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The expression of miR-181a-5p and miR-371b-5p in chondrosarcoma

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Abstract. – OBJECTIVE: Chondrosarcomas are malignant tumors of chondrocytes that affect bones and joints, and it represents the third most common type of primary bone tumors. Chondrosarcoma is difficult to treat because it is relatively resistant to both chemotherapy and radiation. Thus, surgery remains the best available treatment. It is important to find new diagnostic markers and improve treatment options.

BACKGROUND: miRNAs are small non-coding transcripts (19-25 nucleotides) that regulate gene expression via targeting complementary sequences within messenger RNAs (mRNAs). miRNAs have been shown to be involved in regulation of many biochemical pathways. Dysregulated expression of many miRNAs has also been associated with multiple human diseases, such as cancer.

PATIENTS AND METHODS: 18 surgical chondrosarcoma specimens were obtained from patients. RNA extractions were performed from decalcified paraffin embedded tissues. The aim of this study was to investigate the expression levels of miR-181a and miR-371b in patients with chondrosarcoma by using RT-PCR and to evaluate the relationship between these miRNAs and chondrosarcoma.

RESULTS: miR-181a was found to be upregulated in chondrosarcoma specimens whereas no significant alteration was found for miR-371b expression.

CONCLUSIONS: It has been proposed that miRNA expression studies might be used as diagnostic, prognostic marker in cancer. miRNA expression data produced in our study may contribute future chondrosarcoma diagnosis and therapy.

Key Words:
Chondrosarcomas, miRNA, RT-PCR, Gene expression.

Introduction

Chondrosarcoma is a type of cancer arising from the cartilage-producing chondrocytes. It is third most common primary malignancy of bone that affects bones and joints after myeloma and osteosarcoma[1]. Chondrosarcoma is difficult to treat since it is resistant to radiation and chemotherapy. Thus, surgical excision remains the best appropriate treatment[2].

The majority of these tumors grow slowly and rarely metastasize, and they have an excellent prognosis after adequate surgery[1]. They are relatively chemo- and radiotherapy resistant because of a low percentage of dividing cells, poor vascularity and their extracellular matrix. However, a minority of patients present recurrent with metastatic disease, and up to 13% of recurrent chondrosarcoma cases show higher grade than the original neoplasm[3,4].

Histologically, chondrosarcomas are divided into three subgroups; grade 1, 2, and 3 with respect to properties such as cellular atypia and cellularity. In addition, subtypes have been identified such as secondary, myxoid, dedifferentiated, clear cell, mesenchymal, periosteal chondrosarcoma. The most prominent feature of cartilage lesions is the presence of calcifications and opacities on radiographs[4].

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MicroRNAs (miRNAs) are short non-coding RNAs of 19-25 nucleotides in size that modulate the gene expression. The miRNAs alter expression levels of their target genes via base-pairing with complementary sequences within their target mRNA molecules. The alteration of miRNA expression has been shown in several diseases including cancer[5]. It is noted that the miRNAs might have many functions as tumor suppressor genes or oncogenes, and etc.[5].

The miRNAs and cancer association has been shown were in 2002, with chronic lymphocytic leukemia, and consequently with many other types of malignancies[6,7]. The regulation of miRNA expression can contribute to development of cancer during failure of these controls[7]. The miRNAs can be used as a biomarker to detect malignancy. In the future, the miRNA may act as future therapeutic targets.

Patients and Methods

18 surgical chondrosarcoma specimens (both tumor and adjacent normal tissue) were obtained from patients (ranging from ages 12 to 67) in Department of Orthopedics in Baltalimanı Hospital (Istanbul, Turkey) between 2012-2014 years. RNA extraction was performed from decalcified paraffin embedded tissues. This research obtained the approval of the Clinical Research Ethical Committee of Taksim Research and Educational Hospital.

