Upregulation of CENP-H in tongue cancer correlates with poor prognosis and progression

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Abstract

Background: Centromere protein H (CENP-H) is one of the fundamental components of the human active kinetochore. Recently, CENP-H was identified to be associated with tumorigenesis. This study was aimed to investigate the clinicopathologic significance of CENP-H in tongue cancer.

Methods: RT-PCR, real time RT-PCR and Western blot were used to examine the expression of CENP-H in tongue cancer cell lines and biopsies. CENP-H protein level in paraffin-embedded tongue cancer tissues were tested by immunohistochemical staining and undergone statistical analysis. CENP-H-knockdown stable cell line was established by infecting cells with a retroviral vector pSuper-retro-CENP-H-siRNA. The biological function of CENP-H was tested by MTT assay, colony formation assay, and Bromodeoxyuridine (BrdU) incorporation assay.

Results: CENP-H expression was higher in tongue cancer cell lines and cancer tissues (T) than that in normal cell and adjacent noncancerous tongue tissues (N), respectively. It was overexpressed in 55.95% (94/168) of the paraffin-embedded tongue cancer tissues, and there was a strong correlation between CENP-H expression and clinical stage, as well as T classification. CENP-H can predict the prognosis of tongue cancer patients especially those in early stage. Depletion of CENP-H can inhibit the proliferation of tongue cancer cells (Tca8113) and downregulate the expression of Survivin.

Conclusion: These findings suggested that CENP-H involves in the development and progression of tongue cancer. CENP-H might be a valuable prognostic indicator for tongue cancer patients within early stage.
been shown to be deregulated in human cancers, which suggests an important role of kinetochore for chromosome instability and cancer development [6-9].

CENP-H was initially identified in the mouse centromere as a fundamental component of the active centromere [10,11]. Human CENP-H presented at the inner plate of kinetochore throughout the cell cycle, co-localized with CENP-A and CENP-C, and was necessary for the appropriate localization of CENP-C [10-13]. Recent report demonstrated that the CENP-H-I complex was required for the efficient incorporation of newly synthesized CENP-A into centromere [14]. These findings indicate that CENP-H might play an essential role in kinetochore assembly and function throughout the cell cycle. CENP-H is also strongly correlated with human cancer. It’s expression was deregulated in colorectal cancers, and ectopic overexpression of CENP-H induces chromosome instability in diploid cell lines [6]. In addition, CENP-H was deregulated in oral squamous cell carcinomas (SCCs), nasopharyngeal carcinoma (NPC), and esophageal carcinoma [15-17].

The expression of CENP-H in oral SCCs was significantly correlated with the cell proliferation in malignant conditions[17].

Genomic aberrations including aneuploidy in epithelial cells of the oral mucosa indicate high risks of oral cancer and cancer-related mortality [18]. Tongue cancer is one of the most common and serious types of oral cancer with poor prognosis [19,20]. It is of great clinical value to identify efficient proliferation markers and valuable markers that help to find tongue cancer patients at very early stage. In this study, we investigated the expression of CENP-H in tongue cancer and evaluated the role of CENP-H in proliferation of tongue cancer cells.

Methods

Cell cultures

Primary cultured normal tongue mucosa epithelial cells (TEC) were maintained in Keratinocyte-SFM (Gibco, Invitrogen Corp, USA). Tongue cancer cell lines TSCCa and Tca8113 were cultured in RPMI 1640 medium supplemented with 10% fetal calf serum (HyClone, Logan, UT).

