Serum lncRNA *XIST* has the Capacity to Serve as a Diagnostic Biomarker for Nasopharyngeal Carcinoma

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Abstract

Background

LncRNA *XIST* (X-inactive specific transcript) is involved in various tumors. In this study, we aimed to investigate the diagnostic performance of serum lncRNA *XIST* in NPC.

Methods

Serum samples were collected from 117 NPC patients and 80 gender and age matched healthy individuals. The relative expression of serum lncRNA *XIST* was detected by qRT-PCR. Chi-square test was applied to evaluate the relationship between lncRNA *XIST* expression and clinical characteristics. ROC analysis was performed to identify the feasibility of serum *XIST* as a diagnostic biomarker for NPC.

Results

Serum lncRNA *XIST* was higher in NPC patients than that in healthy volunteers ($P < 0.001$). Furthermore, its elevated expression showed positive link with clinical stage ($P = 0.013$), TNM stage ($P = 0.024$), and lymph node metastasis ($P = 0.003$). ROC curve demonstrated that lncRNA *XIST* could discriminate between NPC patients and healthy individuals, with the AUC value of 0.827. The cut-off value of serum *XIST* for NPC diagnosis was 3.735, with the sensitivity of 75.2% and specificity of 83.7%.

Conclusion

LncRNA *XIST* as an oncogene plays promoting roles in aggressive progression of NPC. Serum lncRNA *XIST* may be employed as a diagnostic biomarker for NPC.

Background

Nasopharyngeal carcinoma (NPC) is one of the most common malignancies originating from the mucosal epithelium of the nasopharynx [1]. The incidence of NPC exhibits distinct regional diversity. Its morbidity is high in southeast Asia and Africa, particularly in southern China, while it is relatively rare in other regions of world [2–4]. The tumorigenesis of NPC is a complex process, with the involvement of genetic factors, environmental carcinogens, and Epstein-Barr virus (EBV) infection [5]. Like other types of malignancy, tumor stage at diagnosis is the key factor for outcomes of NPC patients. For patients diagnosed at early stage, the cure rate is high, however, the long-term survival remains dismal among those diagnosed with advanced stages [6]. Early diagnosis represents a great challenge in clinical setting, due its deep location and silent nature in early stage [7, 8]. Until now, early detection of NPC mainly depends on pathological biopsy and imaging. However, these methods are associated with several limitations, such as invasive, high cost, inconvenient, and time consuming, and they are not appropriate for routine screening and surveillance [6]. Consequently, it is urgently need to explore novel and effective diagnostic biomarkers to improve diagnosis and treatment of NPC.

Long non-coding RNAs (lncRNAs) are a group of RNAs with no or limited protein coding capacity which are longer than 200 nucleotides in length [9]. LncRNAs are implicated in various cellular processes, such as alternative splicing genes modification, histone modification, chromatin rearrangement and gene expression regulation [10]. Alteration of lncRNAs may contribute to human diseases, like cancer. Growing evidences have reported that lncRNAs played important roles in tumor initiation, progression and metastasis by regulating oncogenic and tumor-suppressing pathways in tumorigenesis [11]. LncRNA X-inactive specific transcript (*XIST*), a product of *XIST* gene, is a newly identified lncRNA, and plays pivotal roles in regulating X inactivation in mammals [12]. It was reported that lncRNA *XIST* was associated with proliferation, differentiation, and genome maintenance in tumor cells [13]. Dysregulation of lncRNA *XIST* was observed in diverse cancers, such as non-small cell lung cancer, breast cancer, glioblastoma carcinoma and ovarian cancer [13–16]. The function of lnc *XIST* in NPC was also reported in the previous studies. Song et al. suggested that *XIST* was up-regulated in NPC tissue specimens, and promoted the malignant progression of the cancer [17]. Based on the related researches, we deduced that serum *XIST* might be employed as a diagnostic biomarker for NPC.

In this study, the serum level of lncRNA *XIST* in NPC patients was detected, as well as its association with clinical characteristics. Additionally, we investigated the diagnostic performance of lncRNA *XIST* in NPC.

Methods

Patients and specimens collection

A total of 117 newly diagnosed NPC patients were recruited from Chinese PLA General Hospital. The diagnosis was based on pathological examination and tumors were classified according to the TNM cancer staging system (2009) set by the Union of International Cancer Control. 5 mL blood specimens were collected from NPC patients before receiving any treatments, such as radiotherapy or chemotherapy. In addition, 80 gender and age matched healthy volunteers without any malignancies history were enrolled in the study, to serve as healthy controls. Blood specimens were also obtained from the healthy control group. Serum specimens were isolated from the blood samples via centrifugation, then the serum was stored at -80°C until used. The basic data of the patients with NPC were recorded, including age, sex, family tumor history, clinical stage, TNM stage, and lymph node metastasis.

