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EXPRESSION OF hsa-miR-186 IN HUMAN COLON CANCER

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ABSTRACT

Objective: Increasing evidence has shown that microRNAs function as oncogenes or tumor suppressors in human malignancies ,here we explore the expression of hsa-miR-186 in human colorectal cancer tissues.
Materials and Methods: In this experimental study, the expression of hsa-miR-186 in colon cancer tissue and the adjacent tissues in 15 paired were analyzed using real-time quantitative RT-PCR.
Results: The relative expression of miR-186 in the cancer tissues was significantly lower than that in the adjacent tissues (about 9 fold, P=0.008).
Conclusion: As a tumor suppressor gene, hsa-miR-186 is down-regulated in human colon carcinoma tissues and compared with adjasent tissues.

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1. Introduction

Colon cancer is the third most common malignant tumor in the world, and its incidence in iran has been on the rise in recent years. However, migration and invasion of colon cancer occur Attack
mechanism is still limited understanding. Recently, more and more research supports that miRNAs are tumor related [1]. Mature miRNAs are a class of about 22 nt of non-coding small RNAs at the post transcriptional level in the 3'UTR region of the target gene Binding, leading to the degradation of the target gene at the mRNA level or causing protein translation Inhibition [2].
MiRNAs play an important role in many physiological processes Such as cell proliferation, differentiation, apoptosis and the potential to maintain the potential of embryonic stem cells [3-4]. It has been confirmed that miRNAs are regulated as oncogenes or tumor suppressor genes in Cell proliferation and apoptosis play a key role in tumorigenesis [5-7]. The present study examined the expression of miR-186 in colon cancer tissues.

2. Materials and methods

Colorectal tissue samples

Colorectal samples were obtained from patients who had been referred to the Digestive Disease Research Institute (DDRI)-Shariati Hospital-Tehran-Iran, at the time of surgical resection. For each sample, the tumor section was paired with the adjacent normal section in separate cryotubes. The samples were then immediately snap-frozen in liquid nitrogen and stored at −80°C until use. The pathology of tumor samples was confirmed and collected with written consent from each patient. This study was approved by the Clinical Research Ethics Committee of Shariati Hospital, Tehran University of Medical Sciences.

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RNA extraction and CDNA synthesis and quantitative RT-PCR for detection of *hsa-miR-186* in human colon carcinoma tissues

Total RNA was extracted from cells using Trizol reagent according to the manufacturer's protocol (Invitrogen). The RNA quality and yield of extracted RNA was analyzed by using agarose gel electro-phoresis and spectrophotometry, respectively. In order to remove geno-mic DNA contamination, DNaseI treatment was performed prior to cDNA synthesis by the following method: DNAaseI treatment (Takara) at 37 °C for 30 min followed by heat and EDTA inactivation, then cDNAs was synthesized using Prime Script II reverse transcriptase (Takara). For miRNA detection, polyA tail was added to 3′ end of RNAs before cDNA synthesis. Real-time PCR was performed using standard protocols by ABI PRISM 7500 instrument (Applied Biosystems).

Statistical analysis

Relative changes of gene expression were calculated by the levels of ΔΔCt between cancer tissues and noncancerous tissues that were analyzed by the paired Student'st-test to determine statistical significance by the probability of difference between the means.P< 0.05 was considered statistically significant. Values in all the graphs are expressed as means ± SEM. For each experiment, the significance of differences between groups or samples was determined by comparison test (GraphPad Prism 6: GraphPad Software).

Results

Hsa-miR-186 was lowly expressed in human colon carcinoma

Real-time PCR was used to detect 15 pairs of paired colon cancers . In the expression of miR-186, $2^{-\triangle\triangle Ct}$ value by paired sample t test analysis, The results showed that the average expression of miR-186 in colon cancer tissues was, significantly lower than in paracancerous tissues; The expression of miR-186 in colon cancer tissues was significantly lower than that in normal mucosa Weaves (about 9 fold, t = 3.209, P = 0.008, Figure 1)

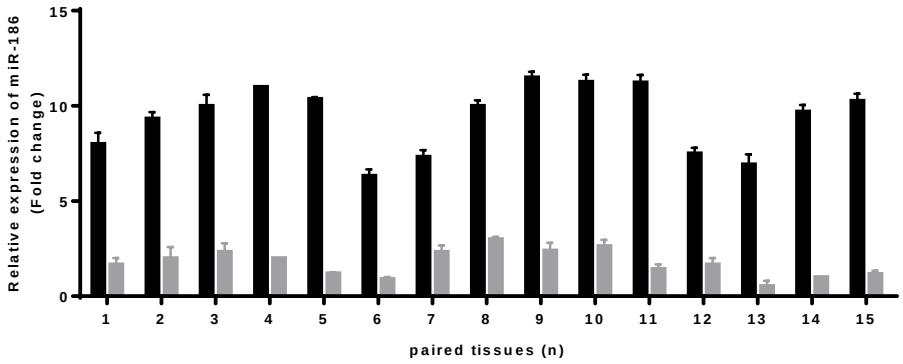

Figure1. Implication of hsa-miR-186 has-miR-186 in colorectal cancer. Realtime-PCR was used to detect miR-186 expression in paired colon cancer tissues.

Discussion

Recent studies have shown that many miRNAs and human tumors (eg Lung cancer, breast cancer, brain tumor, liver cancer, stomach cancer, glioma and lymphoma Etc.) is closely related to the formation [8-11]. Some miRNAs have been identified to be suppressed or promote tumor invasion and metastasis, providing a potential therapeutic target. Bostjancic et al [12] confirmed miR-186 in the heart Disorders in vascular disease, and participate in a variety of physiological and pathological processes; by qRT-PCR analysis .

Studies confirmed that miR-186 increased expression of tumor epithelial cells P2X7 mRNA levels [13]; in addition, miR-186 in lung adenocarcinoma, breast cancer and pancreatic cancer in varying degrees of study decreased [14-16], but in colon cancer Research in IRAN has not been reported yet.

Is hsa-miR-186 also involved in the development and progression of colon cancer? in order to better explore the biological function of miR-186 in colon cancer cells, we used fluorescence quantitative RT-PCR experiments on 15 paired colon cancer groups Weave and adjacent tissue. In colon cancers, suggesting suppression of miR-186 may be present in Colon cancer occurrence and development play a role.so in the follow-up of miRNA as a new target for targeted tumor gene therapy for important experimental basis, may also be a gene therapy for colon cancer One of the new targets.

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