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Role of personalized microRNA-124 Expression in Ovarian Cancer

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Abstract

Introduction: MicroRNA-124 (miR-124) is moderated in some human malignancies and is associated with tumor advancement. But, its expression and clinical importance in ovarian carcinoma is still unclear. Thus, the goal of this study was to feature the clinical importance of personalized miR-124 expression in ovarian carcinoma.

Methods: 94 women ovarian cancer tissues and 26 normal ovarian tissues were accumulated from patients. We used Real-time PCR to quantify the expression of personalized miR-124 in clinical ovarian carcinoma specimen and normal tissues. Moreover, we measured the miR-124 relationship with clinicopathologic characteristics and the ovarian carcinoma survival.

Results: The lesser expression of miR-124 in tumor tissues can be found in compared with normal tissue using PCR method ($P < 0.05$). Our data exhibited that there is a notable association among low expression of miR-12 and clinical staging of ovarian carcinoma ($P = 0.023$). Nevertheless, miR-124 expression was not notably associated with age ($P = 0.671$), differentiation status ($P = 0.512$), lymph node metastasis ($P = 0.415$) and histological subtypes (0.547). Kaplan-Meier survival analysis and log-rank test were applied in present study. These tests showed the less expression on patients had markedly short-term survival time in comparison with high expression group ($P = 0.022$). Multivariate Cox proportional hazards model analysis revealed that less expression of miR-124 and clinical staging were contribute to short-term survival in patients with ovarian carcinoma. The HR of the low miR-124 expression group was calculated to be 2.532 (95% CI: 1.572-9.237, $P = 0.021$), (clinical staging HR: 2.532; 95% CI: 1.321-9.241, $P = 0.032$).

Conclusions: These findings suggested that personalized miR-124 could be considered as an independent prognostic factor for ovarian carcinoma patients. Our findings suggested that low expression of personalized miR-124 has prognostic worthiness in ovarian.

INTRODUCTION

Lately, personalized medicine has mainly involved the systematic utilize of genetic to select remedial care and in parallel, modern personalized medicine is based on targeted remedy [1]. MicroRNAs (miRNAs) are known as small non-coding RNA molecule, which modulate gene expression in a post-transcriptional manner.

Various personalized miRNAs display a key pattern in more biological processes functions including differentiation, apoptosis and cellular proliferation [2-4].

The relationship between miRNA expressions and tumor prognosis has been documented [3-5]. In addition, it has been found that miRNAs can play its role as either oncogenes or tumor suppressors [4-6] Application of molecular research can be beneficial to clarify the functional and clinical importance of a specific miRNA. The role of microRNAs as potential markers therapeutic targets were previously investigated in different kinds of cancers [6]. This cancer is known as one of the most usual diseases gynecological malignancies, which is cause of cancer related mortality among women [7], because more than 70% of patients are in late-stage, with metastasis away at the diagnosis time [8, 9].

It has been presented that microRNA has a potential role in the diagnosis, prognosis and ovarian cancer therapy [10, 11]. Previous studies have been indicated that Micro RNA-124 (miR-124) was epigenetically down regulated in various kinds of cancers [12-14]. Moreover, several studies have been indicated that miR-124 involves in several malignant processes, including, Epithelial-mesenchymal transition and tumor proliferation, and angiogenesis [15-17]. Many miRNAs are implicated in ovarian cancers but the expression level and the possible role of miR-124 in ovarian cancer need to further studies. Since, we travel more into a period of personalized medicine and the elevating availability of targeted treatments. Therefore, we investigated the relationship of personalized miR-124 with clinicopathological evaluations and survival of ovarian carcinoma patients by real-time PCR.

METHODS

Specimens

94 women ovarian tumor tissues and 26 normal ovarian tissues were collected from patients in current study. Whole of samples were stained with H&E for histopathological evaluation and diagnosed by tow pathologists. Clinical data of patients was presented in Table 1. We defined overall survival according to the elapsed time from the surgery to the death.

