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SNHG6 203 Transcript Could be Applied as an Auxiliary Factor for more Precise Staging of Breast Cancer

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Abstract

Background: Nowadays long non-coding RNAs are known as interesting functional part of the transcriptome. LncRNA SNHG6 was reported to be expressed more in breast cancer tissues than non-tumor ones. As a frequent cancer among women, breast cancer treatment needs applied biomarkers for fast prognosis and diagnosis. SNHG6 RNA and its splice variants could be considered as molecular biomarkers for the breast cancer well-timed treatment.

Methods: RNA extraction from 35 breast cancer tissues and their relative non-tumor tissues was done and cDNAs of the RNAs were synthesized and then RT-qPCR was performed. Relative expression of SNHG6 202 and 203 was studied in breast cancer samples.

Results: The expression patterns of SNHG6 202 and 203 variants were different. Difference in the expression pattern of SNHG6 202 was significantly remarkable in relation to the HER2 status of tumor samples. SNHG6 203 was expressed in tumor and non-tumor tissues differentially and the expression difference was significant. Also, this transcript exhibited significant expression difference in different stages of the studied breast tumor samples.

Conclusion: It could be stated that SNHG6 203 transcript might be considered as a prognostic and staging biomarker in breast cancer studies and treatment.

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Introduction

In comparison to the past, long non-coding RNAs are considered as an important functional part of the transcriptome. These transcripts are proved as key regulators of cell functions. LncRNAs are involved in cell cycle progression, apoptosis, cell migration, cell senescence, epithelial to mesenchymal transition and cell viability (1-6). Some lncRNAs contribute to cell cycle progression and apoptosis, so they have the potentiality to participate in cancer progression. In fact, many lncRNAs are expressed more in cancerous tissues than their adjacent non-cancerous ones. For example, ANRIL, as a well-known lncRNA, is up-regulated in nasopharyngeal tumor tissues than non-cancerous ones (7). Up-regulation of these transcripts in tumor tissues than non-tumor ones can be applied for well-

timed treatment of cancers. Small nucleolar RNA host gene 6 (SNHG6), as a controversial long non-coding RNA, has been featured recently. This transcript has a special sequence in its gene. SNHG6 gene has snord87 sequence in its body, which is a small nucleolar RNA (snoRNA). Small nucleolar RNAs modify rRNAs chemically for maturation (8-10). The process of rRNA modification leads to maturation of the rRNAs which is critical for ribosome biogenesis (11-14). Ribosomes are translation apparatus and could play important roles in carcinogenesis (15-17). SNHG6 gene contains one snoRNA sequence in its body and transcription of SNHG6 gene in cancer cells might affect the fate of the mentioned cells which express it (18).

Long non-coding RNA splice variants could have an important role in carcinogenesis (19). So, we decided to study two splice variants of SNHG6 gene. In this research, we investigated the expression pattern of SNHG6 202 and SNHG6 203 in 70 breast tumor tissues and their relative non-tumor adjacent tissues.

This study aimed to introduce a new molecular biomarker as a contributory factor for more accurate prognosis and staging of the breast cancer.

Material and Methods

Patients and specimens

The tumor and non-tumor tissues of 35 breast cancer patients were collected from Iran National Tumor Bank and they were stored in liquid nitrogen. The ethical committee of the Kerman Graduate University of Advanced Technology approved the procedure of experiments. Also, the written informed consent of patients was received from Iran National Tumor Bank.

RNA extraction

For every tissue, RNA extraction was performed according to the Rnx plus manufacturer's instructions. The RNA pellet was dissolved in 30 µl of RNAase free water. The quality and quantity of the extracted RNAs were assessed by agarose gel electrophoresis and nano-drop instrument, respectively.

cDNA synthesizing

According to the thermo fisher scientific (fermentas) manufacturer's instructions, 1 µg of the extracted RNAs was considered for synthesizing of cDNA. The cDNA synthesizing procedure is shown in Table1 briefly.