Vectors and retroviral infection

Silence endogenous CENP-H, RNAi oligonucleotides (5-GGATCCTGCCCTTAAGGAAAT-3) was cloned into the pSuper-retro-puro vector to generate pSuper-retro-CENP-H-siRNA. Retroviral production and infection were performed as described previously[21]. Stable Tca8113 cells expressing CENP-H RNAi were selected for 10 days with

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Table 1: Primer Sequences Used for Reverse Transcription-PCR and Real-time Quantitative RT-PCR (5’ to 3’)

| Gene    | Forward primer     | Reverse primer     | Probe               |
|---------|--------------------|--------------------|---------------------|
| RT-PCR  | CENP-H             | TGCAAGAAAAGCAAATCGAA| ATCCCAAGATTCTCTGCTGTG| FAM-TTCCTTAAGGCCAGGATCT-TAMRA |
|         | GAPDH              | CCACCCATGGCAATTTCCATG| TCTAGACGGGAGTTCACTCCAC| FAM- CATCCTGCCACCAGAGACATG-TAMRA |
| Real-time PCR | CENP-H | CTTTTTTGGGAGTAAAGTCAT | ACAAATGCACAGAAATTTCCAAAT | FAM-TTCCTTAAGGCCAGGATCT-TAMRA |
|         | GAPDH              | GTACTGACCAAGTCCCATG | AGAGGCAGGGATGTTCTG | FAM- CATCCTGCCACCAGAGACATG-TAMRA |

Full gene names: CENP-H, centromere protein H; GAPDH, glyceraldehyde-3-phosphate dehydrogenase

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Figure 1

CENP-H expression was tested in normal tongue cell line and tongue cancer cell lines. (A) Expression of CENP-H protein in normal tongue cell line TEC and cultured tongue cancer cell lines TSCCa and Tca8113. (B) and (C) CENP-H mRNA level analyzed by RT-PCR and Real-time RT-PCR.
0.5 μg/ml puromycin 48 h after infection. After 10 days selection, the Tca8113 cell lysates prepared from the pooled population of cells in sample buffer were fractionated on sodium dodecyl sulfate-polyacrylamide gel electrophoresis for the detection of CENP-H protein level.

Patients and tissue specimens
The present study was performed on 168 cases of paraffin-embedded archived tongue cancer samples obtained from the Department of Pathology, the Second Affiliated Hospital of Sun Yat-sen University (PR China). Prior patients' consents and approval from the Institutional Research Ethics Committee were obtained for the purpose of research. The final study population included 61 female and 107 male patients (age range, 24–82 years). The median follow-up time for overall survival was 63.14 months (range, 3–169 months) for patients who were still alive at the time of the analysis. The resected tumors were

Figure 2
CENP-H expression in human tongue cancer tissues (T) and adjacent tongue tissues (N). (A) Comparative expression levels of CENP-H mRNA in six noncancerous and tongue cancer samples by RT-PCR. GAPDH was used as an internal control. (B) Comparative expression levels of in six noncancerous and tongue cancer samples by Western blot. Expression levels were normalized for α-Tubulin. (C) Real time-PCR analysis of CENP-H expression in each of the T and N tissues. GADPH was used as internal control. Columns, mean from three parallel experiments; bars, SD.
classified according to the current International Union Against Cancer (UICC) tumor-node-metastasis (TNM) classification [22,23].

**RT-PCR and real-time RT-PCR**

RT-PCR and real-time RT-PCR analysis were performed as described previously [24]. The primers and probes for RT-PCR and the real-time RT-PCR were designed with Primer Express v 2.0 (Applied Biosystems, Inc.) and provided in Table 1.

**Western blot**

Western blot analysis was performed as described previously[15,24] using anti-CENP-H (Bethyl Laboratories, Montgomery, Texas, USA), anti-α-Tubulin (Sigma, Saint Louis, Michigan, USA), anti-p21, anti-p27 and anti-Rb antibodies (Cell Signaling, Danvers, Massachusetts, USA).

**Immunohistochemical analysis**

The staining procedures and result measure of CENP-H were done as described previously[15,24]. The cells at each intensity of staining were recorded on a scale of 0 (no staining), 1 (weak staining = light yellow), 2 (moderate staining = yellowish brown), and 3 (strong staining = brown). An intensity score of ≥ 2 with at least 50% of malignant cells with positive CENP-H staining was used to classify tumors with high expression, and < 50% of malignant cells with nuclear staining or < 2 intensity score classified tumors with low expression of CENP-H.

**MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) assay**

Growing cells (5 × 103 per well) were seeded into 96-well plates. Cells were stained with 100 μl sterile MTT dye (0.5 mg/ml, Sigma, St. Louis, Missouri, USA) at each time point, followed by additional incubation for 4 h at 37 °C. After removal of the culture medium from each well, 150 μl of dimethyl sulphoxide (Sigma, St. Louis, MO, USA) was added and thoroughly mixed for 15 min. The optical density was read at 570 nm using a microplate reader (Bio-Rad 3500, Hercules, California, USA), with 655 nm as the reference wavelength. All experiments were performed in triplicate.

**Colonies formation assays**

Cells were seeded in 6-well plates (1×103 cells per well) and cultured for two weeks. The colonies were fixed with methanol for 10 min and stained with 1% crystal violet for 1 min. Each group of cells was performed in triplicate.

**Bromodeoxyuridine (BrdU) incorporation and immunofluorescence**

Cells grown on cover slips (Fisher, Pittsburgh, Pennsylvania, USA) were synchronized by serum starvation (0.5%FBS) for 48 h and then released into serum-containing medium for 4 h. The cells were labelled by incubating in 10-μM bromodeoxyuridine (BrdU) for 1 hour, fixed with 4% paraformaldehyde and stained with anti-BrdUrd antibody (Upstate, Temecula, California, USA). The cover slips were imaged with a con-focal laser-scanning microscope (Axiovert 200 M, Zeiss). At least 500 nuclei were counted to determine the proportion of positive nuclei (BrdU index). All values presented are the means of at least three independent experiments.

**Statistical analysis**

All statistical analyses were performed using the SPSS 13.0 statistical software package. The Mann-Whitney U test and Spearman's correlation coefficient by log-rank test were used to assess the relationship between CENP-H expression and clinicopathologic parameters. Overall survival curves were plotted by the Kaplan-Meier method and were compared by the log-rank test. The Cox proportional hazards regression model was used for multivariate analysis.
Student's t-test was used to compare the values between subgroups in all cases analyzed by real-time RT-PCR. In all cases, a $P$ value of less than 0.05 in all cases was considered statistically significant. All $P$ values were two-tailed.

**Results**

*CENP-H expression is elevated in human tongue cancer cells and primary tongue cancers*

Western blot analyses on normal tongue mucosa epithelial cells (TEC) and two tongue cancer cell lines (TSCCa and Tca8113) revealed that CENP-H protein was highly expressed in cancer cells, while it was only weakly detected in TEC cells (Figure 1A). The RT-PCR results displayed a higher expression of CENP-H mRNA in cancer cell lines than that in normal tongue cells (Figure 1B). Real-time RT-PCR results showed higher level of CENP-H mRNA in comparison with TEC cells, increasing up to 15-fold in both tongue cancer cell lines (Figure 1C). In addition, both CENP-H protein and mRNA were overexpressed in all six cases of tongue cancer biopsies compared with that in the matched adjacent noncancerous tissues (Figure 2A and 2B). The quantitative PCR showed that the

![Image](image_url)

**Figure 3**

*CENP-H protein expression in paraffin-embedded tongue cancer tissue samples and its prognostic value.* (A) Representative images of CENP-H protein expression examined by immunohistochemistry (IHC). CENP-H was only negatively or marginally detectable in non-cancerous tongue tissue (a, 200× and b, 400×), while it was positive in tongue cancer cells (c, 200× and d, 400×). (B) Upper panel: Overall survival of tongue cancer patients with low CENP-H expression versus high CENP-H-expressing tumors plotted with Kaplan-Meier analysis. Lower panel: Statistical significance of the difference between curves of CENP-H high-expression and low-expression patients was compared in stage I and stage II patient subgroups. $P$ values were calculated by log-rank test.
tumor/normal (T/N) ratio of CENP-H mRNA levels were diversity from approximately 4 to 20-fold (Figure 2C). immunohistochemical analysis further confirmed this result (Figure 2D). These observations suggested that high CENP-H expression was associated with the clinical progression of tongue cancer.