Total RNA Isolation and cDNA Conversion

Both tumoral and adjacent normal chondrosarcoma tissues of 18 patients were processed by miRNeasy FFPE Kit (Qiagen GmbH, Hilden, Germany) to obtain total RNA for miRNA expression. miScript II RT Kit (Qiagen GmbH, Hilden, Germany) was used to obtain cDNA for qRT-PCR analysis. Rotor-Gene Q (Qiagen, Hilden, Germany) Thermal Cycler was used to reveal expression of miRNAs. Real-time PCR was performed using Rotor Gene 6000 Real-Time PCR Machine (Qiagen GmbH, Hilden, Germany) with miScript SYBR Green PCR Kit (Qiagen GmbH, Hilden, Germany) for miRNA expression.

Statistical Analysis

To calculate fold changes in miR-181 expressions between tumor and normal samples, RT² Profiler PCR Array Data Analysis version 3.5 (Qiagen GmbH, Hilden, Germany) was used. This analysis program is based on $2^{-\Delta\Delta}$CT method for fold change calculations. $p < 0.05$ was considered statistically significant.

Results

Our study group involves 18 surgical chondrosarcoma specimens (both tumor and adjacent normal tissue) were obtained from patients (ranging from ages 12 to 67) in Department of Orthopaedics in Baltalimanı Hospital (Istanbul, Turkey) between 2012-2014 years. RT-PCR was performed to determine hsa-miR-181a-5p and hsa-miR-371b-5p expression levels. RNAs extracted from decalcified samples were of poor quality and produced inconsistent RT-PCR results. In order to get good quality tissue sections, decalcification of formalin fixed tissue is required before paraffin process. It was shown that decalcification causes RNA fragmentation[8] and we believe this harsh treatment is the reason why results are difficult to interpret correctly. Although mir-181a and 371b expression levels were studied in 18 specimens, only some were able to be evaluated because of this specific reason. 12 samples were analyzed for miR-181a

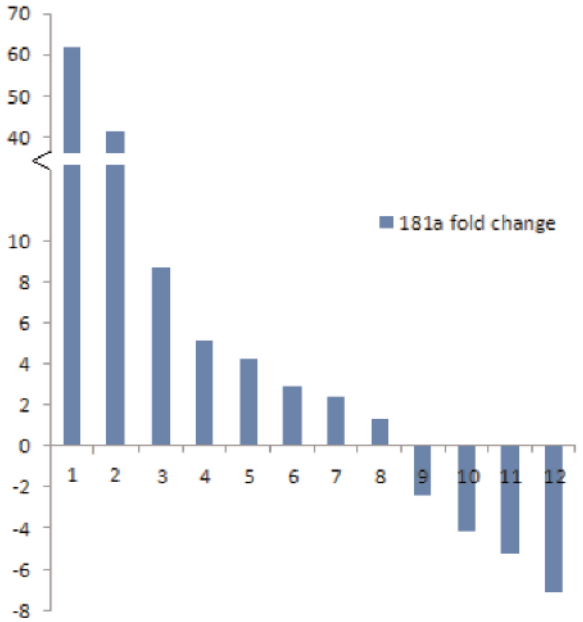

Figure 1. MiR-181a expression levels in chondrosarcoma patients. Real-time PCR was performed using Rotor Gene 6000 Real-Time PCR Machine (Qiagen GmbH, Hilden, Germany) with miScript SYBR Green PCR Kit (Qiagen GmbH, Hilden, Germany).

