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Dicer and Drosha Expression Levels in Ovarian Cancer Tissues

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Abstract

MicroRNA (Mirnas) play crucial roles in diverse cellular processes and associate with various Ovarian Cancers (OC). Dicer and Drosha are two major enzymes in the miRNA maturation process. Dicer and Drosha genes expression investigated OC and analyzed the impact of clinicopathological characteristics on their expression. We investigated the expression of Dicer and Drosha in invasive (n=50), matched adjacent marginal tissue (n=50) using real-time RT-PCR. The expression rate change in the Dicer and Drosha genes was assessed using the 2-ΔΔCT method. The statistical analysis of genes expression levels showed that Dicer and Drosha were up-regulated, significantly increased in tumors compared to marginal tissue. On the other hand, there was no correlation between the expression of Dicer and Drosha to age and tumor size. This article serves as an early laboratory diagnostic and non-invasive prognosis in OC patients. These genes may have a potential role in malignancy and increase tumor stage by influencing miRNA molecule level and serve as markers for disrupted miRNA. Additional research should investigate the different disease stages.

Keywords: DROSHA; DICER; MiRNA; Ovarian cancer

Introduction

Ovarian Cancer (OC) is one of the widespread cancer death causes in women. According to a study in 2016, 1.3 million women showed that endometrioid, clear cell carcinomas have the most significant risk factors in OC, with the results of Wentzensen et al. On the other hand, Smoking was associated decreased risk of clear cell tumors. Negatively influence OC factors, including a high intake of saturated fats and a high body mass index, was studied before. miRNAs are short (19-25 nucleotides), single-stranded, highly conserved non-coding RNA molecules that negatively regulate the post-transcriptionally genes via binding with their 3′-Untranslated Region (UTR) [1-5]. Such binding assists the degradation of mRNA's genes or blocks their translation, which involves sequential cleavages in pri-miRNA by the microprocessor complex (Drosha and DGCR8) and the endonuclease Dicer, before being loaded onto an RNA-induced silencing complex to repress gene expression Figure 1. Although Dicer and Drosha are essential for the production of miRNAs, their expression in cancer cells does not study in-depth [6]. miRNAs could be used as biomarkers to identify patients that might benefit from the addition of targeted agents and could be regulated at the transcriptional level by transcription factors or at the post-transcriptional level by RNA-binding proteins Dicer or Drosha involved in maturation. Comprehensive analysis of more than 1493 small RNA deep sequencing datasets estimated that the human genome transcribes about 1917 miRNA precursor sequences, generating 2654 mature miRNA sequences [7]. Some of the top miRNAs' expression changes were associated with shorter survival in serous OC. This study examined miRNA processing pathway enzymes, such as Dicer and Drosha, to show their influential role in miRNA's function [8].

Method

Study cohort

The Medical Ethics Committee approved this study Azad University of Iran (Approval No. 22030503941002). This study retrospectively analyzed OC patients between 2018 and 2019; in Tabriz and Urmia, which was never investigated before. Tissue samples were taken from individuals aged 40 to 62. Most patients were included in the training group of tumor tissue of OC (n=50), and the marginal cell tissue of OC patients was included in the verification group (n=50). Under the National Statement on Ethical Conduct in Research Involving Humans, a waiver of consent was granted for this study [9-12].

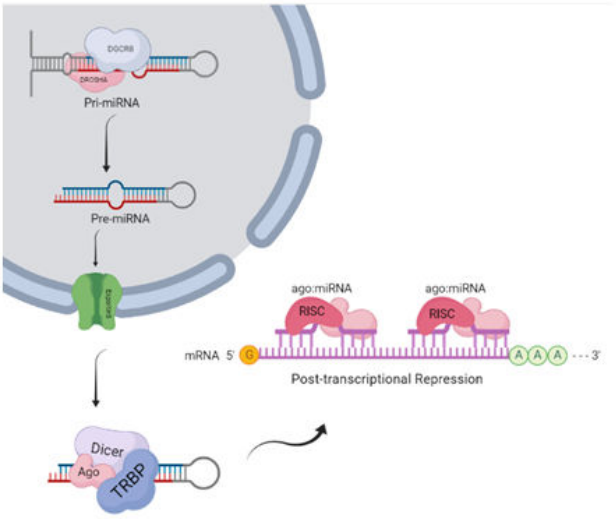

Figure 1: Dicer and Drosha's essential roles in miRNA maturation pathway.