**Clinicopathological significance of CENP-H in human tongue cancer tissues**

55.95% (94/168) of the samples were highly detected by the rabbit-human CENP-H polyclonal antibody (Figure 3A). Signals were mainly observed in the cancerous areas, and no or only weak signals were detected in the normal tissues (Figure 3A). Additional file 1 shows that the immunohistochemical staining signal with CENP-H antibody could be completely blocked by recombinant CENP-H polypeptide. This result indicated that the CENP-H antibody used in the present study specifically recognizes the CENP-H protein.

Mann-Whitney U test showed that CENP-H expression was strongly correlated with clinical stage ($P = 0.005$) and T classification ($P = 0.004$). While no significant association was found between CENP-H level and lymph node metastasis ($P = 0.172$) (Table 2). There were also no significant correlations between the CENP-H expression level and age or gender (data not shown). Kaplan-Meier survival analysis showed a better outcome for patients who with low CENP-H level (Figure 3B, upper panel). The median survival period for patients with high CENP-H expression levels was substantially shorter (53 months) than that for patients with low CENP-H expression levels (76 months) ($P = 0.0006$, log-rank test). Multivariate Cox regression analysis revealed that the relationship between CENP-H expression and overall survival remained unchanged even when adjustments were made for tumor stage (Table 3). Additionally, CENP-H expression and overall survival were significantly correlated in stage I ($n = 38$, $P = 0.0033$) and stage II ($n = 41$, $P = 0.0117$) subgroups of patients (Figure 3B, lower panel). However, no such correlation was observed with regard to a subgroup of patients with stage III (data not shown). These results suggest that CENP-H can predict the prognosis of tongue cancer in patients only in the early stage of the disease.

**Downregulation of CENP-H inhibits proliferation of Tca8113 cells**

The impact of CENP-H expression on tongue cancer proliferation was evaluated in CENP-H knockdown cells (Figure 4). As shown in Figure 4A, the depletion of CENP-H expression caused significantly compromised viability in Tca8113 cells. The population doubling time cells of CENP-H RNAi are significantly shorter as compared with control (Figure 4A, $P < 0.05$). BrdU incorporation assays also demonstrated a significant inhibition of proliferation in Tca8113/CENP-H RNAi cells as compared to the control (Figure 4B, upper panel). The photographs of crystal violet stained Tca8113/control siRNA and Tca8113/CENP-H siRNA. Data were obtained from three independent experiments with similar results. Green:Brdu; Blue:DAPI. Lower: The photographs of crystal violet stained Tca8113/control siRNA and Tca8113/CENP-H siRNA. Data were obtained from three independent experiments with similar results. (C) Cell lysates were prepared for western blot analysis of antibodies against CENP-H and Survivin. α-Tubulin was detected as an internal control.
These results suggested that CENP-H is essential for the control Tca8113 cells (Figure 4B, lower panel, \( P < 0.01 \)). Colony formation assay revealed that Tca8113/CENP-H RNAi cells formed much less and smaller colonies than that of control Tca8113 cells (Figure 4B, lower panel, \( P = 0.01 \)). These results suggested that CENP-H is essential for the proliferation of Tca8113 cells in vitro.

**CENP-H regulates Survivin expression in tongue cancer cells**

As deregulation of the CENP-H expression firmly linked with proliferation of tongue cancer cells, we further investigated the modulate cell cycle factors which could be regulated by CENP-H. Western blot analysis revealed that the expression level of Survivin in CENP-H knockdown cells was significantly downregulated as compared with control cells (Figure 4C).

**Discussion**

Defects in kinetochore function are responsible for chromosome instability and the generation of cancer. Several kinetochore proteins have been shown to be deregulated in human oral SCCs. CENP-F and Survivin expression were elevated in oral SCCs [25]. CENP-H was upregulated in human oral SCCs and CENP-H mRNA expression level was significantly correlated with the clinical stage of this disease. Higher CENP-H mRNA level predicted poor prognosis of oral SCC patients [17]. In the present study, we found that CENP-H was upregulated in oral tongue cancer cells and tongue cancer tissue samples both at transcriptional levels and at translational levels, indicating that CENP-H might play a crucial role in the human tongue cancer. We also found that CENP-H level was positively correlated with the clinical stage and T classification. These results indicate the possible role of CENP-H in progression of oral tongue cancer. Furthermore, we found that CENP-H expression was a significant predictor of poor prognosis for a subgroup of patients with early-stage cancer according to the clinical stage. Together with our results, CENP-H may be a new biomarker of early-stage tongue cancer.

