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Role of MicroRNAs in carcinogenesis that potential for biomarker of endometrial cancer

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HIGHLIGHTS

- Three miRNA may controlled genes that regulate early development of endometrial cancer.
- PTEN is central gene of endometrial cancer that targeted by the miRNA.
- The miRNA and PTEN are potential for a biomarker of early detection of endometrial cancer.

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ABSTRACT

The non-invasive diagnostic tool for early detection of endometrial cancer still limited. The etiology of this disease is believed to be associated with disharmony hormone production. One predominant factor that regulate hormone production is microRNA (miRNAs). Some studies reported that miRNAs play a significant role in the process carcinogenesis. We have identified 12 of miRNAs that potentially have a role in controlling endometrial carcinogenesis pathways. Further analysis suggested that these miRNA targeted genes that regulate the early development of endometrial cancer. These genes cluster into several functional groups involving a process of angiogenesis, apoptosis, cell cycle, cell proliferation and p53 pathways. Some of the genes are PTEN, GSK3b, and TP53, which are a tumor suppressor that control the process of growth arrest, DNA Repair, and Apoptosis. Upregulation of the miRNA may obstruct the cell ability to control the cell cycle. This study was found three miRNA that plays a role in the development of endometrial cancer. The hsa-miR-495 and hsa-miR-152 were repressed in endometrial cancer compared to normal tissue. The microRNA regulate genes that control proliferation and cell survival. Moreover, hsa-miR-181d was upregulated to control expression a tumor suppressor gene, PTEN to protect the cancer cell from apoptosis. Further investigation to validate the function of the miRNA is a warrant for developing biomarkers of endometrial carcinoma.

1. Introduction

Endometrial cancer (EC) cases are still highest malignancy in the female genital tract[1,2]. Epidemiologic studies indicated that risk factor of EC is obesity [3], diabetes [4], excess estrogen and hereditary syndromes [5], and metalloestrogens such as Cd, Pb, Cr and Ni [6]. Only a few people understand that obesity [7,8] and diabetes or hyperglycemia adequate stimulate endometrial cancer [4]. Diagnostic of the malignancy was developed based on the metastasis cell in the lymph node using tomography. However, the method to detect invasion into lymph note using tomography still had a limitation [2]. Further study to unfold new non-invasive method for diagnosing endometriosis based on biomaterial or microRNA from peripheral blood is necessary to do [9]. Moreover, the microRNAs (miRNAs) allegedly involved in carcinogenesis [10], and the expression profile in Endometrial cancer and the healthy people are significantly different [11]. So it is a possible develop biomarker for endometrial cancer based on miRNA profile.

MicroRNAs (miRNAs) are non-coding RNA, ~22 nucleotides in length, and serves to regulate gene expression by inhibiting the translation process, or initiate the process of mRNA degradation. The miRNAs work as endogenous epigenetic regulators of gene expression [12] which plays a role in many diseases [13], including endometrial cancer [14]. The previous report suggested that several microRNA, i.e., hsa-mir-337-3p [14] let-7b, 7d, 7f, and miR-135a

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may have the potential for developing noninvasive biomarkers for endometriosis [15]. The miRNAs let-7 also known involved in the process epithelial-to-mesenchymal transition (EMT) and carcinogenesis [10]. The profile of miRNA expression in endometrial cancer (grade 1) has different compared to controls [11], that warrant for early detection.

Upregulation (miR-221-5p, miR-31-3p, miR-221-3p) and downregulation miRNA (miR-205, miR-200b, miR-200C, miR-141, miR-101, miR-342-3p, let-7g, miR-26b) affected a variety of cell signaling mechanism associated carcinogenesis [16]. The deeper study showed that the pattern of miRNA expression has a change since from early stages of carcinogenesis [11]. The miRNA expression patterns strongly influence the occurrence of endometrial carcinogenesis. This information is crucial to identify biomarkers for developing diagnostic, prognostic and novel targets for a cancer drug. Besides, miRNAs are found in serum, plasma, and saliva [12] which is very easy to detect. Recently, many pharmaceutical companies are investigating the potential of miRNA for diagnostic tools and therapies [12]. This study aims to identify potential miRNA that plays a role in endometrial carcinogenesis process.