This study was approved by the Ethics Committee of Chinese PLA General Hospital. Each patient or family signed informed consent before blood collection.

RNA extraction and quantitative real-time polymerase chain reaction (qRT-PCR)

Total RNA was extracted from serum specimens using Trizol (Invitrogen) according to the manufacturer's instructions. The quality of RNA was evaluated by NanoDrop ND-1000 Spectrophotometer (Agilent, USA) and the RNA samples with the OD A260/A280 ratio close to a value of 2.0 were used for complementary DNA (cDNA) synthesis with the help of PrimeScript™ RT-PCR (Takara, Dalian, China). Quantitative real-time PCR (qRT-PCR) was performed to test the relative expression levels of lncRNA *XIST*. The reaction

was performed using SYBR Premix Ex Taq™ (TaKaRa, Japan) in Applied Biosystems 7900 Fast Real-Time PCR system (Applied Biosystems, Foster City, California, USA). *GAPDH* acted as internal control, The primer sequences were as follows: *XIST* forward: 5'-CTCTCCATTGGGTTCAC-3', reverse: 5'-GCGGCAGGTCTTAAGAGATGAG-3'; *GAPDH* forward: 5'-TGCACCACCAACTGCTTAGC-3', reverse: 5'-GGCATGGACTGTGGTCATGAG-3'. The relative expression of *XIST* was normalized to *GAPDH*, and calculated by $2^{-\Delta\Delta C_t}$ method. Each experiment was repeated at least three times.

Statistical analysis

SPSS 19.0 statistical software was applied for statistical analyses in the current study. The numerical data were presented as mean $\pm$ standard deviation (SD), and analyzed by Student's t-test. χ^2 tests was used to estimate the relationship between the expression of lncRNA *XIST* and clinicopathological characteristics. Receiver operating characteristic (ROC) analysis was performed to determine the diagnostic performance of serum lncRNA *XIST* in NPC. *P* value less than 0.05 was considered statistically significant.

Results

The expression level of lncRNA *XIST*

117 NPC patients including 71 men and 46 women were collected in our study. QRT-PCR was used to estimate the serum levels of lncRNA *XIST*. The result showed that serum lncRNA *XIST* was significantly higher in the patients with NPC than that in healthy volunteers ($P < 0.001$) (Fig. 1).

Relationship between lncRNA *XIST* and clinicopathological characteristics of NPC

In the current study, the patients were divided into high expression group ($n = 68$) and low expression group ($n = 49$), according to their mean serum level of *XIST*. Chi-square test was applied to estimate the potential association of *XIST* level with clinicopathological characteristics. Analysis results demonstrated that the expression of *XIST* was positively associated with advanced clinical stage ($P = 0.013$), TNM stage ($P = 0.024$) and lymph node metastasis ($P = 0.003$). However, no significant correlation was observed between the expression of lncRNA *XIST* and the age, gender, or family tumor history of the NPC patients (all $P > 0.05$) (Table 1).

Table 1
The association of serum lncRNA *XIST* expression with clinicopathological features of NPC patients

Feathers	No.	LncRNA <i>XIST</i> expression		<i>P</i> values
	N = 117	Low (n = 49)	High (n = 68)	
Age (years)				
< 50	51	21	30	0.892
≥ 50	66	28	38	
Gender				
Male	71	34	37	0.102
Female	46	15	31	
Clinical stage				
I-II	68	35	33	0.013
III-IV	49	14	35	
Family tumor history				
Yes	5	2	3	0.931
No	112	47	65	
TNM stage				
I-II	72	36	36	0.024
III-IV	45	13	32	
Lymph node metastasis				
no	62	34	28	0.003
yes	55	15	40	

Diagnostic value of lncRNA *XIST* for NPC

In order to investigate the diagnostic accuracy of serum *XIST* for NPC, ROC curve was constructed in the present study. The AUC value of the curve was 0.827, suggesting that lncRNA *XIST* could distinguish the NPC patients from the healthy controls, with the sensitivity of 75.2% and specificity of 83.7%, respectively. The cutoff value of serum lncRNA *XIST* mRNA for NPC diagnosis was 3.735 (Fig. 2).

