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Serum level of long noncoding RNA H19 as a diagnostic biomarker of multiple myeloma

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ABSTRACT

Circulating long noncoding RNA (lncRNA) H19 has been reported to be a biomarker for cancer monitoring. The purpose of this study was to determine whether serum lncRNA could serve as a novel biomarker for the diagnosis of multiple myeloma (MM) and evaluate its value of clinical application. In our study, the expression of lncRNA H19 was up-regulated in 80 patients with MM and MM cell lines by RT-PCR analysis. Clinicopathological analysis showed the expression level of H19 could assist clinical staging, and the severity of the disease could be roughly determined according to the amount of H19 expressed in the patient serum. This is the first report to show that H19 was expressed in the serum of MM patients, suggesting that upregulation of serum lncRNA H19 may prove to be a novel biomarker for early diagnosis and clinical treatment of MM.

1. Introduction

Multiple myeloma (MM) is a hematological malignancy characterized by abnormal proliferation of monoclonal plasma cells in bone marrow. These monoclonal plasma cells secrete monoclonal immunoglobulin detectable in serum and/or urine, contributing to various end-organ damages including anemia, hypercalcemia, renal insufficiency and osteolytic bone disease. More importantly, MM is a biologically complex disorder characterized by a large clonal heterogeneity as reflected by a wide range of genetic alterations and manifested in a patient-to-patient variation in overall survival and response to treatment. This MM-associated heterogeneity has certainly limited the clinical benefits of currently available and personalized treatment strategies. Therefore, seeking effective biomarkers and therapeutic targets is essential for early diagnosis and clinical treatment of MM.

Noncoding RNAs (ncRNAs) include microRNAs (miRNAs) and long noncoding RNAs (lncRNAs) [1,2]. miRNAs are short (typically 18–23 nucleotides) single-stranded RNAs that cause posttranscriptional gene silencing by inducing mRNA degradation or repressing translation upon binding to the 3′-untranslated region (UTR) of their target mRNAs [3]. LncRNAs are transcripts > 200 nucleotides in length which have also been shown to regulate gene expression [4]. LncRNAs are highly conserved throughout mammalian evolution and abnormally expressed in tumor tissues [5].

H19 lncRNA is a 2.3-kb capped, spliced and polyadenylated noncoding RNA transcribed from the conserved imprinted H19/igf2 gene cluster located on human chromosome 11p5.5 [6,7]. The locus has been intensively analyzed as a model system for genomic imprinting. However, the biological functions of the H19 gene product await further elucidation. H19 is known to repress embryonic placental growth and trans-regulate the network of imprinted genes during fetal development [8,9], but its role in cancer development can be either tumor-suppressive or oncogenic, depending on the cellular content and tumor type [10–12]. It is reported that H19 is abundantly expressed in most human cancers, suggesting that H19 may play a role in oncogenesis [13,14].

Previous studies have indicated that lncRNAs exist in human body fluids [15–18] and have revealed their potential utility as diagnostic biomarkers [19]. Dysregulation of some lncRNAs including OIP5-AS1, MALAT1, MEG3, PCAT1, KIAA0495, UCA1 and CRNDE was reported to be associated with MM [20–26]. However, the function of these lncRNAs in MM and their clinical significance remain incompletely understood. Aberrant H19 expression has also been linked to diverse human cancers. The association between H19 and MM remains undetermined. Circulating biomarkers are easily accessible, requiring only non-invasive sampling methods for detection, and the detection of lncRNAs in serum has been studied intensively [27–29]. Additionally, accumulated circulating lncRNAs have been demonstrated to have a diagnostic value, indicating that lncRNAs hold great potential as

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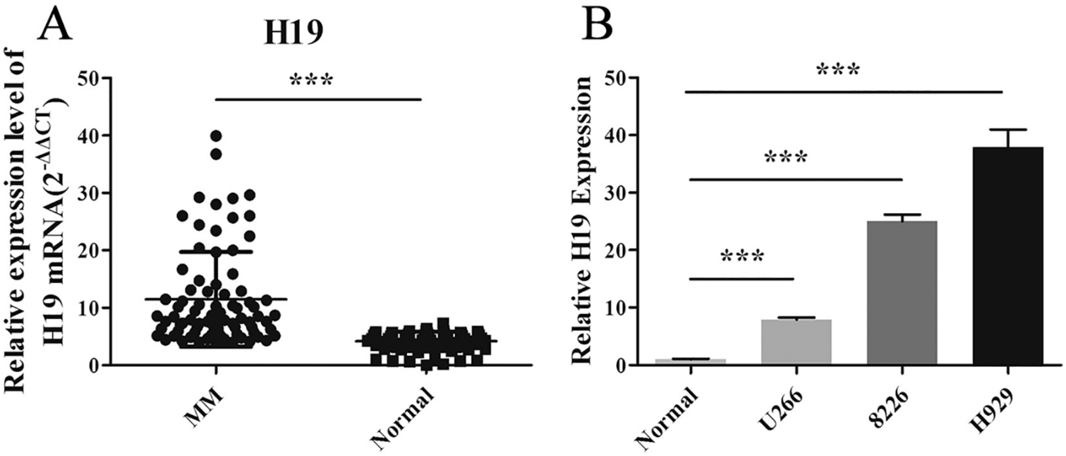

Fig. 1. H19 were detectable in serum and cell lines. (A) H19 expression was examined by real-time PCR and normalized to 18S expression in 80 MM patients compared with 67 healthy controls. (B) Real-time PCR was used to measure H19 expression in MM cells. Values were expressed as mean ± SD, $^*P < 0.05$.

powerful tumor biomarkers. However, no systematic study of serum lncRNAs in MM samples has been performed to date.

