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Gene expression profiles of ovarian low-grade serous carcinoma resemble those of fallopian tube epithelium

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HIGHLIGHTS

- Fallopian tube is likely the cellular source of ovarian low-grade serous carcinoma.
- Ovarian surface tumors are unlikely derived from the ovary itself.
- Serous tumors and tubal unique genes are mainly morphogenesis related.
- FOXA2, CDH1, PAX8, and FOLR1 highly expressed in ovarian serous tumors.
- ARX and FCN1 mainly expressed in ovarian surface and peritoneal cells.

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ABSTRACT

Objective. The cell of origin of ovarian low-grade serous carcinoma (LGSC) remains unclarified. Our recent morphologic and immunophenotypic study suggests that most LGSCs may be derived from the fallopian tube. The purpose of the current study was to gain further insight into the origin of LGSC at the molecular level.

Methods. RNA-seq analysis was performed on a total of 31 tissue samples including LGSC ($n = 6$), serous borderline tumors (SBT, $n = 6$), fallopian tube epithelia (FTE, $n = 5$), ovarian surface epithelia (OSE, $n = 4$), and human peritoneal mesothelia (HPM, $n = 4$). HGSC cases ($n = 6$) served as a positive control. Gene expression profiles were compared and analyzed. To validate the findings from the gene expression array study, we selected the highly differentially expressed genes (PAX8, CDH1, FOXA2, and ARX) as well as those corresponding proteins and examined their expression levels in tissue samples of ovarian serous tumors, fallopian tube, ovarian surface epithelia, and peritoneal mesothelia.

Results. Dendrograms revealed that OSE samples clustered with HPM, while ovarian serous tumors, including LGSC, SBT and high-grade serous carcinoma (HGSC), clustered with FTE. Furthermore, LGSC showed a significantly closer relationship with FTE than with OSE and HPM samples. PAX8, CDH1, and FOXA2 were highly and specifically expressed in serous tumors and FTE samples but not in OSE samples. In contrast, ARX was mainly expressed in OSE samples but not in FTE and serous tumors.

Conclusions. The findings of the current study provide further evidence at a molecular level that the fallopian tube is likely the cellular source of LGSC. This finding may enable new prevention strategies, improve early detection, and allow novel therapies to be tested.

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1. Introduction

Ovarian cancer is the most lethal gynecological malignancy. In 2016, the estimated number of deaths in America due to the disease was approximately 14,240 [1]. Although many advances have been made in early diagnostic techniques, surgical techniques and adjuvant therapy,

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the overall survival of ovarian cancer patients has remained unchanged for over 50 years. The high fatality rate associated with ovarian cancer is not only due to challenges in early stage diagnosis and development of drug resistance but also attributed to uncertainty regarding cancer origin and pathogenesis. Ovarian epithelial cancer (OEC), which accounts for 80–90% of all ovarian neoplasia, is the most common type. Traditionally, based on epidemiologic studies and pathological observations, many investigators have opined that OEC was one disease process. With advanced molecular and biological techniques, we have now come to understand that OEC is a complex and heterogeneous disease. It is composed of diverse subtypes with widely differing clinicopathological features and behavior.

According to histological and morphological differences, OEC is divided into several subtypes: serous, mucinous, endometrioid, clear cell, and others. Of these, serous carcinomas are the most common, representing ~70% of OEC [2]. In 2004, based on a series of morphological and molecular genetic studies, Kurman and his colleagues proposed a dualistic model that categorizes various types of ovarian cancer into two groups, type I and type II [3]. Serous carcinoma, the most common type, is divided into high-grade and low-grade in this model, but these grades have recently been recognized as completely distinct types of gynecological tumor rather than different grades of the same neoplasm, and there are drastic clinicopathological and molecular differences between the two. Traditionally, it was thought that high-grade serous carcinoma (HGSC) originated from ovarian surface epithelium (OSE), but no reliable precursors were identified in the ovary. However, in the patients with HGSC, simultaneous occult noninvasive and invasive carcinomas are detected in the fallopian tubes, especially in the fimbriae [4–7]. This finding suggests that the fallopian tube is the likely primary site of most HGSCs. In addition, the precancerous lesions, serous tubal intraepithelial carcinomas (STICs), share identical TP53 mutations with HGSC, indicating a clonal relationship between the two [8,9]. Furthermore, gene expression profiles and patterns of HGSCs are more similar to those of normal fallopian tube epithelium than to normal OSE [10,11]. Finally, establishment of an HGSC model transformed from fallopian tube secretory epithelium provides proof-of-principle evidence that secretory cells could be the cellular origin of HGSC [12,13]. Overall, the majority of HGSCs originate from fallopian tube.