Table 1: Correlation between miR-124 Expression and Clinicopathological Features of Patients with in Ovarian Carcinoma

Parameters	Case no.	miR-124 expression		P value
		Low	High	
Age				0.671
≤50	40	22	18	
>50	54	32	22	
Differentiation status				0.512
High or medium	38	18	20	
Low	56	33	21	
Clinical stage				0.023
Stage I	21	7	14	
Stage II	37	30	7	
Stage III	42	38	4	
Lymph node metastasis				0.415
Yes	40	26	18	
No	54	34	20	
Histological subtypes				0.547
Serous	46	25	19	
Mucinous	7	4	3	
Endometrioid	6	4	2	
Clear cell	16	9	7	
Other	19	11	8	

Table 2: Multivariate Analysis with a Cox Proportional Hazards Model between Clinicopathological Factors

Clinicopathological Characteristics	HR	95% CI	P-value
Age	0.812	0.436-2.214	0.612
Clinical stage	2.613	1.321-9.241	0.032
Differentiation status	0.741	0.916-3.326	0.615
Lymph node metastasis	0.632	0.532-2.126	0.572
Histological subtypes	1.228	0.643-2.176	0.514
miR-124 level	2.532	1.572-9.237	0.021

Quantitative Real-time PCR

We used TRIzol reagent (Invitrogen, Carlsbad, California, USA) to extracted total RNA according to manufacturer's protocol.

Furthermore, PrimeScript Reagent Kit (Promega, Madison, WI, USA) was used to synthesize cDNA, Real-time PCR was carried out using an SYBR Green PCR Master Mix (Applied Biosystems) by system of Rotor-gene 6000 (Qiagen). We utilized the comparative cycle threshold (CT) method to calculate modifications in expression. The relative amount of miR-124 was

normalized with respect to U6 RNA. Moreover, for miR-124, $2^{\Delta\Delta}$Ct method were utilized to calculated the fold-change among tumor tissues and normal tissue control, that $^{\Delta\Delta}Ct = {}^{\Delta}Ct$ (target-reference in tumor samples) - $^{\Delta}Ct$ (target-reference in normal samples). The Comparative $2^{-\Delta\Delta}$Ct analysis was utilized for investigation of the miRNAs expression in cancer tissue compared to their non-tumorous controls.

Statistical Analysis

This approach was conducted by the SPSS version 19.0 software (SPSS Inc., Chicago, IL, USA). The differences in miR-124 expression among ovarian tumor tissues and normal ovarian tissues were assessed using Student's t-test.

Moreover, Correlations between and the clinicopathological characteristics were evaluated using chi-square test and Fisher's exact test. Differences in survival between two groups were analyzed using the log-rank test and Kaplan-Meier method. In addition, independent prognostic factors linked to patient survival were analyzed by multivariate Cox regression analyses. Differences were considered to be statistically notable at $P<0.05$

RESULTS

Less Expression of miR-124 in Tumor Tissues

Quantitative real-time PCR indicated that lesser expression in tumor tissues in comparison with normal tissues (Fig 1; $P < 0.05$). In present study, the patients divided in tow group including high (41 cases) and low expression (53 cases) groups (according to median level of expression of patients that was 0.40).

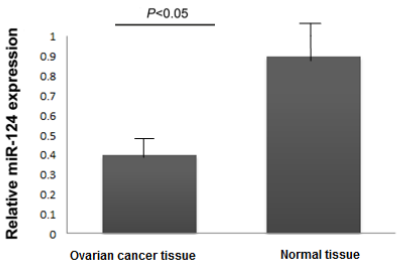

Figure 1: Lower Expression was seen in Tumor Tissues in Comparison with Normal Tissues using by Quantitative Real-time PCR

Expression Levels of miR-124 and its Relationship with Clinicopathological Feature

In current study, the level of miR-124 expression with a number of clinicopathological feature were evaluated. Our findings revealed that there is a significant association between low expression of miR-12 and clinical staging of ovarian carcinoma ($P = 0.023$). Nevertheless, miR-124 expression was not associated with age ($P = 0.671$), Differentiation status ($P = 0.512$), Lymph node metastasis ($P = 0.415$) and Histological subtypes (0.547), (Table 1).

Survival Analysis

Kaplan-Meier survival analysis and log-rank test were performed in present study. These tests showed the low expression in patients had markedly short-term survival time in comparison with high expression group, (log-rank test P= 0.022; Fig 2). Multivariate Cox proportional hazards model analysis was employed to analyze the prognostic value of miR-124 expression and different clinicopathological features in survival of ovarian cancer patients (Table 2).