Discussion

Nasopharyngeal carcinoma (NPC) represents a malignant head and neck cancer which is characterized by highly invasive and metastatic [18]. Although the mechanisms underlying NPC remains unclear, it is general considered that NPC is affected by many factors, such as EB Virus infection, genetic alterations, dietary habit, as well as environmental factors [19, 20]. Timely and appropriate treatments are pivotal for outcomes of NPC patients. Unfortunately, most NPC patients are diagnosed in advanced stage due to the silent nature of NPC in the early stage and lack of effective surveillance tools [8]. Diagnosis at metastatic stage may lead to high recurrence rate, therapeutic failures and poor prognosis [21]. Therefore, the sensitive and efficiency biomarker for early diagnosis are urgently needed to improve the treatments and prognosis of NPC patients.

LncRNA *XIST* (X-inactive specific transcript) is a newly authenticated lncRNA deriving from *XIST* gene. Previous studies have proved that lncRNA *XIST* was involved in tumorigenesis based on its functional roles in cell proliferation, differentiation, and genome maintenance [22]. Alteration of *XIST* might lead to overall epigenetic instability, thus resulting in tumorigenesis [23]. For examples, the decreased expression of lncRNA *XIST* could contribute to the disappearance of the Barr body in breast cancer cells that might provide a new insight in the mechanisms of the disease [24]. Yildirim E et al. indicated that abnormal expression of lncRNA *XIST* was associated with X chromosome reactivation and overall changes of genome, thus promoting the progression of cancers [25]. Given its functional roles in tumorigenesis, lncRNA *XIST* was confirmed as a biomarker for several cancers. For example, the study carried out by Tantai and his colleagues demonstrated that serum lncRNA *XIST* might be employed as a diagnostic biomarker for non-small cell lung cancer patients [26]. Therefore, we deduced that lncRNA *XIST* might be a potential diagnostic biomarker for NPC.

In the present study, we studied the expression level of serum lncRNA *XIST* in NPC patients. Results suggested that the expression level of lncRNA *XIST* was up-regulated in NPC patients compared with that in healthy volunteers. Moreover, its elevated expression was significantly associated with clinical stage, TNM stage and lymph node metastasis. All the data revealed that lncRNA *XIST* as an oncogene was implicated in aggressive progression of NPC. It was reported that the oncogenic function of *XIST* in NPC might dependent on its regulatory role in expression of *E2F3*. *XIST* could up-regulate the expression of *E2F3* via spongeing *miR-34a-5p* [17]. However, we did not investigate the specific oncogenic mechanisms of lncRNA *XIST* in NPC in this study. Further researches were still needed.

Early diagnosis is key for outcomes of NPC patients. At present, the commonly used methods for NPC diagnosis include pathological biopsy and imaging. However, the clinical value of the methods are limited by invasive procedures, high cost, and time consuming [6]. In order to improve the diagnosis of NPC, various researches were devoted to explore novel and non-invasive biomarkers for screening of the cancer. A study of 529 subjects demonstrated that serum levels of cytokeratin 19 fragment 21.1 (*CYFRA21-1*) were significantly different between NPC patients and non-cancerous subjects that might be employed as a biomarker for the disease, moreover, its expression was closely correlated with EBV antibodies [27]. The predictive function of non-coding RNAs in NPC was also reported in the previous tumor investigation. For example, lncRNA actin filament associated protein 1 antisense RNA1 (*AFAP1-*

AS7) was proved to play promoting roles in metastasis of NPC that might be a potential prognostic biomarker and therapeutic target for the cancer [28]. In this study, we estimated the diagnostic performance of lncRNA *XIST* in NPC. ROC curve showed that serum lncRNA *XIST* could distinguish NPC cases from healthy individuals with high sensitivity and specificity. Taken together, the altered molecules during tumorigenesis might provide novel biomarkers for early diagnosis and prognosis prediction of malignancy. Nevertheless, considering the relatively small sample size in the current study, well-designed researches with large sample size were still required to identify the results obtained in our study.

Conclusion

In conclusion, lncRNA *XIST* is up-regulated in NPC patients, and positively correlates with aggressive clinical characteristics. lncRNA *XIST* may be a potential diagnostic biomarker for NPC patients.

Declarations

Disclosure

The authors report no conflicts of interest in this work.

Author Information

Ethics approval and consent to participate

This study was supported by the Ethics Committee of Chinese PLA General Hospital and also has been carried out in accordance with the World Medical Association Declaration of Helsinki.

Consent for publication

The subjects had been informed the objective. Certainly, written consents were signed by every subject in this study.

