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Research paper

MiR-205 promotes motility of ovarian cancer cells via targeting ZEB1

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ABSTRACT

Aim: To investigate the clinical significance of microRNA-205 (miR-205) and zinc finger E-box binding homeobox 1 (ZEB1) in epithelial ovarian cancer (EOC) and the underlying mechanisms by which they are involved into tumorigenesis.
Methods: Expression levels of miR-205 and ZEB1 mRNA in EOC tissues were detected by quantitative real-time PCR. Their associations with clinicopathological features of EOC patients were statistically analyzed. Luciferase reporter assay was used to confirm target associations between miR-205 and ZEB1. After that, the functions of miR-205–ZEB1 axis on cell migration and invasion were further determined by transwell assay in vitro.
Results: Expression levels of miR-205 ($P = 0.0001$) and ZEB1 mRNA ($P < 0.0001$) in clinical EOC tissues were significantly higher and lower than those in normal tissues, respectively. Interestingly, there was a negative correlation between miR-205 and ZEB1 mRNA expression in EOC tissues ($P = 0.01$). Additionally, miR-205-upregulation and/or ZEB1-downregulation were significantly associated with high pathological grade and advanced clinical stage of EOC patients (all $P < 0.05$). Meantime, luciferase reporter assays identified ZEB1 as a direct target of miR-205 in EOC cells. Moreover, miR-205 blockage inhibited, whereas miR-205 mimics promoted the motility of EOC cells in vitro. Importantly, all the alterations of the above cellular phenotypes by blocking or enhancing of miR-205 could be alleviated by subsequent suppression or re-introduction of its target ZEB1, respectively.
Conclusion: MiR-205, acting as an oncogenic miRNA, may promote the clinical progression of EOC patients and enhance the cellular motility in vitro by directly and negatively regulating ZEB1, providing a potential therapeutic strategy for suppression of EOC metastasis.

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1. Introduction

Epithelial ovarian cancer (EOC), the fifth most fatal cancer and a highly metastatic disease for women, is originated from the ovarian surface, the nearby distal fallopian tube epithelium, or from inclusion cysts in the ovarian parenchyma (Vargas-Hernández et al., 2014). Annually, EOC caused an estimated 125,000 deaths worldwide (Clarke et al., 2014). Due to the lack of definitive early symptoms and efficient diagnostic markers, the majority of EOC patients are diagnosed at the advance stage of the disease, which typically has a 5-year survival less than 30% (Doufekas and Olaitan, 2014). If the EOC patients could be diagnosed and treated at the early stage, the 5-year survival can reach over 90% (Hacker, 2013). The above statistical results imply that the clinical outcome of EOC patients may be critically dependent on the clinical stage at patients' diagnosis. Therefore, it is extremely necessary to identify novel, specific and sensitive biomarkers for early diagnosis and prognosis of EOC patients.

Accumulating evidence shows that the carcinogenesis and cancer progression of EOC may be related to multistep changes in the genome, in particular the expression and function of various microRNAs (miRNAs), a class of small, endogenous non-coding RNA molecules (Ha and Kim, 2014). MiRNAs play a central role in post-transcriptional gene regulation by binding to a target site in the 3′-UTR of target mRNAs, and function as key regulators in various biological processes, including cell development, growth, differentiation and division (van Rooij and Kauppinen, 2014). Pathologically, miRNAs have been reported to be involved in tumor initiation and progression, and the associations between miRNAs and cancer have become a research focus. The abnormal expression of miRNAs in various cancer types has been widely reported in recent years. According to the functions of their target genes, miRNAs act as either oncogenes or tumor suppressors (Hata and Lieberman, 2015; Huang et al., 2014). They regulate the key signaling pathways in different cancer cells, and changes in their expression can greatly influence the protein expression patterns, leading to a series of malignant phenotypes.

Metastasis is the major cause of cancer-related deaths for EOC patients. Growing evidence shows that it is promoted by the epithelial-

Abbreviations: miRNA, microRNA; miR-205, microRNA-205; ZEB1, zinc finger E-box binding homeobox 1; EOC, epithelial ovarian cancer; EMT, epithelial-to-mesenchymal transition.

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to-mesenchymal transition (EMT) of primary cancer cells (Díaz-López et al., 2014; Zaravinos, 2015). Since elucidating the underlying mechanisms of metastasis occurred during the progression of EOC will make significant contributions toward combating this disease, a number of studies have identified several crucial regulators in the EMT, such as miR-125a, the miR-200 family of microRNAs (miR-141, miR-200a, b, c, and miR-429), miR-34, miR-205, zinc finger E-box binding homeobox 1 (ZEB1) and zinc finger E-box binding homeobox 2 (ZEB2) (Mezzanzanica et al., 2010; Cowden Dahl et al., 2009; Park et al., 2008; Corney et al., 2010; Gao et al., 2014). Especially, the plasma miR-205 has been indicated as a biomarker for ovarian cancer detection that complements CA-125, and the upregulation of miR-205 has also been reported to promote the invasion and proliferation of ovarian cancer cells (Zheng et al., 2013); ZEB1 has been identified as an activator of EMT in EOC cells (Jin et al., 2014), and the regulatory effect of miR-205 on ZEB1 expression has been confirmed based on various cancer cells, including breast cancer cells and papillary urothelial tumors of the urinary bladder (Zhang et al., 2014; Wiklund et al., 2011). In the current study, to investigate the clinical significance of miR-205 and ZEB1 in human EOC and to clarify the underlying mechanisms by which they are involved into tumorigenic processes of this disease, quantitative real-time PCR was performed to detect the expression levels of miR-205 and ZEB1 mRNA in human EOC tissues, and statistical analysis was used to evaluate their associations with clinicopathological features of EOC patients; Then, luciferase reporter assay was used to confirm target associations between miR-205 and ZEB1. After that, the functions of the miR-205–ZEB1 axis on cell migration and invasion were further determined by transwell assay in vitro.

