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Review Article

The role of MicroRNA molecules and MicroRNA-regulating machinery in the pathogenesis and progression of epithelial ovarian cancer

Xiyin Wang [a], Mircea Ivan [b,c], Shannon M. Hawkins [a,d,*]

[a] Department of Obstetrics and Gynecology, Indiana University School of Medicine, Indianapolis, IN, United States
[b] Department of Medicine, Indiana University School of Medicine, Indianapolis, IN, United States
[c] Department of Microbiology and Immunology, Indiana University School of Medicine, Indianapolis, IN, United States
[d] Department of Biochemistry and Molecular Biology, Indiana University School of Medicine, Indianapolis, IN, United States

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ABSTRACT

MicroRNA molecules are small, single-stranded RNA molecules that function to regulate networks of genes. They play important roles in normal female reproductive tract biology, as well as in the pathogenesis and progression of epithelial ovarian cancer. DROSHA, DICER, and Argonaute proteins are components of the microRNA-regulatory machinery and mediate microRNA production and function. This review discusses aberrant expression of microRNA molecules and microRNA-regulating machinery associated with clinical features of epithelial ovarian cancer. Understanding the regulation of microRNA molecule production and function may facilitate the development of novel diagnostic and therapeutic strategies to improve the prognosis of women with epithelial ovarian cancer. Additionally, understanding microRNA molecules and microRNA-regulatory machinery associations with clinical features may influence prevention and early detection efforts.

Contents

1. Introduction

Ovarian cancer is the most lethal gynecologic malignancy and will claim more than 14,000 lives in 2017 in the United States [1]. Epithelial

* Corresponding author at: Indiana University School of Medicine, Department of Obstetrics and Gynecology, 550 N. University Blvd, UH 2440, Indianapolis, IN 46202, United States.
E-mail address: shhawkin@iu.edu (S.M. Hawkins).

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ovarian cancer (EOC) is the most prevalent type of ovarian cancer, accounting for 90% of all ovarian cancers. It is characterized by distinct histological phenotypes including serous, endometrioid, clear-cell, and mucinous. Each histotype is thought to arise from distinct precursor lesions of the female reproductive tract [2]. Molecularly, the landscape of each individual EOC histotype is distinct at the gene expression and genomic DNA level, allowing novel means to classify tumors beyond traditional histology [3,4].

Early stage EOC has a 5-year survival of 92%, while late stage disease has a 5-year survival of only 29%. Unfortunately, 79% of women with EOC have late stage disease, defined as regional or distant metastasis, based on SEER data from 2006 to 2012 [1]. Improved screening approaches to detect early stage disease and novel histotype- or molecular-marker specific therapies for the treatment of late stage disease are urgently needed. This review highlights clinical associations of microRNA molecules and microRNA machinery, including DROSHA, DICER, and Argonaute proteins, in EOC identified since our last review [5]. The clinical relevance of these potential new biomarkers as prognostic, diagnostic, and therapeutic molecules are discussed.

2. Genesis of mature microRNA molecules

2.1. MicroRNA-regulating machinery

RNA polymerase II transcribes microRNA molecules from genomic DNA into a primary microRNA molecule (pri-miRNA). Pri-miRNA molecules are typically greater than 200 nucleotides in length with a characteristic stem loop structure. Furthermore, microRNA clusters containing multiple stem loop structures, each coding for a mature microRNA molecule, can be in the kilobase size range. Pri-miRNA molecules are recognized by DROSHA, an RNAse III, which cuts the double-stranded RNA into ~70-nucleotide precursor microRNA (pre-miRNA) in the nucleus. Pre-miRNA molecules are exported to the cytoplasm and are processed by DICER, an RNAse III, into two unique single-stranded mature microRNA molecules representing each side of the stem loop structure. Mature microRNA molecules are loaded onto the Argonaute-containing RNA-induced silencing complex (RISC). Within this structure, mature microRNA molecules function to repress gene expression by complementary binding of the 3′ untranslated region (UTR) of the target gene to the miRNA seed sequence, nucleotides 2–8 of the mature miRNA molecule, leading to transcript degradation, and subsequent gene product loss [6]. Studies have shown that microRNA-target genes play an important role in EOC cancer biology [7]. Thus, microRNA molecules and their biogenesis regulation as mediated by microRNA machinery is clinically important.