2. Materials and methods

2.1. Identification for miRNA that involved in endometrial carcinogenesis pathway

A total 296 of miRNA that has been validated by Griffiths-Jones lab at the Faculty of Life Sciences, University of Manchester, were collected from the miRNA database (miRBase) [17]. The role of the miRNA on the molecular mechanism of endometrial carcinogenesis was analyzed using mirPath, DIANA-microT-CDS, and combined with a meta-analysis based on a database in the Kyoto Encyclopedia of Genes and Genomes (KEGG) [18]. Among 296 miRNA were successfully collected, only 37 miRNA have been analyzed the role in endometrial carcinogenesis using PantherDb.

2.2. Network analysis of miRNA-target genes

Interaction among the miRNA-target genes was analyzed using STRINGdb version 9.1 based on PubMed database, Genomic Context, High-throughput Experiments and Coexpression [19]. The network then filtered based on the molecular pathways of KEGG database. This analysis is intended to examine the function of genes on the molecular mechanism of endometrial cancer.

2.3. Functional analysis of miRNA-target genes on endometrial carcinogénesis pathways

MirPath analyzed the function of miRNA-target genes in the pathway Endometrial Carcinogenesis and verified by KEGG pathways [20]. KEGG pathway is derived from Kanehisa Lab, Kyoto University, Japan [21]. This analysis is used to determine the position and role of these genes to control endometrial carcinogenesis pathway. The position and function of genes in a pathway are crucial to know the mechanism of intervention or regulation of miRNA in endometrial carcinogenesis.

2.4. Microarray microRNA analysis

The expression level of twelve miRNA that has been predicted involve in endometrium cancer were analyzed from microRNA microarray (Array Express database; EBI). Micro-RNA expression was collected from 5 samples of endometrium tissue stage I (GSM875024, GSM875027, GSM875029, GSM875035, and GSM875037) and three samples of the normal endometrium (GSM875033, GSM875034, and GSM875037). The expression level of miRNA was compared between endometrium cancer tissue and normal endometrium tissue.

3. Result and discussion

We have employed MirPath to analyze involvement microRNA in the various cellular pathways. The results showed that the 12 miRNA might involve in endometrial cancer, prostate cancer, glioma, and leukemia pathway. However the highest role of the miRNA involved in endometrial cancer pathway (Fig. 1). The data suggested that the miRNA has a crucial role in the process of formation of endometrial carcinogenesis. This data correspond with previous reports that microRNAs can trigger expression gene imbalance that causes various diseases [14]. The levels of circulating let-7b and miR-135A were statistically Significantly decreased in women with endometriosis compared with controls [15]. The miR-513A-5p allegedly regulates progesterone receptors (PRs), which is associated with the incidence of breast cancer [22]. Wherefore the human endometrium is responsive to sex steroid hormone [23] and regulated by miRNA [13] that explained the use of oral contraceptives confers long-term protection against endometrial cancer [24].

The twelve miRNA regulate 27 genes (Table 1), further gene function analysis by Panther DB showed the genes are involving