In this study, we found that H19 expression was upregulated in MM serum and cell lines. High expression of H19 was associated with clinicopathological characteristics and poor prognosis in MM patients. We also determined its prognostic role in MM, which might dramatically improve the therapeutic strategy of MM.

2. Results

2.1. H19 is upregulated in an independent validation set of serum samples and cell lines

Serum H19 levels were examined on a large scale for our validation study using sera from 80 MM patients and 67 healthy controls. The results showed that H19 expression in MM patients was significantly higher than that in controls (Fig. 1A, $P < 0.0001$). To further test the hypothesis that serum lncRNAs were primarily released or leaked from the tumor cells, H19 expression levels were investigated using RT-qPCR in MM cell lines U266, 8226 and H929, and CD138+ plasma cells of normal human bone marrow. The H19 expression in H929, 8226 and U266 cells was significantly higher than that in the healthy control cells (Fig. 1B).

2.2. Methodological evaluation of H19 detection

The above results indicate the possibility of clinical application of H19. To better apply H19 to clinical laboratory analysis, we carried out methodological evaluation by firstly evaluating the linearity of H19 using serial ten-fold dilutions of H19 cDNA. The R^2 of the H19 standard curve was 0.9977, and the regression equation was $y = -3.5675\times + 21.5525$, indicating that qPCR could be used to detect different concentrations of serum H19 (Fig. 2A). In the same way, the reference was also processed and analyzed (Fig. 2B). The intra-assay coefficient of variation and the inter assay coefficient of variation of H19 were also satisfactory (Table 1). The results of PCR show that the melting curve and melting peak are single peak and have good specificity (Fig. 2C). Additionally, the PCR products were further detected by agarose gel electrophoresis (Fig. 2D). The result indicated that the PCR method had high specificity and sensitivity.

2.3. H19 expression and clinical parameters in MM

To assess whether the expression level of H19 was related to clinical parameters, we assigned the patients to two groups according to clinical parameters to analyze the difference in H19 expression. As shown in Table 2, the expression level of H19 in the bone injury group (fracture or osteodynia) ($n = 41$) was significantly higher ($P < 0.001$) than that in the bone injury-free group ($n = 39$). In addition, the expression level of H19 in the high total protein group ($n = 52$) was significantly higher than that in normal group ($n = 28$) ($P < 0.05$). However, there was no significant correlation with other clinicopathological features such as age, gender, M protein, light chain, HGB, albumin, globulin, urine protein, and renal injury ($P > 0.05$).

2.4. Further study on clinical application of serum H19 to clinical staging

To further evaluate the possibility of clinical application of serum H19, we studied the clinical stages of the 80 MM patients and found that the expression levels of H19 were associated with Durie–Salmon (DS) and the International Scoring System (ISS) stage of MM. According to the DS stage system, the expression of H19 in stage I patients was significantly lower ($P < 0.05$; Fig. 3A) than that in stage III patients, but there was no significant difference between stage I and stage II, and between stages II and III (both $P > 0.05$). According to the ISS stage system, the expression level of H19 in patients with stage I was significantly lower than that in stage II and III ($P < 0.05$; Fig. 3B), but there was no significance in the expression of H19 between patients with stage II and III ($P > 0.05$). Our results provide evidence that serum H19 levels could be used to assist clinical staging of MM patients.

2.5. The use of H19 for monitoring tumor dynamics in MM patients

As circulating lncRNAs release or leak from tumor cells, they should revert to a normal level after resection of the tumor [30]. First, we analyzed the expression levels of H19 before and after treatment in 7 newly diagnosed patients. As shown in Fig. 4A and B, the amount of H19 expression decreased in varying degrees. Subsequently, we increased the sample capacity and monitored the change of serum H19 expression in 50 newly diagnosed patients after 30-day chemotherapy. As expected, serum level of H19 was significantly decreased after treatment as compared with that before treatment (Fig. 4C). Importantly, we found that H19 did not decline but increased to some extent in bortezomizomi resistant patients (Fig. 4D).

2.6. Evaluation of H19 in serum as a MM-related predictor

To investigate the characteristics of H19 as a potential biomarker for MM, ROC curves and the AUC of ROC curves were performed on data from the 80 MM patients and 67 healthy controls. Representation of the data using an ROC plot showed strong separation between the two groups, with an AUC of 0.888 (0.836–0.939). The sensitivity and