2. Materials and methods

2.1. Patients and tissue samples

This study was approved by the Ethics Committee of First Affiliated Hospital of Chinese PLA General Hospital, China. All the clinical samples were obtained from First Affiliated Hospital of Chinese PLA General Hospital, China. Written informed consent was obtained from all patients.

A total of 110 EOC tissues and 20 normal ovarian tissues were selected from the archives of First Affiliated Hospital of Chinese PLA General Hospital, China. All 110 EOC tissues were collected during debulking surgery from March 2010 to February 2013 and 20 normal ovarian tissues were obtained from women who underwent hysterectomies for benign disease from February 2013 to December 2013. All tissue specimens were snap-frozen in liquid nitrogen and stored at −80 °C following surgery for quantitative real-time PCR assay. Surgical staging of EOC patients was established based on the International Federation of Gynecology and Obstetrics (FIGO) system. None of EOC patients were treated with radiotherapy, chemotherapy, or hormonal therapy before surgery. The clinicopathological features of 110 EOC patients were summarized in Table 1.

2.2. Cell culture

Human serous papillary cystic adenocarcinoma cell line SKOV3 was purchased from American Tissue Type Collection (Manassas, VA) and cultured in RPMI-1640 medium supplemented with 10% fetal calf serum (GIBCO) in a humidified atmosphere of 5% CO_2 at 37 °C.

2.3. Quantitative real-time PCR

Total RNA was extracted from EOC cells and tissues using TRIzol reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. A total of 20 μg of total RNA was subjected to reverse transcription. Single-stranded cDNA was synthesized with the PrimeScript Reagent Kit (Promega, Madison, WI, USA). The cDNA was used for the amplification of mature miR-205, ZEB1 and the endogenous controls, U6 and β-actin, by PCR, which was performed using the SYBR Green PCR Master Mix (Applied Biosystems, Foster City, CA, USA) on an ABI 7300HT real-time PCR system (Applied Biosystems, Foster City, CA, USA). The PCR primers used in this study were as follows: miR-205 forward, 5′-TCC TTC ATT CCA CCG GAG TCT G-3′ and reverse 5′-GCG AGC ACA GAA TTA ATA CGA C-3′; U6 forward, 5′-ATT GGA ACG ATA CAG AGA AGA TT-3′ and reverse, 5′-GGA ACG CTT CAC GAA TTT G-3′; ZEB1 forward 5′-CAG GCA GAT GAA GCA GGA TG-3′ and reverse 5′-CAG CAG TGT CTT GTT GTT GTA G-3′; and β-actin forward 5′-GGC GGC ACC ACC ATG TAC CCT-3′ and reverse 5′-AGG GGC CGG ACT CGT CAT ACT-3′. Expression data of miR-205 and ZEB1 were respectively normalized to the internal control (U6) and β-actin, and the relative expression levels were evaluated using the $2^{-\Delta\Delta CT}$ method (Livak and Schmittgen, 2001).

2.4. Cell transfection

MiR-205 mimic, miRNA control (miRCon), anti-miR-205, anit-miRNA control (anti-miRCon), ZEB1 siRNA oligonucleotides (si-ZEB1), non-specific scrambled control (si-Con), ZEB1 expression vector (ex-ZEB1) and control vector (ex-Con) were synthesized by RiboBio (Guangzhou, China). Then, EOC cells were harvested and plated onto 6-well plates with 70–80% confluence overnight before the transfection.

Table 1
Association of miR-205/ZEB1 expression with clinicopathological features of epithelial ovarian cancer tissues.

Features	No. of patients	MiR-205-high (n, %)	P	ZEB1-low	P	MiR-205-high/ZEB1-low	P
Age							
<55	45	20 (44.44)	NS	20 (44.44)	NS	12 (26.67)	NS
≥55	65	38 (58.46)		35 (53.85)		18 (27.69)	
Histological type							
Serous	80	48 (60.00)	NS	45 (56.25)	NS	25 (31.25)	NS
Non-serous	20	10 (50.00)		10 (50.00)		5 (25.00)	
Pathological grade							
1–2	22	8 (36.36)	0.01	8 (36.36)	0.01	0 (0)	0.006
3	88	50 (56.82)		47 (53.41)		30 (34.09)	
Clinical stage							
I–II	32	10 (31.25)	0.008	10 (31.25)	0.008	0 (0)	0.006
III–IV	78	48 (61.54)		45 (57.69)		30 (38.46)	
Residual tumor after surgery							
<1 cm	70	35 (50.00)	NS	33 (47.14)	NS	18 (25.71)	NS
≥1 cm	40	23 (57.50)		22 (55.00)		12 (30.00)	

Note: 'NS' refers to the difference without statistical significance.

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