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Increased expression of the lnc H19 gene in the plasma of people with breast cancer

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Abstract

The majority of ncRNAs are known as long non-coding RNAs (lncRNAs) whose length exceeds 200 nucleotides. H19, a lncRNA, is the transcription product of the H19 gene, an oncogene in breast cancer, and is highly expressed in cancer tissues compared with normal tissues. The expression level of H19 is associated with the oncogenesis, proliferation, invasion, metastasis, and drug resistance of breast cancer. H19 expression levels were detected in breast cancer plasma using qRT-Real-Time PCR assay in 50 breast cancer samples and 50 healthy control samples. The results showed that the expression of this gene in both the tissue and the plasma of patients increased compared to that of healthy individuals.

INTRODUCTION

The Lnc H19 gene is located in an imprinted region of chromosome 11 near the insulin-like growth factor 2 (IGF2) gene. This gene is only expressed from the maternally-inherited chromosome (1). The product of this gene is a long non-coding RNA which functions as a tumor suppressor. H19 promotes biological processes such as apoptosis, angiogenesis, inflammation, and cell death. Furthermore, gene ontology (GO) analyses predict that H19 is connected with neurogenesis, angiogenesis, and inflammation through DNA transcription, RNA folding, methylation, and gene imprinting (2). The aberrant expression of H19 is associated with multiple diseases, including carcinoma, sarcoma, type 2 diabetes, and hypertrophic cardiomyopathy (3). Abnormal expression of H19 has been demonstrated in a variety of cancer cells, such as gastric cancer, pancreatic cancer, liver cancer and breast cancer cells, affecting the development and progression of cancer through various mechanisms (4). H19 is an estrogen-regulated transcript, and a wide array of studies have confirmed that it regulates cell differentiation and proliferation. In addition, research into its application in the clinical setting has revealed that H19 has the potential to serve as a biomarker for cancer and shows promise as a novel therapeutic option in oncotherapy (5). Breast cancer is a malignant tumor deriving from the epithelium of the mammary gland. Metastasis is the leading cause of mortality in patients with breast cancer. The H19 gene is an oncogene in breast cancer and is highly expressed in cancer tissues compared with normal tissues (6). The expression level of H19 is associated with the oncogenesis, proliferation, invasion, metastasis, and drug resistance of breast cancer, but the underlying molecular mechanisms differ greatly (7). The molecular mechanisms underlying H19-associated carcinogenesis may involve several aspects. The H19 promoter is activated by the E2F transcription factor 1 (E2F1), which promote cell cycle progression (particularly in the S-phase) of MCF-7 cells. H19 knockdown increases the DNMT3B-mediated methylation of Nctc1, a gene encoding lncRNAs, within the Igf2-H19-Nctc1 locus (8). Thus, H19 alters DNA methylation and leads to breast tumorigenesis. A new lncRNA within the H19/IGF2 locus known as 91H is an antisense gene to H19. Furthermore, it has been shown to regulate H19 and IGF2 expression levels by epigenetic modifications and increase the tumorigenic properties of MDA-MB-231 cells both *in vitro* and *in vivo* (9). In summary, H19 plays an oncogenic role in breast tumorigenesis. Serum H19 levels may possess clinical significance in the early diagnosis, treatment, and prognosis of breast cancer. The current study investigated whether H19 is detectable in the plasma of breast cancer patients compared with matched healthy controls using qRT-Real-Time PCR assay.