Table 1. The steps and conditions of cDNA synthesizing

DNAase treatment	**1µl DNAase enzyme +1µl buffer + 1µg RNA + up to 10 µl nuclease free water**	**37°c**	**30 minutes**
DNAase inactivation	1µl EDTA 50mM	65°c	10 minutes
Random hexamer addition	1µl random hexamer	65°c	5 minutes
RT complex addition	(1µl Reverse transcriptase enzyme, 4 µl Reverse transcriptase enzyme buffer, 2 µl dntp mix and 0.5 µl RNAase inhibitor)	25°c 42°c	10 minutes 60 minutes
RT enzyme inactivation		72°c	10 minutes

RT-qPCR

Relative expression of the studied variants was evaluated by ABI real time PCR instrument. The expression data of the variants were normalized by β actin as a housekeeping gene. RT-qPCR was carried out in 10µl reaction. 0.5 µl of template, 0.5µl of reverse and forward primers and 5 µl of YTA qPCR master mix were mixed and nuclease free water was added up to 10 µl. The sequences of the primers are shown in Table 2, and thermal cycles of qPCR were performed based on Table 3.

Table 2. Sequences of applied primers

	Forward primer	Reverse primer
β Actin	ACTCTCTTCCAGCCTTCCTTCCT	ACTGACAGCACTGTGTTGGCGTA
SNHG6 203	GAGTGCCTAAGAGCTGTCTTCC	GCCGCGTGATCCTAGTAGTT
SNHG6 202	CCAGTGCTTTGCAGTCAGGATTC	GCCGCGTGATCCTAGTAGTT

Table 3. qPCR conditions of SNHG6 203 and 202

	Initial denaturation	Denaturation in cycles	Annealing and extension
SNHG6 203	95°c for 40 sec	95°c for 5 sec	62°c for 40 sec
SNHG6 202	95°c for 40 sec	95°c for 5 sec	62°c for 40 sec

Statistical analysis

The normalized relative expression of SNHG6 202 and 203 were analyzed by unpaired T test and the significance level was considered at P<0.05. To determine the specificity and sensitivity of SNHG6 203 as a molecular biomarker, ROC curve analyses were carried out. P value < 0.05 was considered as statistically significant. Graphpad prism version 6 software was applied for statistical analysis.

Results

The expression patterns of SNHG6 202 and 203 in HER2 status of the tumor samples were different

Studying the expression of the two SNHG6 splice variants in breast tumor tissues in HER2 status revealed that the expression patterns of these splice variants were reverse. SNHG6 202 expression was more in HER2 positive breast tumor samples than HER2 negative ones. on the other hand, the variant of 203 demonstrated more expression level in HER2 negative samples than positive ones (Figure 1).

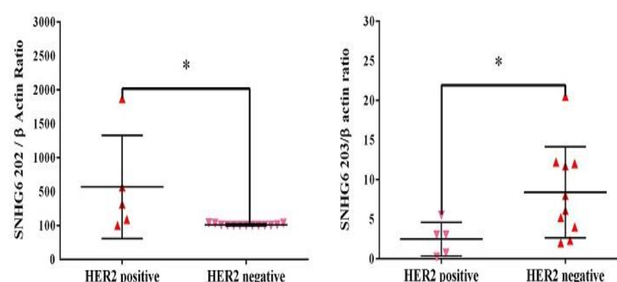

Figure 1. SNHG6 202 was expressed in HER2 positive breast tumor samples more than HER2 negative ones. On the other hand SNHG6 203 was expressed in HER2 negative breast tumor samples more than positive ones.

SNHG6 203 expression in P53 negative tumors was more than positive ones

The expression level of SNHG6 203 variant was higher in P53 negative breast tumors than P53 positive ones (Figure2). SNHG6 202 splice variant demonstrated a similar expression pattern, but the difference was not significant.