Extraction of RNA

RNA was extracted from OC tissue specimen (stored at -80°C) with RNeasy Mini Kit (Qiagen, Hilden, Germany) following the manufacturer's instruction to gain the RNA from 20-30 mg of tissue specimens [13]. Respectively, the integrity and quality of RNA, confirmed by agarose gel electrophoresed on 2% agarose gel, and absorbance readings at 260 nm. According to the manufacturer's recommendation, the Revert FastLane Cell cDNA kit (Qiagen, Hilden, Germany) synthesized the cDNA in a final volume of 20 µl. According to the cDNA synthesis kit protocol, an adverse control

reaction -RT and NTe and a positive control reaction were used to evaluate the accuracy of cDNA synthesis. This reaction is vital for detecting DNA contamination in extracted RNA samples [14]. Due to the lack of the enzyme, cDNA would not have synthesized (NT), so the genes were amplified using cDNA. Therefore, no bandages saw during the reproduction of the studied genes using this cDNA, and the band indicates contamination in DNA. Therefore, the band's existence was indicating DNA contamination [15].

Real-time PCR

Total RNA was transcribed using cDNA reverse transcription kit; after that, Real-time PCR analysis was performed in duplicate using TaqMan® Universal PCR mix manufacturers' instructions, with results quantified on a 7500 real-time PCR system (Life Technologies). The expression of Dicer and Drosha were quantified using Taqman Gene Expression Assays. The Dicer and Drosha relative expressions were normalized to the GAPDH housekeeping gene (ΔCt) and expressed as the fold change as described previously. We have verified that GAPDH is equally expressed among the different tissues analyzed in this study [16].

Statistical analysis

Changes in genes expression were assessed using the 2-ΔΔCT method(Pffafi, 2001). All data analysis was performed using GraphPad Prism(version 8.0.2, GraphPad Software Inc., La Jolla, CA, USA). To show a statistical difference, $P<0.05$ had considered (11). A two-tailed test was used to verify a statistically significant difference in Dicer and Drosha expression between the two groups.

Results

The Dicer and Drosha expression were quantitated in all 50 OCs by real-time RT-PCR. The relative mRNA expression of Dicer and Drosha were significantly higher between marginal and tumor tissues (respectively $p=0.014$, $p=0.028$). The results demonstrate no significant relationship between the age of patients and the expression changes of studied genes ($p=0.66$). We also found that Dicer and Drosha mRNA levels in °C cells have associations with outcomes in patients with ovarian cancer [17].

Discussion

Our results have shown that Significant up-regulation of Dicer and Drosha expression were observed in ovarian cancer compared to marginal and tumor tissue, in agreement with the results of Merrit Due to our investigation and other research about communication of Dicer and Drosha with ovarian cancer, such as Pampalaski et al, we point some critical investigation about that, including the influence of up-regulated Dicer and Drosha in survival decreased and their potential to be malignancy in ovarian tissue [18-21]. According to the Zhang et al. examination, EIF2C2, Dicer, and Drosha are more highly expressed in bladder carcinoma, promote bladder cancer, and suggest a poor Furthermore, Kim et al, mentioned that Drosha and Dicer significantly increased colorectal carcinogenesis. Furthermore, Kim investigate that levels of Dicer and Drosha and DGCR8 have a positive correlation between altered mRNA expression in TCGA Papillary Thyroid Carcinoma (PTC) samples observed. On the other hand, Vaksman et al, said that Dicer and Drosha are two major enzymes in the miRNA maturation process. The expression of Dicer and Drosha suggests a possible mechanism for modifying miRNA in malignancies. Recently, Quidamo et al, showed that miRNAs play fundamental roles in diverse cellular processes and links to various cancers [22-24].