METHODS AND MATERIALS

The study population included 40 people with breast cancer (patients) and 50 healthy people (controls). Samples (50 ml) of peripheral blood were taken from each participant. All patients in this study were diagnosed with primary breast cancer based on histopathological examination and were not subjected to any preoperative radiotherapy or chemotherapy; they were also determined to be without other malignant diseases or injuries before sample collection. The patients' ages ranged from 47 to 72 years, and the median was 59 years. The healthy participants had not been exposed to any potentially harmful chemicals and had no malignant diseases, acute diseases, or injuries at the time of sample collection. The ages of healthy participants ranged from 29 to 66 years with the median age of 47 years. Immediately after blood sampling, the samples were centrifuged at 3000 rpm for 15 min at room temperature to prevent contamination of cellular nucleic acids. The supernatant was transferred to a microtube and stored at −80 ℃ until further use. Total RNA was extracted from plasma using the Plasma/Serum RNA Purification minikit (Norgen Biotek, Canada). Reverse transcription reaction was carried out using PrimeScript TM RT reagent kit with gDNA eraser (Takara, Japan) in accordance with the manufacturer's instructions. Real-time PCR was then performed using RealQ Plus Master mix green (Ampliqon, Denmark) as implemented in Rotorgene Corbett 6000 Real-Time. The sequences of the PCR primers for H19 and β-actin are shown in Table 1. β-actin was selected as an internal control.

The expression level of H19 was obtained using the 2^-ΔΔCt method.

Table 1. Primer Sequences

Primer	Sequence
Lnc H19	F: 5'-TGCTGCACTTTACAACCACTG-3' R: 5'-ATGGTGTCTTTGATGTTGGGC-3'
B-actin	F: 5'-TCCTCTCCCAAGTCCACACA-3' R: 5'-GCACGAAGGCTCATCATTCA-3'

RESULTS

In this study, qRT-Real-Time PCR was performed to analyze 40 breast cancer and 50 control plasma samples. It was found that H19 expression was significantly higher in breast cancer plasma relative to the control plasma (Fig. 1, $p = 0.004$). The data indicates that H19 is overexpressed in the circulating plasma of human breast cancer patients.

DISCUSSION

The majority of ncRNAs are known as long non-coding RNAs (lncRNAs) whose length exceeds 200 nucleotides. LncRNAs were first regarded as the "noise" of gene transcription (10). Because of their position relative to the protein-coding genes, lncRNAs can be roughly divided into antisense lncRNAs, enhancer lncRNAs, large intergenic non-coding RNAs, bidirectional lncRNAs, and intronic transcript (11). LncRNAs can regulate gene expression via multiple mechanisms at the epigenetic, transcriptional, and post-transcriptional levels. They participate in the regulation of a variety of cell activities, such as cell differentiation, proliferation, invasion, apoptosis, and autophagy (12) by interacting with RNAs, DNAs, or proteins. The H19 gene is located in 11p15.5, an imprinted region of chromosome 11, adjacent to the insulin-like growth factor 2 (IGF2) gene and is expressed only from the maternally inherited chromosome; IGF2, however, is expressed only from the paternally inherited chromosome (13). H19, a lncRNA, is the transcription product of the H19 gene, and diversified transcript variants exist due to alternative splicing (14). A recent case-control study in China revealed that high H19 expression levels were associated with an increased risk of breast carcinogenesis in both codominant and dominant models, and the association was more apparent in patients with estrogen receptor-positive (ER+), human epidermal growth factor receptor 2-negative (HER2-), and ER+-HER2-negative (HER2-) molecular subtypes (15). Numerous studies have indicated that circulating nucleic acids are detectable in the human peripheral circulation system. Matouk et al. indicated that H19 is highly expressed in all common metastatic sites, regardless of primary tumor origin, and its expression is tightly correlated with BC metastatic potential (1-17). Vennin et al. demonstrated that the overexpression of H19/miR-675 plays a pivotal role in enhancing the proliferation and migration of BC cells and increasing tumor growth and metastasis (18). Therefore, the detection of circulating nucleic acids in plasma or serum could be utilized as a non-invasive diagnostic tool for human cancer (19-20). The current study evaluated changes in lncRNA H19 gene expression in the plasma of breast cancer patients as a non-invasive biomarker. The results showed that H19 expression was significantly higher in breast cancer plasma relative to control plasma ($p = 0.004$). The data indicates that H19 is overexpressed in the circulating plasma of human breast cancer cases. The results of this study are consistent with the results of the study by Zhang et al., who evaluated the expression of the lncH19 gene

in the tissue and plasma of breast cancer patients (21). Their results indicated that the expression of this gene in both the tissue and the plasma of patients was increased compared to that in healthy individuals. It is recommended that this study be performed on a larger scale and that the results be compared with the clinical characteristics of individuals.

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