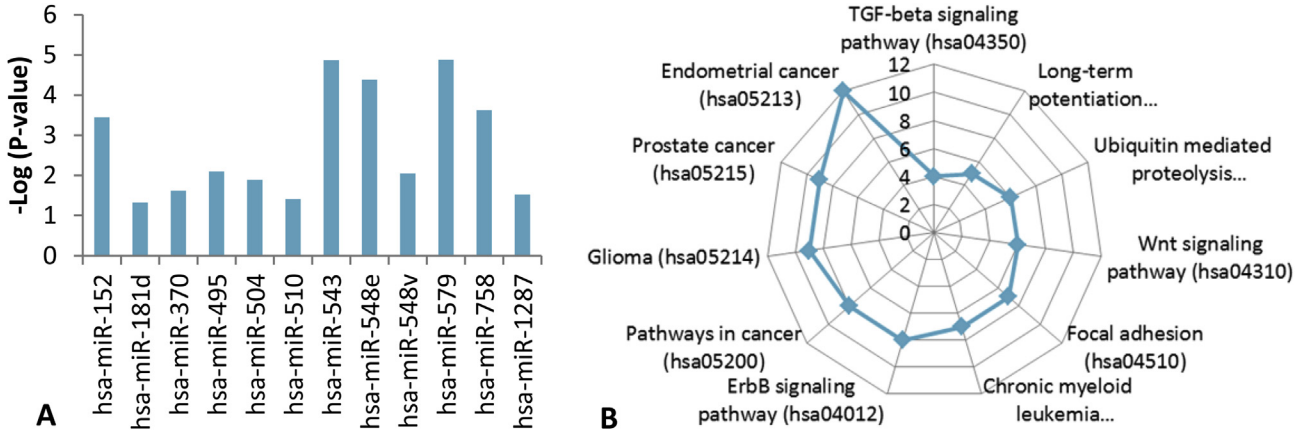

Fig. 1. The twelve miRNA might regulate Endometrial Cancer pathway (**A**). The miRNA also controlled several pathway including prostate cancer, glioma, and leukemia. The miRNA have the highest correlation with Endometrial Cancer pathway (**B**).

Table 1
The target genes of miRNA.

miRNA	P-Value	Gene target											
hsa-miR-152	0.000	SOS2	NRAS	APC	PIK3R3	SOS1	PTEN						
hsa-miR-181d	0.047	PIK3R3	SOS1	PTEN									
hsa-miR-370	0.024	APC	RAF1	PIK3CA									
hsa-miR-495	0.008	GSK3B	NRAS	TCF4	PIK3CB	PIK3R3	CCND1	AXIN2	PIK3R1	SOS1	PIK3CA	FOXO3	PTEN
hsa-miR-504	0.013	TP53	AKT3										
hsa-miR-510	0.039	NRAS	TCF4	EGFR	CDH1								
hsa-miR-543	0.000	NRAS	TCF4	PIK3CB	KRAS	CTNNB1	PDK1	PIK3CA	FOXO3	PTEN	MAPK1		
hsa-miR-548e	0.000	GSK3B	SOS2	TCF4	PIK3CB	KRAS	PIK3R3	CCND1	PIK3R1	SOS1	AKT3	PIK3CA	PTEN
hsa-miR-548v	0.009	TCF4	AKT3	PTEN									
hsa-miR-579	0.000	GSK3B	NRAS	TCF4	APC	PIK3CB	EGFR	CTNNA1	PDK1	PIK3R1	PIK3CA	FOXO3	PTEN
hsa-miR-758	0.000	TCF7L1	PIK3R1	PIK3CA	MAP2K1	MAPK1	ILK						
hsa-miR-1287	0.030	APC	PIK3CB	EGFR									

several pathways such as the process of apoptosis, angiogenesis, cell proliferation and cell survival (Fig. 2). This data suggests the genes have an important role in endometrial carcinogenesis. The miRNA regulate genes that involved in a central role in cell growth, apoptosis, and angiogenesis. These results corroborated previous studies that some cancer-associated fibroblasts (CAFS) promote tumorigenesis was regulated by miRNA, which controlled cell differentiation, migration, proliferation [16]. On the other hand, hsa-miR-337-3p has downregulated in endometrial cancers in white ethnic [14].

The position and function of target genes in the endometrial carcinogenesis pathway were mapped based on KEGG pathway database. The data indicated that these genes have a role in the early development of endometrial cancer, i.e., atypical endometrial hyperplasia and endometrial adenocarcinoma (in the low grade). These genes clustered into three functional groups involving a process of angiogenesis, apoptosis, cell cycle, cell proliferation and p53 pathways. The group 1 consisted EGFR, PTEN, PIK3CA, ILK, AKT3 and FOXO3 that Regulate Cell Survival. Group 2 included SOS1, KRAS, RAF1, MAP2K3, GSK3b, AXIN2, LEF1 and CCND1 that control Cell Growth and Proliferation. The last group is a TP53 gene that controls the process of growth arrest, DNA repair and apoptosis (Fig. 3).