In particular, 11 genes and 12 miRNAs in the integrated network are associated with ovarian cancer. Low Dicer and Drosha expression associated with poor prognosis in ovarian cancer, Ear-nose-and-throat tumors, and hematological malignancies and contributed to epithelial ovarian cancer progression by elevating PDIA3 expression also low Dicer expression role of miRNAs in the pathogenesis of breast, ovarian, and prostate cancer will provide insights for the use of miRNAs as a biomarker or therapeutic agent for the cancers. Dicer deregulated in LPS. Ovarian Cancer does not show significant symptoms in its early stages. It was possible to diagnose it in the advanced stages of the tumor, so it has a high mortality rate. A better understanding of the role of microRNA expression in ovarian cancer may provide a new array for the OC's detection, diagnosis, and therapy. They also discussed miRNA levels in ovarian disease effect, epigenetic alterations, and oncogenic mutations [25]. Emphasis gives to the role of particular miRNAs by Copy Number Variations (CNVs) in altering the gene expression in human ovarian cancers with the potential to provide diagnostic, prognostic, and therapeutic targets. The first report characterizes Dicer's Expression and function in the tumor stroma and highlights its pro-metastatic role in this context. Additionally, they suggest that the Dicer-miR6780b-NFκB cascade is an attractive target choice in stroma-oriented OC therapy. Drug resistance overcoming in ovarian cancer may be affected by Dicer's decreasing activity in the level of proteins invoived in apoptosis pathway.

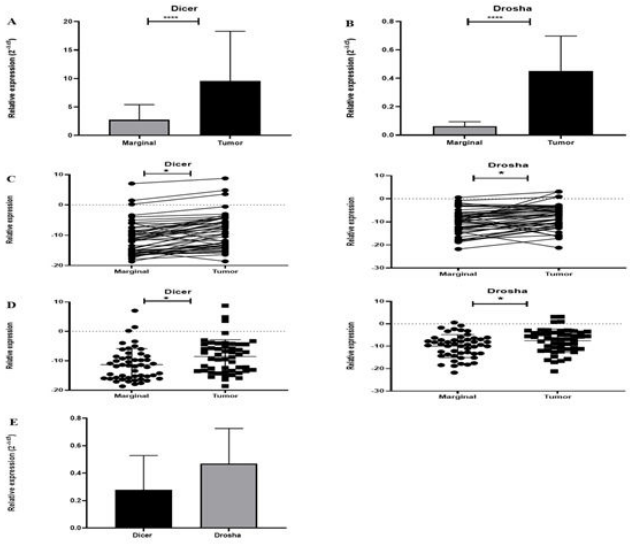

Figure 2: Dicer and Drosha expression of ovarian cancer: A) Relative qualification of Dicer B) Relative qualification of Drosha by real-time RT-PCR C) Relative qualification Dicer and Drosha in marginal and tumor samples. Results are shown as the target's relative normalized expression D) Relative qualification in tumor and marginal samples 0307 drosha *P=0.19 E) Relative qualification of Dicer and Drosha in tumor samples.

Conclusion

Up-regulated expression of miRNAs biogenesis machinery enzymes (Dicer and Drosha) during ovarian cancer can alter the miRNA expression involved in the pathogenesis of malignancy. This

article serves as an early laboratory diagnostic and non-invasive prognosis in ovarian cancer patients by increasing survival levels. Above all, previous studies, including up-regulated, down-regulated expression in Dicer and Drosha, suggest that these genes are a potential role in malignancy and increase tumor stage by influencing miRNA molecule level; additional research should investigate the different disease stages.

Competing Interests

The authors declare they have no competing interests.

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Availability of Data and Material

The data that support the findings of this study are available from correspondence. However, restrictions apply to the availability of these data, which were used under license for the current study, and so are not publicly available. Data are, however, available from the authors upon reasonable request and with permission of correspondence.

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