Research Ethics Committee with the decision numbered 16 dated 07.08.2018.

References

1. Hotta K, Fujiwara Y, Kiura K, Takigawa N, Tabata M, Ueoka H, et al. Relationship between response and survival in more than 50,000 patients with advanced non-small cell lung cancer treated with systemic chemotherapy in 143 phase III trials. J Thorac Oncol. 2007;2(5):402-7.
2. Jemal A, Bray F, Center MM, Ferlay J, Ward E, Forman D. Global cancer statistics. CA Cancer J Clin. 2011;61(2):69-90.
3. Müdürlüğü HSG. Türkiye Kanser İstatistikleri 2015. Türkiye Cumhuriyeti Sağlık Bakanlığı; 2018.
4. Codd AS, Kanaseki T, Torigo T, Tabi Z. Cancer stem cells as targets for immunotherapy. Immunology. 2018;153(3):304-14.
5. Singh SK, Clarke ID, Hide T, Dirks PB. Cancer stem cells in nervous system tumors. Oncogene. 2004;23(43):7267-73.
6. Dragu DL, Necula LG, Bleotu C, Diaconu CC, Chivu-Economescu M. Therapies targeting cancer stem cells: Current trends and future challenges. World J Stem Cells. 2015;7(9):1185-201.
7. de Sousa e Melo F, Kurtova AV, Harnoss JM, Kljavin N, Hoeck JD, Hung J, et al. A distinct role for Lgr5(+) stem cells in primary and metastatic colon cancer. Nature. 2017;543(7647):676-80.
8. Prabavathy D, Swarnalatha Y, Ramadoss N. Lung cancer stem cells-origin, characteristics and therapy. Stem Cell Investig. 2018;5:6.
9. Visvader JE, Lindeman GJ. Cancer stem cells in solid tumours: accumulating evidence and unresolved questions. Nat Rev Cancer. 2008;8(10):755-68.
10. Corbeil D, Marzesco AM, Fargeas CA, Huttner WB. Prominin-1: a distinct cholesterol-binding membrane protein and the organisation of the apical plasma membrane of epithelial cells. Subcell Biochem. 2010;51:399-423.
11. Hardavella G, George R, Sethi T. Lung cancer stem cells-characteristics, phenotype. Transl Lung Cancer Res. 2016;5(3):272-9.
12. Tsai RY, McKay RD. A nucleolar mechanism controlling cell proliferation in stem cells and cancer cells. Genes Dev. 2002;16(23):2991-3003.
13. Çalışkan E. Glioblastoma multiforme tümör hücrelerinde nükleostemin ifadesinin tanı ve prognozda etkinliği [Master thesis]: Ankara Üniversitesi Biyoteknoloji Enstitüsü; 2011.

14. Tsai RY, McKay RD. A multistep, GTP-driven mechanism controlling the dynamic cycling of nucleostemin. J Cell Biol. 2005;168(2):179-84.
15. Ma H, Pederson T. Depletion of the nucleolar protein nucleostemin causes G1 cell cycle arrest via the p53 pathway. Mol Biol Cell. 2007;18(7):2630-5.
16. Dai MS, Sun XX, Lu H. Aberrant expression of nucleostemin activates p53 and induces cell cycle arrest via inhibition of MDM2. Mol Cell Biol. 2008;28(13):4365-76.
17. Hsu JK, Lin T, Tsai RY. Nucleostemin prevents telomere damage by promoting PML-IV recruitment to SUMOylated TRF1. J Cell Biol. 2012;197(5):613-24.
18. Uema N, Ooshio T, Harada K, Naito M, Naka K, Hoshii T, et al. Abundant nucleostemin expression supports the undifferentiated properties of germ cell tumors. Am J Pathol. 2013;183(2):592-603.
19. Kobayashi T, Masutomi K, Tamura K, Moriya T, Yamasaki T, Fujiwara Y, et al. Nucleostemin expression in invasive breast cancer. BMC Cancer. 2014;14:215.
20. Soslow RA, Dannenberg AJ, Rush D, Woerner BM, Khan KN, Masferrer J, et al. COX-2 is expressed in human pulmonary, colonic, and mammary tumors. Cancer. 2000;89(12):2637-45.
21. Eramo A, Lotti F, Sette G, Pilozzi E, Biffoni M, Di Virgilio A, et al. Identification and expansion of the tumorigenic lung cancer stem cell population. Cell Death Differ. 2008;15(3):504-14.
22. Herpel E, Jensen K, Muley T, Warth A, Schnabel PA, Meister M, et al. The cancer stem cell antigens CD133, BCRP1/ABCG2 and CD117/c-KIT are not associated with prognosis in resected early-stage non-small cell lung cancer. Anticancer Res. 2011;31(12):4491-500.
23. Mardani A, Gheytanchi E, Mousavie SH, Madjd Jabari Z, Shooshtarizadeh T. Clinical Significance of Cancer Stem Cell Markers CD133 and CXCR4 in Osteosarcomas. Asian Pac J Cancer Prev. 2020;21(1):67-73.
24. Park E, Park SY, Sun PL, Jin Y, Kim JE, Jheon S, et al. Prognostic significance of stem cell-related marker expression and its correlation with histologic subtypes in lung adenocarcinoma. Oncotarget. 2016;7(27):42502-12.
25. Li X, Liu X, Cui D, Wu X, Qian R. Clinical significance of nucleostemin and proliferating cell nuclear antigen protein expression in non-small cell lung cancer. J buon. 2015;20(4):1088-93.
26. Sami MM, Hachim MY, Hachim IY, Elbarkouky AH, López-Ozuna VM. Nucleostemin expression in breast cancer is a marker of more aggressive phenotype and unfavorable patients' outcome: A STROBE-compliant article. Medicine (Baltimore). 2019;98(9):e14744.
27. Fan Y, Liu Z, Zhao S, Lou F, Nilsson S, Ekman P, et al. Nucleostemin mRNA is expressed in both normal and malignant renal tissues. Br J Cancer. 2006;94(11):1658-62.
28. Liu RL, Zhang ZH, Zhao WM, Wang M, Qi SY, Li J, et al. Expression of nucleostemin in prostate cancer and its effect on the proliferation of PC-3 cells. Chin Med J (Engl). 2008;121(4):299-304.
29. Nakajima TE, Yoshida H, Okamoto N, Nagashima K, Taniguchi H, Yamada Y, et al. Nucleostemin and TWIST as predictive markers for recurrence after neoadjuvant chemotherapy for esophageal carcinoma. Cancer Sci. 2012;103(2):233-8.
30. Ye F, Zhou C, Cheng Q, Shen J, Chen H. Stem-cell-abundant proteins Nanog, Nucleostemin and Musashi1 are highly expressed in malignant cervical epithelial cells. BMC Cancer. 2008;8:108.
31. You Y, Li X, Zheng J, Wu Y, He Y, Du W, et al. Transcript level of nucleostemin in newly diagnosed acute myeloid leukemia patients. Leuk Res. 2013;37(12):1636-41.
32. Bao Z, Wang Y, Yang L, Wang L, Zhu L, Ban N, et al. Nucleostemin promotes the proliferation of human glioma via Wnt/β-Catenin pathway. Neuropathology. 2016;36(3):237-49.
33. Wei B, Huang Q, Zhong X. Upregulation of nucleostemin in colorectal cancer and its effects on cell malignancy. Onco Targets Ther. 2015;8:1805-14.