Recently, several studies have documented that deregulation of kinetochore proteins frequently occur in cancer development and progression [6,14-17,26-28]. Shigeishi et al. reported that CENP-H was deregulated in oral SCCs and closely linked to the increased or abnormal cell proliferation in malignant conditions [17]. Since our results showed that CENP-H was deregulated in tongue cancer, we consider whether change of CENP-H expression level can affect the growth of tongue cancer cells. In fact, we found that downregulation of CENP-H significantly inhibits the proliferation of tongue cancer cells.

We further investigated the potential mechanism by which CENP-H inhibits the proliferation rate of tongue cancer cells (Tca8113). We found that the expression level of Survivin in CENP-H-knockdown Tca8113 cells was significantly downregulated as compared with control cells. As an essential chromosome passenger protein, Survivin exhibits a dynamic interaction with centromeres, concentrated at the inner centromere at metaphase [29]. Survivin also belongs to the inhibitor of apoptosis protein family and functions as an essential regulator of cell division and apoptosis, and it ensuring continued cell proliferation and cell survival in unfavorable milieus [30-32]. Survivin is overexpressed in most oral SCCs and its high expression can predict poor prognosis of oral SCCs patients [33]. Additionally, expression of Survivin is an early event during oral carcinogenesis [34]. In the present study, we found that depletion of CENP-H can downregulate the expression of Survivin protein. Thus, the clinical and biological significance of CENP-H and Survivin oral cancer including tongue cancer suggested that both deregulation of Survivin and CENP-H were early event in development of this kind of cancer.

In summary, the present study not only demonstrated the possible role of CENP-H in the development and progression of tongue cancer, but also suggested the possibility of using CENP-H as a prognostic indicator for tongue cancer patients within early stage. To our knowledge, this is the first report showing that ectopic expression of CENP-H could significantly enhance proliferation of tongue cancer cells though upregulation of Survivin expression. However, the molecular mechanisms by which CENP-H upregulate Survivin expression need to be investigated in future.

**Conclusion**

In conclusion, expression of CENP-H was associated with clinical stage and T classification of tongue cancer, as well as poor prognosis of tongue cancer patients. Down-regulation of CENP-H can inhibit the proliferation of tongue cancer cells. These findings suggested that CENP-H play an important role in development and progression of tongue cancer. It also might be a valuable prognostic biomarker for early stage tongue cancer patients.

**Competing interests**

The authors declare that they have no competing interests.

**Authors’ contributions**

WL carried out cell cultures, establishment of stable cell lines, proliferation functional assays, and preparation of manuscript. CY and DW participated in RT-PCR and immunohistochemistry, as well as data analysis. LX and GW have been involved in western blot analysis and data interpretation. LZ participated in critical revision of the manuscript. MZ participated in the study design and coordination and helped to revise the manuscript. LS and JL conceived of the study, participated in experimental
design and coordination, and involved in data analysis and helped to draft the manuscript. All authors read and approved the final manuscript.

Additional material

Additional file 1
Validation for the specificity of CENP-H antibody. Tongue cancer sections were incubated with CENP-H antibody alone or previously co-incubated and thereby blocked with recombinant CENP-H polypeptide.
Click here for file [http://www.biomedcentral.com/content-supplementary/1756-9966-28-74-S1.doc]

Acknowledgements
Grant support: Science and Technology Bureau Foundation of Guang Zhou (200821-E201). National Natural Science Foundation of China grants, 30470666, and 30570701, 30670803, 30770836. The Ministry of Science and Technology of China grant 2004CB518708, the National Natural Science Foundation of Guangdong Province, China, grants 4009427 and S001749, and a key grant from 985-II project. Municipal Science and Technology of China grant 2004CB518708, the National Natural Science Foundation of China grants, 5001749, and a key grant from 985-II project. Municipal Science and Technology Bureau Foundation of Guang Zhou (20060302).

