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Original contribution

Down-regulation of miR-145 and miR-143 might be associated with DNA methyltransferase 3B overexpression and worse prognosis in endometrioid carcinomas☆,☆☆

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Summary The aim of this study was to determine the clinicopathologic significance of miR-145 and miR-143 down-regulation in endometrial cancers. The microRNA profiles were analyzed by microRNA microarray. The expression levels of miR-145 and miR-143 in 73 endometrial cancers were further determined by quantitative real-time polymerase chain reaction. Potential targets of miR-145/143 were defined. The status of DNA methyltransferase 3B (DNMT3B), mutL homologs 1, and phosphatase and tensin homolog was assessed using immunohistochemistry. miR-145 and miR-143 frequently co–down-regulated in endometrial cancers, but the expression levels varied greatly between endometrioid carcinomas (ECs) and non-ECs (NECs); they were significantly lower in ECs than in NECs ($P < .05$). *DNMT3B* was defined as a potential target of miR-145/143 by Internet algorithms. In ECs, DNMT3B overexpression occurred more often in the miR-145 and miR-143 down-regulation subgroups, and the correlation between DNMT3B and miR-145 status reached statistical significance ($P = .021$), whereas such phenomena were not present in NECs ($P > .05$). In univariate analysis, the combination of DNMT3B overexpression and miR-145 or miR-143 down-regulation was more powerful in predicting shorter survival ($P < .05$) than use of the biomarkers individually ($P > .05$). In multivariate analysis, such combination was not an independent predictor of disease-free survival ($P > .05$). Our findings suggest that the target and function of miR-145 and miR-143 may differ in ECs versus NECs. *DNMT3B* might be a potential target of miR-145 and miR-143 in ECs. Furthermore, the combined miR-145 or miR-143 and DNMT3B status may have a prognostic impact on ECs.

Abbreviations: ECs, endometrioid carcinomas; NECs, non-endometrioid carcinomas; miR (miRNA), microRNA; qRT-PCR, quantitative real-time polymerase chain reaction; TLDA, TaqMan low-density arrays; DNMT3B, DNA methyltransferase 3B; MMR, DNA mismatch repair; hMLH1, human MutL homologs 1; PTEN, phosphatase and tensin homolog; HER-2, human epidermal growth factor receptor 2; LOH, loss of heterozygosity; IHC, immunohistochemistry; FFPE, formalin-fixed, paraffin-embedded; IRS, immunoreactive score; RQ, relative quantity.

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1. Introduction

Endometrial carcinoma is the most common malignancy of the female genital tract. The most common basis for determining the risk of aggressive disease has been the categorization of endometrial cancer into 2 subtypes. Type I tumors (approximately 80%) are endometrioid carcinomas (ECs) and usually are low grade and follow a favorable course. In contrast, type II (10%-20%) tumors are non-ECs (NECs) with high grade and poor prognosis. However, the prognostic value of this distinction is limited; up to 20% of type I cancers recur, but almost half of type II cancers do not, particularly when the tumors are classified as stage I or II [1,2].

MicroRNAs (miRNAs) are small noncoding RNAs that control approximately 30% of expressed human genes at the posttranscriptional level. Mature miRNAs are typically 19 to 25 nucleotides in length and have been found to bind to the 3′UTR of target messenger RNA (mRNA) and inhibit translation by blocking mRNA transcription or causing mRNA degradation [3,4]. Under normal physiological conditions, individual miRNAs show strict tissue- and development stage–specific expression patterns. In contrast, miRNAs display unique expression profiles, depending on clinical features in several cancers including breast cancer, lung cancer, and chronic lymphocytic leukemia [5-7].

miR-145 and miR-143 have frequently been reported as being down-regulated in various malignancies and considered to act as broad tumor suppressors [8-10]. However, to our knowledge, miR-145 and miR-143 expressions in endometrial cancers have not been extensively studied. Both miR-145 and miR-143 have complementary sites in the 3′UTR and coding region of DNA methyltransferase 3B (*DNMT3B*), which is essential for de novo methylation and believed to be responsible for the aberrant methylation observed in many cancers [11,12]. Studies indicated that silencing of the tumor suppressor genes by a DNA methylation mechanism plays an important role in endometrial carcinogenesis [13-15]. Meanwhile, DNMT3B has been found to up-regulate in various malignancies including endometrial cancer and associates with worse clinicopathologic variables [12,15-17]. Interestingly, when we used the microarray platform to screen for differentially expressed miRNAs in endometrial cancers, we found that miR-145 and miR-143 were significantly down-regulated. Given the above data, we hypothesized that the down-regulation of miR-145 and miR-143 may be associated with DNMT3B up-regulation in endometrial cancer and may possess prognostic use.

2. Materials and methods

2.1. Patients and samples

Formalin-fixed, paraffin-embedded (FFPE) tissue samples of 107 endometrial carcinomas (85 ECs, 22 serous adenocarcinomas) collected between 2001 and 2012 were obtained from the Surgical Pathology files of the First Hospital, Peking University, China. The clinicopathologic features of all of the patients are shown in Table 1. Seventy-three samples (58 ECs, 15 NECs) with sufficient viable tissue available for RNA extraction were selected for the detection of miRNA expression. Ten unmatched proliferative endometrial samples served as normal controls. In addition, inclusion criteria for all cases included the absence of any treatment before surgery. The endometrial carcinoma cases were reviewed and classified using the 2003 World Health Organization criteria [18]. Tumors were staged according to the 2009 International Federation of Gynecology and Obstetrics guidelines [19]. In the 73 cases selected for miRNA expression analysis, follow-up information was obtained for 60 patients (49 ECs, 11 NECs). Fifty-four (90.0%) patients were alive without clinical evidence of tumor at a median interval of 23.5 months (range, 4-111 months). The study was approved by the institutional ethics committee.

2.2. RNA extraction and miRNA microarray

The hematoxylin and eosin slides were checked by a pathologist to identify the tumor region. Total RNA samples were then extracted from the corresponding FFPE tissues

Table 1 Clinicopathologic features of 107 endometrial carcinomas

Variable	No. of cases
Age (median, 54 years; range, 37-76 y)	
Premenopause	44 (41.1%)
Postmenopause	63 (58.9%)
Histologic type	
ECs	85 (79.4%)
NECs	22 (20.6%)
Grade (ECs)	
1	30 (35.3%)
2	39 (45.9%)
3	16 (18.8%)
Myometrial invasion [a]	
<1/2	77 (73.3%)
≥1/2	28 (26.7%)
Lymph node metastases [b]	
No	42 (87.5%)
Yes	6 (12.5%)
Vessel invasion [a]	
No	87 (82.9%)
Yes	18 (17.1%)
Stage [a]	
I	74 (70.5%)
II	17 (16.2%)
III	13 (12.4%)
IV	1 (0.9%)

[a] One hundred five patients received hysterectomy.
[b] Forty-eight patients received lymphadenectomy.