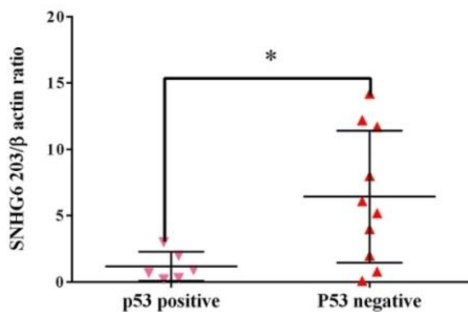

Figure 2. SNHG6 203 was expressed in P53 negative breast tumor tissues more than P53 positive ones.

Progesterone negative breast tumor samples had a higher expression level of SNHG6 203 than positive ones

Assessment of SNHG6 203 expression in progesterone receptor status of the samples showed that variant 203 was expressed in progesterone negative tumors more than positive ones (Figure 3). SNHG6 203 had higher expression in progesterone negative breast tumors, although variant of 202 was expressed in progesterone positive tumors more than negative ones, but the difference was not significant.

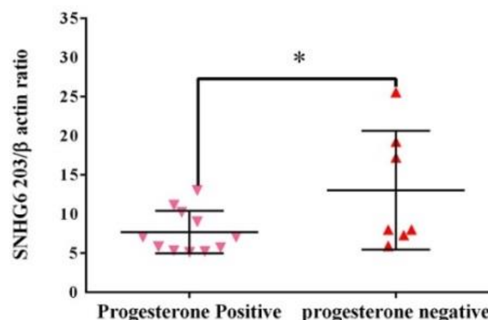

Figure 3. progesterone negative breast tumor samples had more expression of SNHG6 203 than progesterone positive ones.

SNHG6 203 was expressed in breast tumor tissues more than non-cancerous ones

SNHG6 203 was expressed significantly in breast tumor tissues than non-cancerous ones (Figure 4). Significant difference was not observed for variant 202. Additionally, ROC curve analysis of tumor and non-cancerous tissues demonstrated that SNHG6 203 had a significant AUC = 0.74 (p = 0.027) (Figure 5).

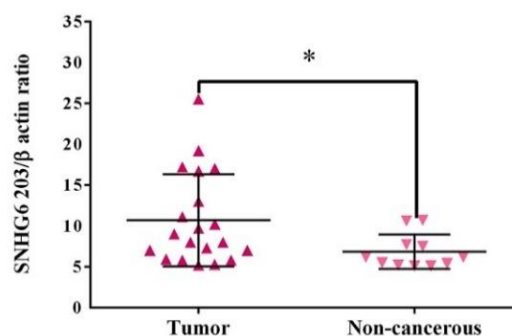

Figure 4. Higher expression of SNHG6 203 in breast tumor samples than non-cancerous ones was proved by RT-qPCR.

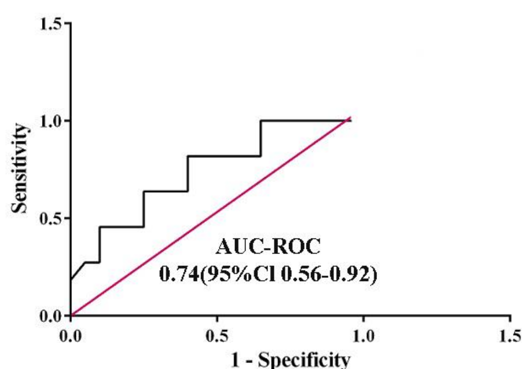

Figure 5. ROC-curve analysis of SNHG6 203 expression for cancerous and non-cancerous breast tissues demonstrated SNHG6 203 has high specificity and sensitivity as a tumor biomarker.