Then we analyzed the significant role of the twelve miRNA in endometrial carcinogenesis pathway. The results indicated that five miRNA (hsa-miR-579, hsa-miR548e, hsa-miR-543, hsa-mir-152, and hsa-miR-459) were dominant and targeted ten or more genes with the smallest p-value among others (Fig. 4). Further, we investigated the expression level of the five miRNA on microRNA array data from endometrium cancer and normal tissue. The result showed that among the five miRNA only three microRNA that has different expression between cancer and normal tissue. The hsa-miR-495 and hsa-miR-152 were repressed, but hsa-miR-181d was upregulated in Endometrium cancer compared to normal tissue. It can be estimated that the miRNA is a crucial factor in the carcinogenesis of the endometrium.

The repression of hsa-miR-495 and hsa-miR-152 will upregulated several genes such as APC, PIK3CB, PIK3R3, CCND1, AXIN2, PIK3R1, SOS1, PIK3CA, and FOXO3. The genes regulate cell proliferation and survival that lead to cancer. However, the upregulation of hsa-miR-181d will downregulate expression of PTEN gene. PTEN is a tumor suppressor gene [25], which is a central role in the process of endometrial carcinogenesis. Therefore, the miRNA that regulates PTEN are very potential for developing a biomarker of early detection of endometrial cancer [11]. The finding is a warrant for further investigation to develop non-invasive detection for the endometriosis.

4. Conclusion

This study found five miRNA predominant controlled genes that regulate early development of endometrial cancer. The three

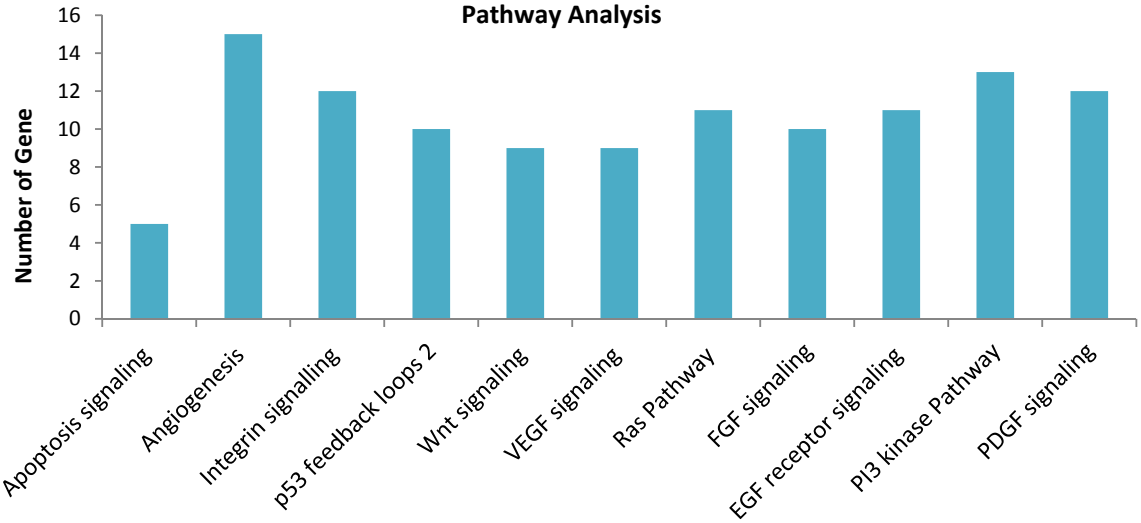

Fig. 2. Functional analysis of target genes. The twelve miRNA targeted over than a dozen genes (Tabel, the uper panel) that involved several pathways including angiogenesis, apoptosis, cell cycle, cell proliferation and p53 (Histogram, bottom panel).