Data availability

Data sharing is not applicable to this article as no datasets were generated or analysed during the current study.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

L.W. design of the work; H.S. the acquisition, analysis, H.S., S.Y. interpretation of data; L.W., H.S. the creation of new software used in the work; L.W., H.S., S.Y. have drafted the work or substantively revised

it. All authors read and approved the final manuscript.

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References

1. Zhao Y, Liu ZG, Tang J, Zou RF, Chen XY, Jiang GM, Qiu YF, Wang H: **High expression of Sox10 correlates with tumor aggressiveness and poor prognosis in human nasopharyngeal carcinoma.** *OncoTargets and therapy* 2016, **9**:1671-1677.
2. Chan AT: **Nasopharyngeal carcinoma.** *Annals of oncology : official journal of the European Society for Medical Oncology / ESMO* 2010, **21 Suppl 7**:vii308-312.
3. Lui VW, Lau CP, Ho K, Ng MH, Cheng SH, Tsao SW, Tsang CM, Lei KI, Chan AT, Mok TS: **Anti-invasion, anti-proliferation and anoikis-sensitization activities of lapatinib in nasopharyngeal carcinoma cells.** *Investigational new drugs* 2011, **29**(6):1241-1252.
4. Zhao YY, Tian Y, Zhang J, Xu F, Yang YP, Huang Y, Zhao HY, Zhang JW, Xue C, Lam MH *et al*: **Effects of an oral allosteric AKT inhibitor (MK-2206) on human nasopharyngeal cancer in vitro and in vivo.** *Drug design, development and therapy* 2014, **8**:1827-1837.
5. Dai W, Zheng H, Cheung AK, Lung ML: **Genetic and epigenetic landscape of nasopharyngeal carcinoma.** *Chinese clinical oncology* 2016, **5**(2):16.
6. Qiu S, Xu Y, Huang L, Zheng W, Huang C, Huang S, Lin J, Lin D, Feng S, Chen R *et al*: **Non-invasive detection of nasopharyngeal carcinoma using saliva surface-enhanced Raman spectroscopy.** *Oncology letters* 2016, **11**(1):884-890.
7. Lee AW, Ng WT, Chan YH, Sze H, Chan C, Lam TH: **The battle against nasopharyngeal cancer.** *Radiotherapy and oncology : journal of the European Society for Therapeutic Radiology and Oncology* 2012, **104**(3):272-278.
8. Lee AW, Lin JC, Ng WT: **Current management of nasopharyngeal cancer.** *Seminars in radiation oncology* 2012, **22**(3):233-244.
9. Pandey GK, Kanduri C: **Long noncoding RNAs and neuroblastoma.** *Oncotarget* 2015, **6**(21):18265-18275.
10. Vikram R, Ramachandran R, Abdul KS: **Functional significance of long non-coding RNAs in breast cancer.** *Breast Cancer* 2014, **21**(5):515-521.
11. Tong YS, Wang XW, Zhou XL, Liu ZH, Yang TX, Shi WH, Xie HW, Lv J, Wu QQ, Cao XF: **Identification of the long non-coding RNA POU3F3 in plasma as a novel biomarker for diagnosis of esophageal squamous cell carcinoma.** *Molecular cancer* 2015, **14**:3.
12. Brown CJ, Ballabio A, Rupert JL, Lafreniere RG, Grompe M, Tonlorenzi R, Willard HF: **A gene from the region of the human X inactivation centre is expressed exclusively from the inactive X chromosome.** *Nature* 1991, **349**(6304):38-44.