Compared to HGSC, the origin of low-grade serous carcinoma (LGSC) is less clear thus far. The finite number of LGSC cases appears to be the main research obstacle. Recently, we performed morphological and immunophenotypic evaluation of LGSC and its putative precursors to gain some insights into its origin. Because LGSC develops through a stepwise cascade from ovarian epithelial inclusions (OEIs), the origination of OEIs may provide clues to the LGSC origin. Our results showed that most (78%) of the OEIs displayed a tubal phenotype and suggested that most OEIs as well as LGSCs are likely to have originated from tubal epithelia [14]. In addition, another two studies by Kurman and Laury provide additional histological support for the fallopian tubal origin of LGSC [15,16]. However, thus far, there are no molecular studies, including investigations of possible tissue origins of LGSC. Accordingly, gene expression profiles of LGSC, serous borderline tumors (SBT), HGSC and their likely origins (FTE, OSE or human peritoneal mesothelia (HPM)) were analyzed to further explore the origin of LGSC at a molecular level. This analysis could have a significant impact on preventative and therapeutic aspects of LGSC.

2. Material and methods

2.1. Tissue specimens

A total of eighteen flash-frozen specimens of primary epithelial ovarian serous tumors, including 6 HGSCs, 6 LGSCs, and 6 SBTs, were collected at the time of the initial staging operation at Qilu Hospital of Shandong University. The tumor diagnoses were all verified by concurrent pathological review by two gynecological pathologists. Given that the majority of HGSCs have been shown to originate from the fallopian tube, we selected HGSC as the positive control in the current study. Briefly, 5-μm frozen sections were sliced, adhered onto slides, and stained with H&E for review to ensure histology and the proportion of tumor cells (>70%). The remaining samples were immediately stored in liquid nitrogen for subsequent array. In addition, a total of four OSE brushings and four HPM brushings were obtained using a cytobrush from the normal ovaries and peritoneum of donors during surgery for other benign gynecological conditions at Qilu Hospital of Shandong University. To enhance the reliability of our study, HPM was included in the research because OSE is recognized as the continuation of peritoneal mesothelium. At the same time, five FTE tissues were obtained from the distal fimbria of control cases undergoing salpingo-oophorectomy in order to maximize the proportion of epithelial cells. All tubal sections were confirmed by routine microscopy. The stromal component in the fimbria of each tubal sample was <10%. The normal fallopian tube, ovarian surface epithelia, and peritoneal mesothelial were obtained from 9 patients without evidence of cancer. The 9 patients included leiomyomata ($n = 6$), endometrial hyperplasia ($n = 1$), cervical high-grade squamous intraepithelial lesion ($n = 1$), and ovarian corpus luteum cyst ($n = 1$). For quality assurance of the samples, the cytobrush with normal cells and tissues from the fallopian tube were suspended in TRIzol immediately and stored at −80 °C. There was no evidence of endometriosis after review of the pathology of the LGSC cases we studied, and therefore, we did not take endometriosis into consideration as a potential origin. All samples were collected with the approval of the Ethics Committee at Qilu Hospital of Shandong University.

2.2. RNA extraction and RNA-seq analysis

Total RNA from all 31 samples was separately isolated and extracted using TRIzol reagent (Invitrogen Corporation, USA) according to the manufacturer's protocol. Before RNA-seq, the quality of all RNA samples was determined by agarose gel electrophoresis and an Agilent 2100 bioanalyzer. Then, mRNA enrichment and fragmentation, reverse transcription, cDNA synthesis, end repair, addition of A and adaptor, and PCR enrichment were performed by Novogene Co. Ltd. Following RNA-seq using the Illumina HiSeq platform, TopHat2 was used to align paired-end clean reads to the reference genome [17]. One of the HGSC samples was excluded because of a low proportion of total reads mapped (<80%). Then, HTSeq v0.6.1 was applied to count the read numbers mapped for each gene. Next, RPKM (Reads Per Kilobase of exon model per Million mapped reads) of each gene was calculated based on the length of the gene and reads count mapped to this gene. RPKM takes the influence of sequencing depth and gene length into consideration and is currently the most common method for estimating gene expression levels [18].

2.3. Clustering analysis and Pearson correlation analysis

Differential expression analysis of 6 groups was performed using Kruskal-Wallis contrasts in Statistical Package Social Sciences software 19.0 (SPSS Inc., USA). Unsupervised hierarchical clustering of differentially expressed genes among six groups was performed with the function of heatmap.2 in R (version 3.3.2) on all 30 qualified transcript samples (5 HGSC, 6 LGSC, 6 SBT, 5 FTE, 4 OSE, and 4 HPM) to verify the quality of samples and determine their overall similarity. To allow for log adjustment, genes with 0 RPKM were assigned a value of 0.01. To test the correlation of each ovarian tumor and normal control, we performed differential expression analysis among three normal groups through Kruskal-Wallis contrasts. Based on the differentially expressed genes, unsupervised hierarchical clustering was also conducted as above among six sample groups. To ascertain the origin of LGSC more accurately, we performed another unsupervised hierarchical cluster analysis only in LGSC samples and the three control groups. Then, to further identify the origin of LGSC, we performed rank-sum analysis and

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