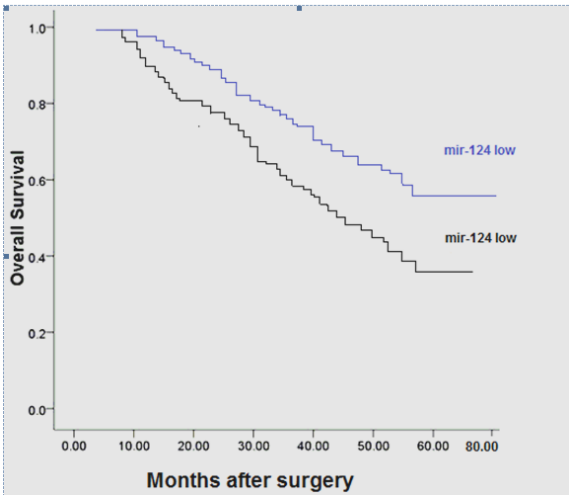

Figure 2: Low miR-124 Expression was Significantly Associated with Shorter Overall Survival in Ovarian Carcinoma in Comparison with High Expression Patients (Log-rank Test P= 0.022)

The result revealed that low miR-124 expression and clinical staging was associated with short-term survival in ovarian cancer patients and other factors that were evaluated had no remarkable prognostic value for survival in ovarian carcinoma (Table 2). The HR of the low miR-124 expression group was calculated to be 2.532 (95% CI: 1.572-9.237, P= 0.021), data indiacted that miR-124 could be considered as an independent prognostic factor for ovarian carcinoma patients.

DISCUSSION

Various personalized miRNAs have been found that can play an important role in many biological functions including cellular proliferation, differentiation, and apoptosis [1-3]. Thus, it worth noting that identifying the involved mechanisms of personalized miRNAs may result in improvement of understanding of these molecules and the complexity of cancer progression. Multiple researches have shown that there is a notable correlation among different miRNA expressions and tumor prognosis [3, 18]. We used quantitative real-time PCR to assess the expression level of miR-124 in ovarian carcinoma patients and normal tissues. Our data demonstrated that miR-124 is reduced in ovarian

carcinoma tissue patients in comparison with normal tissue, indicating that it can play its role as tumor suppressor. Indeed, reduction in expression of miR-124 may be responsible for the progression. Zhang et al, (2013) have been indicated that miR-124 acts as tumor suppressor in ovarian cancer cells [19]. There are different studies that revealed down regulation of Micro RNA-124 in various types of [12, 20, 21]. The finding s of mentioned studies implies that miR-124 has potential role in prognosis prediction and even in cancer treatment. There is a study that has focused on the role miR-124 in ovarian cancer. Zhang et al (2013) have been seen that down-regulation of miR-124 is in ovarian cancer cell lines [19] and tumor tissues that are in line with our findings in current study.

Moreover, down-regulation of miR-124a was previously reported in ovarian cancer tissues in comparison with normal tissue [8]. Our findings revealed that there is a notable correlation among low expression of miR-124 and clinical staging of ovarian carcinoma. (It has been reported that reduction of miR-124 expression may result in the migration and invasion of epithelial ovarian cancer cells and also down-regulation of SphK1, which play its role as direct functional target of miR-124. It is noteworthy that reduction of SphK1 expression could participate to distant metastases in epithelial ovarian cancer cells [19]. It should be noted that However, there are not further studies to explain the association between expression of miR-124 and clinicopathological feature in ovarian cancer. Thus, many studies are needed to clarify it. Recent researches have been reported that down regulation of miR-124 contributes to in the regulating invasion and metastasis in mammary tumors and other cancers patients [13, 20, 22]. Mentioned studies indicated the potential use of miR-124 in prognosis and cancer prognosis [4, 12, 21].

Kaplan-Meier survival analysis and log-rank test were applied in present study. These tests revealed that patients with low expression had markedly short-term survival time in comparison with high expression group. Multivariate Cox proportional hazards model analysis revealed that low miR-124 expression and clinical staging were related to short-term survival in patients with ovarian carcinoma. Liang-liang et al (2015) have been indicated shorter overall survival with low miR-124 expression in comparison with patients with high miR-124 expression level in breast cancer patients, indicating that miR-124 may play its potential role as a prognostic marker [21].

CONCLUSIONS

This study suggested that prognostic value in less expression of miR-124 on ovarian carcinoma and may minister as a prognostic factor. It is worth noting that further studies are required for this purpose.

Conflict of Interest

We declare that we have no conflict of interest.

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