13. Fang J, Sun CC, Gong C: **Long noncoding RNA XIST acts as an oncogene in non-small cell lung cancer by epigenetically repressing KLF2 expression.** *Biochemical and biophysical research communications* 2016, **478**(2):811-817.
14. Salvador MA, Wicinski J, Cabaud O, Toiron Y, Finetti P, Josselin E, Lelievre H, Kraus-Berthier L, Depil S, Bertucci F *et al*: **The histone deacetylase inhibitor abexinostat induces cancer stem cells differentiation in breast cancer with low Xist expression.** *Clinical cancer research : an official journal of the American Association for Cancer Research* 2013, **19**(23):6520-6531.
15. Yao Y, Ma J, Xue Y, Wang P, Li Z, Liu J, Chen L, Xi Z, Teng H, Wang Z *et al*: **Knockdown of long non-coding RNA XIST exerts tumor-suppressive functions in human glioblastoma stem cells by up-regulating miR-152.** *Cancer letters* 2015, **359**(1):75-86.
16. Ren C, Li X, Wang T, Wang G, Zhao C, Liang T, Zhu Y, Li M, Yang C, Zhao Y *et al*: **Functions and Mechanisms of Long Noncoding RNAs in Ovarian Cancer.** *International journal of gynecological cancer : official journal of the International Gynecological Cancer Society* 2015, **25**(4):566-569.
17. Song P, Ye LF, Zhang C, Peng T, Zhou XH: **Long non-coding RNA XIST exerts oncogenic functions in human nasopharyngeal carcinoma by targeting miR-34a-5p.** *Gene* 2016, **592**(1):8-14.
18. Liu L, Wang ZH, Han J, Tang C, Chen N, Lin Z, Peng PJ: **Everolimus enhances cellular cytotoxicity of lapatinib via the eukaryotic elongation factor-2 kinase pathway in nasopharyngeal carcinoma cells.** *OncoTargets and therapy* 2016, **9**:6195-6201.
19. Chien SY, Hsieh MJ, Chen CJ, Yang SF, Chen MK: **Nobiletin inhibits invasion and migration of human nasopharyngeal carcinoma cell lines by involving ERK1/2 and transcriptional inhibition of MMP-2.** *Expert opinion on therapeutic targets* 2015, **19**(3):307-320.
20. Zhong X, Shi C, Gong J, Guo B, Li M, Xu H: **Experimental study of nasopharyngeal carcinoma radionuclide imaging and therapy using transferred human sodium/iodide symporter gene.** *PloS one* 2015, **10**(1):e0117053.
21. Tu Z, Chen Q, Zhang JT, Jiang X, Xia Y, Chan HC: **CFTR is a potential marker for nasopharyngeal carcinoma prognosis and metastasis.** *Oncotarget* 2016.
22. Chen DL, Ju HQ, Lu YX, Chen LZ, Zeng ZL, Zhang DS, Luo HY, Wang F, Qiu MZ, Wang DS *et al*: **Long non-coding RNA XIST regulates gastric cancer progression by acting as a molecular sponge of miR-101 to modulate EZH2 expression.** *Journal of experimental & clinical cancer research : CR* 2016, **35**(1):142.
23. Weakley SM, Wang H, Yao Q, Chen C: **Expression and function of a large non-coding RNA gene XIST in human cancer.** *World journal of surgery* 2011, **35**(8):1751-1756.
24. Chaligne R, Popova T, Mendoza-Parra MA, Saleem MA, Gentien D, Ban K, Piolot T, Leroy O, Mariani O, Gronemeyer H *et al*: **The inactive X chromosome is epigenetically unstable and transcriptionally labile in breast cancer.** *Genome research* 2015, **25**(4):488-503.
25. Yildirim E, Kirby JE, Brown DE, Mercier FE, Sadreyev RI, Scadden DT, Lee JT: **Xist RNA is a potent suppressor of hematologic cancer in mice.** *Cell* 2013, **152**(4):727-742.

26. Tantai J, Hu D, Yang Y, Geng J: **Combined identification of long non-coding RNA XIST and HIF1A-AS1 in serum as an effective screening for non-small cell lung cancer.** *International journal of clinical and experimental pathology* 2015, **8**(7):7887-7895.

27. Song XM, Wang ZJ, Cao WJ, Ji L, Chen F, Wang SZ: **The value of circulating CYFRA21-1 expression in patients with nasopharyngeal carcinoma: a study of 529 subjects.** *International journal of clinical oncology* 2016.

28. Bo H, Gong Z, Zhang W, Li X, Zeng Y, Liao Q, Chen P, Shi L, Lian Y, Jing Y *et al*: **Upregulated long non-coding RNA AFAP1-AS1 expression is associated with progression and poor prognosis of nasopharyngeal carcinoma.** *Oncotarget* 2015, **6**(24):20404-20418.

Figures

Figure 1

Serum level of lncRNA XIST was evaluated by qRT-PCR in NPC patients and healthy controls. The results suggested that serum lncRNA XIST was significantly increased in NPC patients compared with healthy controls. ***: suggested $P < 0.001$.

Figure 2

ROC curve was constructed based on the serum levels of lncRNA XIST in NPC patients and healthy individuals. The curve demonstrated that lncRNA XIST could discriminate between NPC patients and healthy individuals, with the AUC value of 0.827. The cut-off value of lncRNA XIST for NPC diagnosis was 3.735, with the sensitivity of 75.2% and the specificity of 83.7%.