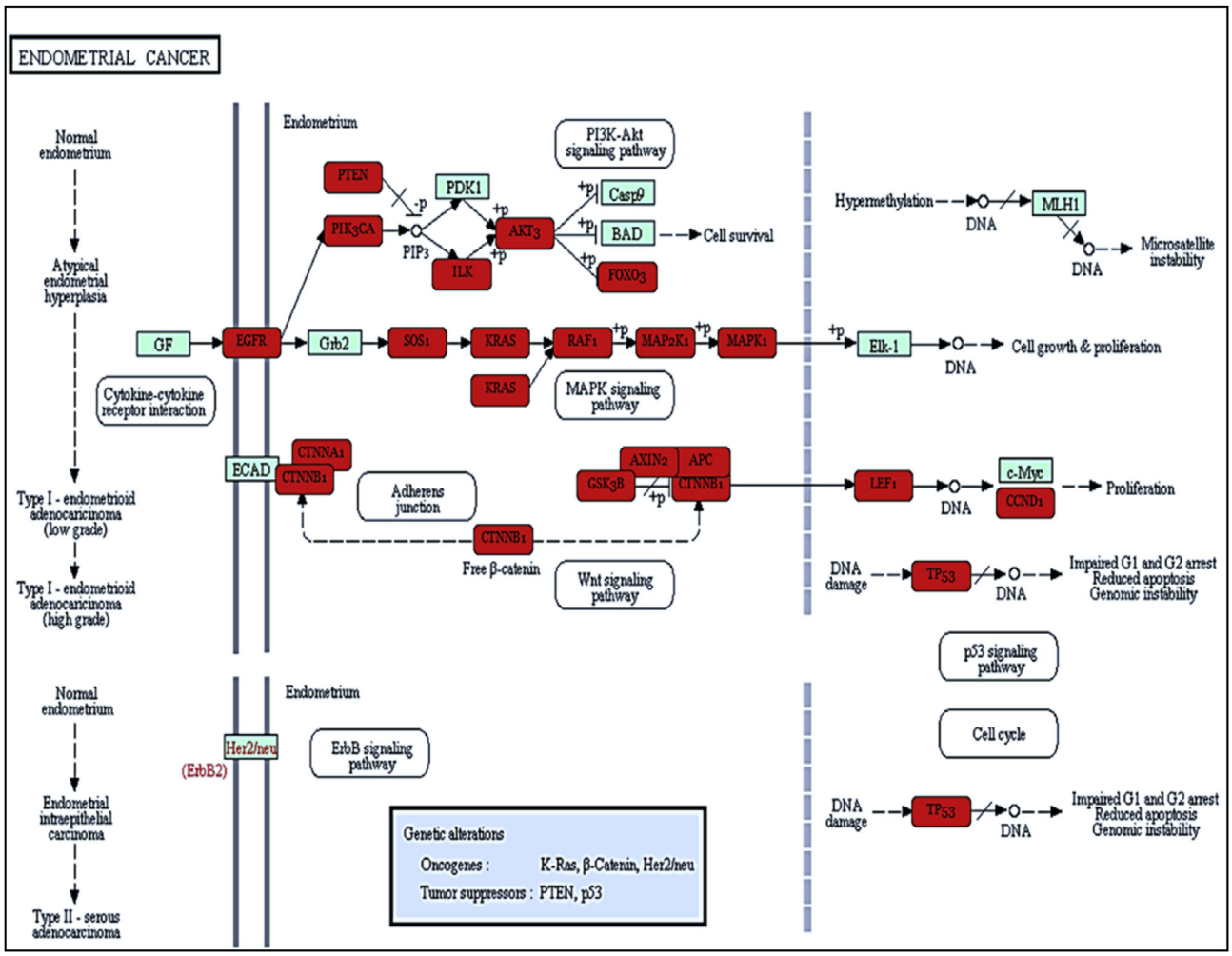

Fig. 3. The position of miRNA targeted genes (red box) in endometrial carcinogenesis pathway (KEGG). The genes controlled in the early development of endometrial cancer by regulating Cell Survival, Cell Growth and Proliferation, Process of Growth Arrest, DNA repair, and apoptosis.