References
1. Fukagawa T, Assembly of kinetochores in vertebrate cells. Exp Cell Res 2004, 296:21-27.
2. Cleveland DW, Mao Y, Sullivan KF: Centromeres and kinetochores: from epigenetics to mitotic checkpoint signaling. Cell 2003, 113:407-421.
3. Westermann S, Cheeseman IM, Anderson S, Yates JR 3rd, Drubin DG, Barnes G: Architecture of the budding yeast kinetochore reveals a conserved molecular core. J Cell Biol 2003, 163:215-222.
4. Cheeseman IM, Drubin DG, Barnes G: Simple centromere, complex kinetochore: linking spindle microtubules and centromeric DNA in budding yeast. J Cell Biol 2002, 157:199-203.
5. De Wulf P, McAinsh AD, Sorger PK: Hierarchical assembly of the budding yeast kinetochore from multiple subcomplexes. Genes Dev 2003, 17:2902-2912.
6. Tomonaga T, Matsushita K, Ishibashi M, Nezu M, Shimada H, Ochiai T, Yoda K, Nomura F: Centromere protein H is up-regulated in primary human colorectal cancer and its overexpression induces aneuploidy. Cancer Res 2005, 65:4683-4689.
7. Barbani S, Ioannou M, Kouvavas E, Karasavvidou F, Nakou M, Papamichalou G: INCENP (Inner Centromere Protein) is Overexpressed in High Grade Non-Hodgkin B-cell Lymphomas. Pathol Oncol Res 2009, 13:11-17.
8. Erlanson M, Casiano CA, Tan EM, Lindh J, Roos G, Landberg G: Immunohistochemical analysis of the proliferation associated nuclear antigen CENP-F in non-Hodgkin's lymphoma. Mod Pathol 1999, 12:69-74.
9. Shigehita H, Mizuta K, Higashikawa K, Yoneda S, Ono S, Kamata N: Correlation of CENP-F gene expression with proliferation activity in human salivary gland tumors. Oral Oncol 2005, 41:716-722.
10. Sugata N, Munekata E, Todokoro K: Characterization of a novel kinetochore protein, CENP-H. J Biol Chem 1999, 274:27343-27346.
11. Fukagawa T, Mikami Y, Nishihashi A, Regnier V, Haraguchi T, Hiroaki Y, Sugata N, Todokoro K, Brown W, Ikemura T: CENP-H, a constitutive centromere component, is required for centromere targeting of CENP-C in vertebrate cells. Embio J 2001, 20:4603-4617.
12. Sugata N, Li S, Earnshaw WC, Yoda K, Maskos H, Munekata E, Warburton PE, Todokoro K: Human CENP-H multimers colocalize with CENP-A and CENP-C at active centromere – kinetochore complexes. Hum Mol Genet 2000, 9:2919-2926.
13. Cheeseman IM, Hori T, Fukagawa T, Desai A: KNL1 and the CENP-H/I/K Complex Coordinate Directly Kinetochore Assembly in Vertebrates. Mol Biol Cell 2008, 19:587-594.
14. Hori T, Okada M, Maenaka K, Fukagawa T: CENP-O class proteins form a stable complex and are required for proper kinetochore function. Mol Biol Cell 2008, 19:843-854.
15. Liao WT, Song LB, Zhang HZ, Zhang L, Liu WL, Feng Y, Guo BH, Mai HQ, Cao SM, Li MZ, Qin HD, Zeng YX, Zeng MS: Centromere protein H is a novel prognostic marker for nasopharyngeal carcinoma progression and overall patient survival. Clin Cancer Res 2007, 13:508-514.
16. Guo XZ, Zhang G, Wang JY, Liu WL, Wang F, Dong JQ, Xu LH, Cao JY, Song LB, Zeng MS: Prognostic relevance of Centromere protein H expression in esophageal carcinoma. BMC Cancer 2008, 8:233.
17. Shigehita H, Higashikawa K, Ono S, Mizuta K, Ninomiya Y, Yoneda S, Taki Kamata N: Increased expression of CENP-H gene in human oral squamous cell carcinomas harboring high-proliferative activity. Oncol Rep 2006, 16:1071-1075.