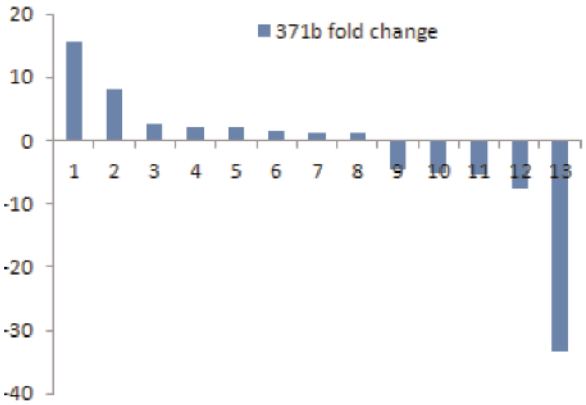

Figure 2. MiR-371b expression levels in chondrosarcoma patients. Real-time PCR was performed using Rotor Gene 6000 Real-Time PCR Machine (Qiagen GmbH, Hilden, Germany) with miScript SYBR Green PCR Kit (Qiagen GmbH, Hilden, Germany).

expression level and 13 samples for miR-371b. miR-181a expression was found to be up-regulated in 8 samples compared to non-tumorous articular chondrocytes and it was down-regulated in remaining 4 samples (Figure 1). Similarly, miR-371b expression level was higher in 8 samples and lower in 5 samples compared to the control (Figure 2).

Discussion

In our study, the expression levels of miR-181a and miR-371b were altered in chondrosarcoma tissue samples compared to non-tumorous articular chondrocytes. Dysregulation of miRNA expressions might affect a wide variety of biological functions in normal chondrocytes and cause their malignant transformation[9].

MicroRNA-181a (miR-181a) has roles in many biological processes such as cell proliferation, cellular invasion and apoptosis[10-12]. The expression levels of miR-181 family were found altered in various human cancers and they were significantly associated with prognosis[13]. miR-181a has been reported to be downregulated in lung cancer[14] but overexpressed in breast cancer[15], gastric cancer[11] and oral squamous cell carcinoma[16]. It was also found that miR-181a is expressed at high levels in chondrosarcoma[9]. Various studies show that miR-181a could affect cancer cell growth, such as gastric cancer[11], osteosarcoma[17] and hepatocellular carcinoma (HCC)[18]. We found that miR-181a was upregulated which was consistent with the literature in which miR-181a expression was elevated in chondrosarcoma[9].

Functional effects of miR-181a upregulation are varied and include modulation of epithelial to mesenchymal transition (EMT) in ovarian carcinoma[19], enhanced chemoresistance to cisplatin by targeting protein kinase C, delta, a promoter of apoptosis[20], and promotion of proliferation and inhibition of apoptosis in gastric cancer by targeting the tumor suppressor ataxia telangiectasia mutated (ATM)[21]. In chondrosarcoma cells, it was shown to be involved in the hypoxic regulation of enhanced VEGF expression[22]. miR-181a expression has been demonstrated to be regulated by various signaling pathways, including estrogen, TGF-β, MYC, STAT3 and Wnt/ -catenin, suggesting a potential role in regulating cancer relevant signaling networks[15-16,23-25].

Conclusions

According to miRtarbase database miR-181a was found that target eight genes; Bcl-2, PROX1, CDKN1B, BCL2L11, HRAS, RNF2, RALA and KLF6 which all have been validated by reporter assay, western blot and qPCR[26]. No validated target was found for miR-371b and no expression data exist in chondrosarcoma as well. miR-371b expression level was down-regulated in some samples, whereas up-regulated in others. In other words, no significant alteration was detected in our samples. As we mentioned above, decalcification of formalin fixed tissue is required in order to get good-quality tissue sections before paraffin processing. It was shown that decalcification causes RNA fragmentation 8 and we believe this harsh treatment may alter miRNA levels. We recommend remembering possibility of RNA fragmentation due to decalcification procedure and analyzing expression data in chondrosarcoma studies accordingly.

Further study should be performed with more chondrosarcoma patients. Besides, functional studies should be carried out to elucidate the association between these miR-181a, miR-371b and chondrosarcoma. It has been proposed that miRNA expression studies might be used as diagnostic, prognostic marker in cancer[5], and therapeutic target for cancer treatment[7]. miRNA expression data produced in our study may contribute future chondrosarcoma diagnosis and therapy.

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Conflict of Interest

The Authors declare that they have no conflict of interests.

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