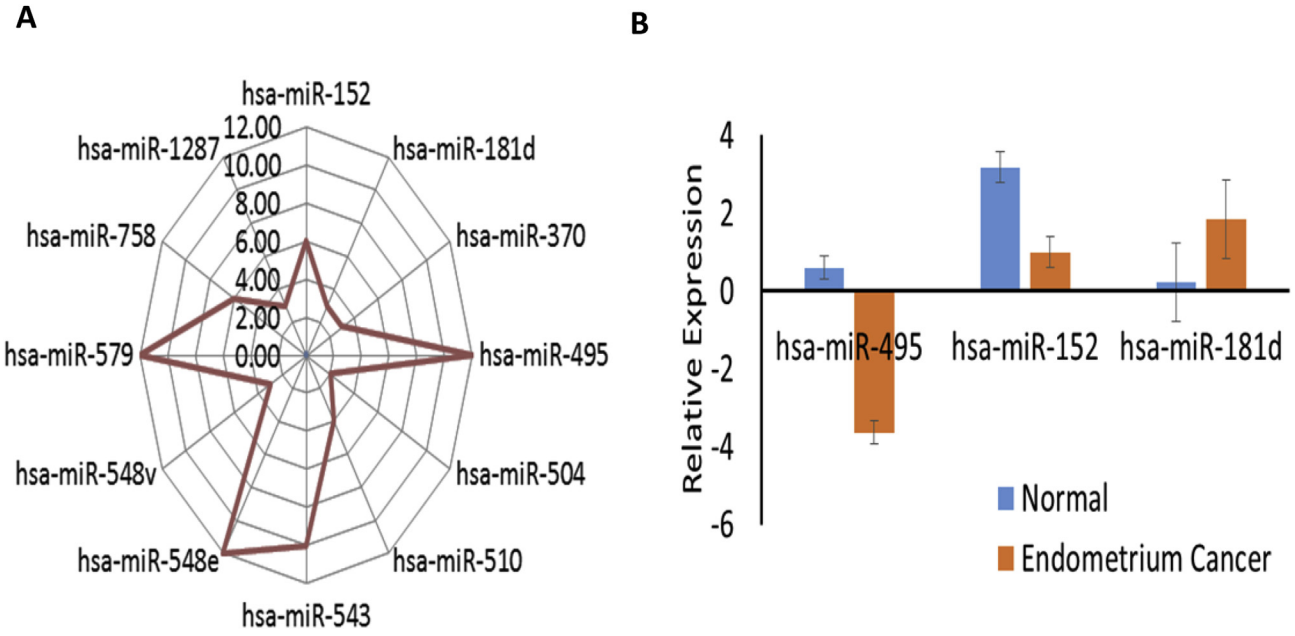

Fig. 4. The expression level of miRNA in Normal and Endometrium Cancer. The five miRNA (hsa-miR-579-HSA, hsa-miR-548e, hsa-miR-543, hsa-miR152, and miR-459-HSA) dominated endometrial cancer pathways (**A**), but only three of them that has significant change the expression level of endometrium cancer compare to normal tissue (**B**). The two miRNA (hsa-miR-495 and hsa-miR-152) were repressed. Hence, hsa-miR-181d was upregulated in Endometrium cancer.

microRNA; hsa-miR-495, hsa-miR-152, and hsa-miR-181d have changed expression level in endometrium cancer compare to normal tissue that may play a pivotal role in controlling endometrial carcinogenesis. Further investigation to elucidate the function of the miRNA is a warrant for developing a biomarker of early detection of endometrial cancer.

Conflict of interest

Authors declare to have no conflict of interest in the research of this article.

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Ethical approval

This research does not need any ethical clearance.

Author contribution

Widodo conduct the research and finishing manuscript.
MS Djati designed the study.
MR write draft of manuscript.

Guarantor

Dr. Widodo.

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