2.2. Primer on microRNA nomenclature

Understanding how microRNA molecules are named is important for understanding how closely related microRNA molecules are to each other in the context of clinical associations and molecular functions. MicroRNA molecules are sequentially named as they are discovered. For example, miR-21 was discovered and annotated in miRBase prior to miR-1307. Identical mature microRNA molecules of identical sequence may originate from different genomic loci with different primary microRNA molecule sequence due to the RNA processing. For example, miR-196a-1 and miR-196a-2 have identical mature microRNA sequence but originate from different genomic locations (*i.e.*, chromosome 17 *versus* chromosome 12). This is different from microRNA molecules that have a closely related mature microRNA sequence such as miR-10a and miR-10b, which have a different mature sequence and are derived from different genomic locations. MiR-10a-5p and miR-10b-5p share the same seed sequence but different in one nucleotide in the mature sequence [8].

DICER processes each precursor microRNA into two mature molecules, with reverse complement sequence. Traditionally, the microRNA molecule with the greatest abundance was assigned the microRNA name (*i.e.*, miR-29c), while the mature microRNA molecule on the other arm was called the * form (*i.e.*, miR-29c*) [8]. This nomenclature, based on abundance, was phased out, and a new nomenclature, based on the location of the mature microRNA on the 5′ or 3′ strand, was phased in. Thus, the miRNA-3p forms are not necessarily less abundant or less functional. The nomenclature is now based on sequence location on the stem look. Mature microRNA molecules are grouped into families based on identical seed sequence, which are nucleotides 2–8 of the mature molecule. This sequence serves to function in complementary binding to the 3'UTR, leading to downstream effects of transcript repression [8]. MiRNA-5p and miRNA-3p molecules do not typically fall within the same microRNA family as they have a reverse complementary sequence. For example, the miR-10-5p family is comprised of miR-10a-5p and miR-10b-5p while miR-10a-3p and miR-10b-3p are each members of their own family. Finally, isomiRs are mature microRNA molecules that differ from the mature sequence by 1–2 nucleotides. For example, the miR-21 + CA isomiR is formed from a unique tailing and trimming mechanism in proliferative diseases such as endometriosis and endometrial cancer. It contains the miR-21 mature sequence plus an additional two nucleotides [9]. While isomiRs have not yet been described in EOC, these unique molecules represent an opportunity to be utilized as biomarkers.

3. Clinical implications

3.1. Aberrant expression of microRNA-biogenesis machinery components in EOC

Multiple studies have examined the relative expression of DICER and DROSHA in EOC compared to control tissues. Choice of control tissue for comparison is critical in relative expression studies. Each histotype of ovarian cancer may arise from distinct precursor cells [2]. For example, high-grade serous EOC may arise from the fallopian tube or ovarian surface epithelium [10–12]. Endometrioid and clear-cell EOC may arise from a benign transformation of endometriosis [13,14]. For these reasons, we will define histotype and control tissues used for each study.

Using 50 cases of high-grade serous ovarian cancer, Flavin et al. [15] revealed a significant upregulation of *DROSHA* and a trend towards upregulation in *DICER* by QPCR compared to normal ovary. Similarly, by immunohistochemistry in 37 samples, DICER showed a significant upregulation in high-grade serous ovarian cancer. High DICER expression was associated with an absence of lymph node metastasis and a low proliferation index. DICER expression did not correlate with disease free or overall survival [15]. The authors did not comment on DROSHA expression by immunohistochemistry, nor did they comment on the association of DROSHA with clinical factors. Additionally, only high-grade serous EOC tumor samples were examined.

Merritt et al. [16] examined *DICER* and *DROSHA* in 111 samples of EOC (2 endometrioid, 109 serous, 93 high grade, 18 low grade) by QPCR with validation by immunohistochemistry. Benign ovarian surface epithelium was used as a control. Using the bimodal expression of *DICER* and *DROSHA* in their dataset to classify tumors into low and high expression for *DICER* and *DROSHA*, they examined clinical associations. Low *DICER* expression was associated with advanced-stage disease and reduced median survival. Low *DROSHA* expression was associated with suboptimal cytoreduction and reduced median survival. Death from ovarian cancer was statistically associated with low levels of both *DICER* and *DROSHA* while high levels of both *DICER* and *DROSHA* were associated with increased median survival. Low *DICER* was a predictor of poor prognosis (hazard ratio, 2.10; 95% CI, 1.15 to 3.85) but low *DROSHA* was not. However, low *DICER* and low *DROSHA* was a predictor of death (hazard ratio, 4.00; 95% CI, 1.82 to 9.09). Cells with low *DICER* expression could not process shRNA, an important potential therapeutic consideration [16]. A similar study using semi-quantitative RT PCR showed that *DICER* expression was downregulated in both benign

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