18. Rethmi SC, Gollin SM: Chromosomal instability in oral cancer progression. J Dent Res 2007, 86:490-496.
19. Greenberg JS, Fowler R, Gomez J, Mo V, Roberts D, El Naggar AK, Myers JN: Extent of extracapsular spread: a critical prognostic factor in oral tongue cancer. Cancer 2003, 97:1464-1470.
20. Haddadin KJ, Soutar DS, Webster MH, Robertson AG, Oliver RJ, MacDonald DG: Natural history and patterns of recurrence of tongue tumours. Br J Plast Surg 2000, 53:279-285.
21. Song LB, Zeng MS, Liao WT, Zhang L, Mo HY, Liu WL, Shao JY, Wu QL, Li MZ, Xia YF, Fu LW, Huang WL, Dimri GP, Band V, Zeng YX: Bmi-1 is a novel molecular marker of nasopharyngeal carcinoma progression and immortalizes primary human nasopharyngeal epithelial cells. Cancer Res 2006, 66:6225-6232.
22. Patel SG, Shah JP: TNM staging of the cancers of the head and neck: striving for uniformity among diversity. CA Cancer J Clin 2005, 55:242-258.
23. O'Sullivan B, Shah J: New TNM staging criteria for head and neck tumors. Semin Surg Oncol 2003, 21:30-42.
24. Liao WT, Wang X, Xu LH, Kong QL, Yu CP, Li MZ, Shi L, Zeng MS, Song LB: Centromere protein H is a novel prognostic marker for human nonsmall cell lung cancer progression and overall patient survival. Cancer 2009, 115:107-117.
25. Jane C, Nerurkar AV, Shah J, Song LB. Survivin is required for stable checkpoint activation in taxol-treated HeLa cells. J Cell Biochem Med 2006, 95:396-401.
26. Kops Gj, Weaver BA. Cleveland DW: On the road to cancer: aneuploidy and the mitotic checkpoint. Nat Rev Cancer 2005, 5:773-785.
27. de la Guardia C, Casiano CA, Trinidad-Pinedo J, Baez A: CENP-F gene amplification and overexpression in head and neck squamous cell carcinomas. Head Neck 2001, 23:104-112.
28. Clark GM, Allred DC, Hilsenbeck SG, Chamness GC, Osborne CK, Jones D, Lee WH: Mitosin (a new proliferation marker) correlates with clinical outcome in node-negative breast cancer. Cancer Res 1997, 57:5305-5308.
29. Carvalho A, Carmona M, Sambade C, Earnshaw WC, Wheatley SP: Survivin is required for stable checkpoint activation in taxol-treated HeLa cells. J Cell Sci 2003, 116:2987-2998.
30. Lens SM, Vager G, Medema RH: The case for Survivin as a mitotic regulator. Curr Opin Cell Biol 2005, 17:166-172.
31. Altieri DC: Survivin, versatile modulation of cell division and apoptosis in cancer. Oncogene 2003, 22:8581-8589.
32. Li F, Ambrosini G, Chu EY, Plescia J, Tognin S, Marchisio PC, Altieri DC: Control of apoptosis and mitotic spindle checkpoint by survivin. Nature 1998, 396:580-584.
33. Lo Muzio L, Farina A, Rubini C, Pezzetti F, Stabellini G, Laino G, Santarelli A, Pannone G, Bufo R, de Lillo A, Carinci F: Survivin as a prognostic factor in squamous cell carcinoma of the oral cavity. J Oral Pathol Med 2005, 34:395-401.
34. Lo Muzio L, Pannone G, Leonardi R, Staibano S, Mignogna MD, De Rosa G, Kudo Y, Takata T, Altieri DC: Survivin, a potential early predictor of tumor progression in the oral mucosa. J Dent Res 2003, 82:923-928.

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