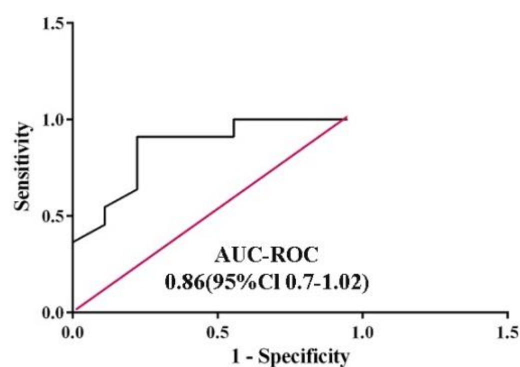

Figure 7. ROC-curve analysis of SNHG6 203 expression for high and low stages of breast tumor tissues showed this transcript has high specificity and sensitivity for breast cancer staging.

SNHG6 203 had more expression in high stage breast tumors than low stage ones

SNHG6 203 variant expression in high stage breast tumors was greater than low stage ones (Figure 6). It was contrary to non-significant data that we gained for SNHG6 202 variant. The ROC curve analysis of different stages (high and low stages) of breast tumors declared a significant ($p = 0.007$) AUC for SNHG6 203 transcript (Figure 7).

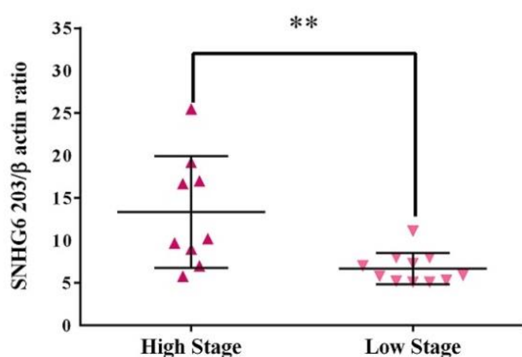

Figure 6. SNHG6 203 was expressed more in high stages breast tumors than low stage ones.

Discussion

RNA splicing as a molecular mechanism causes diversity in RNAs which are long enough. Expression of some long non-coding RNAs splice variants by some cells could alter fate of them (19-21). SNHG6 as an oncogene transcript (18,22,23) has five splice variants.

SNHG6 203 as a SNHG6 gene variant has not been studied in breast cancer yet. SNHG6 203 has been proved as an oncogenic variant of SNHG6 gene in hepatocellular carcinoma. SNHG6 203 is up-regulated in hepatocellular carcinoma and has a significant role in apoptosis prevention of tumor cells in this cancer. Also, SNHG6 203 over expression induces tumors in nude mice (21). Our findings demonstrated that the expression pattern of SNHG6 203 in breast tumor and non-tumor tissues is completely similar to the expression pattern of this transcript in hepatocellular carcinoma. In the present research, we recognized that the expression pattern of SNHG6 203 varied significantly in breast tumor and non-tumor tissues.

SNHG6 203 expression in the breast tumor tissues was significantly more than non-cancerous ones (Figure 4). This expression difference could position this transcript as a

biomarker for distinguishing breast tumor tissues from non-cancerous ones. The SNHG6 203 significant expression difference between these two groups of tissues might be useful as a contributory prognosis factor for breast cancer.

Tumor stage elucidates the aggressiveness of tumor cells and the possibility of patients' survival. So, tumor staging is a crucial step in cancer treatment. Every contributing factor for well-timed tumor staging will result in more confident treatment of cancer. SNHG6 203 was expressed significantly more in high stage breast tumors than low stage ones. In this regard, it can be a suitable molecular biomarker for more effective breast cancer staging and treatment. Therefore, the expression pattern of SNHG6 203 in low and high stages of breast tumors could be applied for well-timed breast cancer treatment (Figure 6). finally , findings obtained from the ROC curve analyses indicate that SNHG6 203 can be considered as a contributory factor for breast cancer prognosis and staging (Figure 5 & 7).

Conclusion

Our findings demonstrated that SNHG6 203 has significant expression differences in tumor and non-cancerous breast tissues as well as in different stages of breast tumors. Consequently, SNHG6 203 can be applied as a contributory factor for well-timed prognosis and staging of breast tumors, the important item that is crucial for more confident treatment of the breast cancer.

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