Pelvic high-grade serous carcinoma in BRCA1 and BRCA2 mutation carriers

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Chapter 7

MICRORNA EXPRESSION IN PELVIC HIGH-GRADE SEROUS CANCER ETIOPATHOGENESIS: A SYSTEMATIC REVIEW OF THE LITERATURE

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ABSTRACT

BACKGROUND

Pelvic high-grade serous cancer (PHGSC) is the most frequently diagnosed and most lethal subtype of female genital cancer. The knowledge concerning etiology and early development of PHGSC is still incomplete, although there is accumulating evidence that PHGSC originates from fallopian tube epithelium. MicroRNAs (miRNAs) regulate complex patterns of gene expression, and may be linked to the repression of tumor suppressor genes or the upregulation of oncogenes involved in PHGSC.

OBJECTIVE

To provide an overview of aberrantly expressed miRNAs in PHGSC compared to benign ovarian or tubal counterparts.

METHODS

We performed a systematic review of the literature and included the published original studies that investigated the expression of miRNAs in PHGSC, by means of a predefined registration form. Study quality was assessed by the following criteria: description of bias, homogeneousity, study group, baseline characteristics, method of miRNA expression and data analysis.

RESULTS

A total of 6 studies met the inclusion criteria and were included, of which 5 studies had used benign control tissue (ovarian or tubal tissue or cell line). The median quality score was 6.2 (range, 5-8). Twenty-nine miRNAs were reported to be differentially expressed in PHGSC by ≥2 studies. Upregulation of miR-182 and miR-200c was reported in three independent studies, and upregulation of miR-17, miR-20b, miR-106a, miR-141, miR-146b, miR-183, miR-200a, miR-203, miR-205 and miR-422a in two independent studies. Downregulation of miR-126, miR-143 and miR-195 was reported in three independent studies, and downregulation of let-7b and c, miR-10b, miR-29b, miR-34c, miR-125b, miR-133a and b, miR-140, miR-145, miR-153, miR-202, miR-485-5p and miR-497 in two independent studies.

CONCLUSIONS

In this review it was found that the upregulated miR-200 family, miR-183 family, and the miR-143/145 cluster in PHGSC might play an essential role as tumor suppressor miRNAs, weather the downregulated let-7 family and miR-34 may be linked to enhanced levels of oncogenes.

INTRODUCTION

In the western world, epithelial ovarian cancer is the leading cause of death among patients with gynecological malignancies.[1] Ovarian cancer is a very heterogeneous disease, and develops from epithelium to four distinct histological types, i.e.: serous, endometrioid, mucinous and clear cell. Additionally, the extent of differentiation of the tumor can vary from low- to high-grade. Low-grade serous ovarian cancers ('type I tumors') are known to develop in a stepwise fashion, often associated with a serous borderline component.[2-4] However, for high-grade serous ovarian cancers ('type II tumors') – currently known as pelvic high-grade serous cancers (PHGSC) – there is accumulating evidence that the majority (if not all) originate from the distal fallopian tube, with serous tubal intraepithelial cancer (STIC) as the putative precursor lesion.[5, 6]

PHGSC accounts for 70% of all epithelial ovarian cancers and a disproportionate number of deaths as patients are more likely to present with advanced stage disease.[7] PHGSC can occur in women with and without a genetic predisposition and *BRCA1/2* mutation carriers are at increased risk to develop PHGSC.[8, 9] Despite enormous efforts that have been made in the field of cancer research, the knowledge concerning etiology and early development of PHGSC is still incomplete.

MiRNAs are short non-coding RNA molecules of 20~25 nucleotides, which play an important role in post-transcriptional gene regulation.[10, 11] By directing of the RNA induced silencing complex (RISC) to the target mRNA transcript, the miRNAs induces either inhibition of translation or degradation of the target transcript.[10, 12] MiRNAs regulate fundamental processes such as cell growth, differentiation, proliferation, and apoptosis, and have been implicated in the initiation and progression of human cancers.[13, 14] The development and progression of tumors can be promoted by quantitative and qualitative (mutational) changes in miRNAs and their targeted binding sites.[10, 11, 15, 16]

Studies of miRNA expression patterns showed a higher miRNA expression in various cancers compared to their normal counterparts.[17-19] The upregulated miRNAs have been linked to the repression of tumor suppressor genes, whereas the downregulated miRNAs are linked to enhanced levels of oncogenes.[20, 21] Recently, the miRNA expression patterns of a number of human epithelial ovarian cancers have been described and several differentially expressed miRNAs have been identified in cancer compared to normal ovarian or tubal tissue, or cell lines derived from ovarian or tubal surface epithelium.[18, 19, 22, 23]

This review provides an overview of the miRNAs that are aberrantly expressed (up- or downregulated) in PHGSC specifically, and when available, describes how these miRNA patterns relate to miRNA expression in normal ovarian or tubal counterparts.

METHODS

SEARCH STRATEGY AND SELECTION CRITERIA

We performed a MEDLINE, PubMed and EMBASE search for studies investigating the expression of miRNAs in PHGSC. The search string included four MESH terms and 12 additional words for which a title search was performed (Table 1). The reference lists of all selected publications and reviews were hand-searched to identify missing relevant publications.

TABLE 1. Search performed in MEDLINE, Pubmed and EMBASE (2007-2012)

MESH terms:	'microRNA' **AND** 'ovarian neoplasms' **OR** 'pelvic neoplasms' **OR** 'fallopian tube neoplasms'
Additional words used for title search:	microRNAs **OR** miRNA **OR** miRNAs **AND** ovarian carcinoma* **OR** ovarian cancer* **OR** tubal cancer* **OR** tubal carcinoma* **OR** fallopian tube cancer* **OR** fallopian tube carcinoma* **OR** pelvic cancer* **OR** pelvic carcinoma* **AND** serous

Studies were included in the analysis if they met the following criteria: (1) patients included had primary and newly diagnosed pelvic, ovarian or fallopian tube cancer; (2) cancer histology was serous; (3) differentiation was high-grade; (4) genome-wide expression profiling or profiling of specific miRNAs was performed; (5) the study reported a list of the differential expressed miRNAs in the text or in a (supplementary) table. Studies were excluded when PHGSC patients had received chemotherapy before tissue collection, when miRNA expression was investigated in serum, blood or blood products instead of tissue of PHGSC patients, or when only miRNAs associated with prognosis were reported (chemosensitivity, survival, metastases) to provide insight in PHGSC biology. In case a single study was reported on multiple occasions, only the report with the largest patient group or the most complete data was included. Only independent studies were included to prevent data of differentially expressed miRNAs to be published multiple times by the same institute. Studies published in languages other than English or German were excluded from the meta-analysis. Reviews and non-original articles were excluded.

Two researchers (WR and JtB) independently examined abstracts to decide whether full-text articles should be obtained fulfilling the in- and exclusion criteria (Figure 1). Cases of disagreement were resolved by discussing the title and abstract. Full-test articles were examined and excluded if a more detailed examination revealed that they did not meet the in- and exclusion criteria.

FIGURE 1. Flow-chart of study inclusion
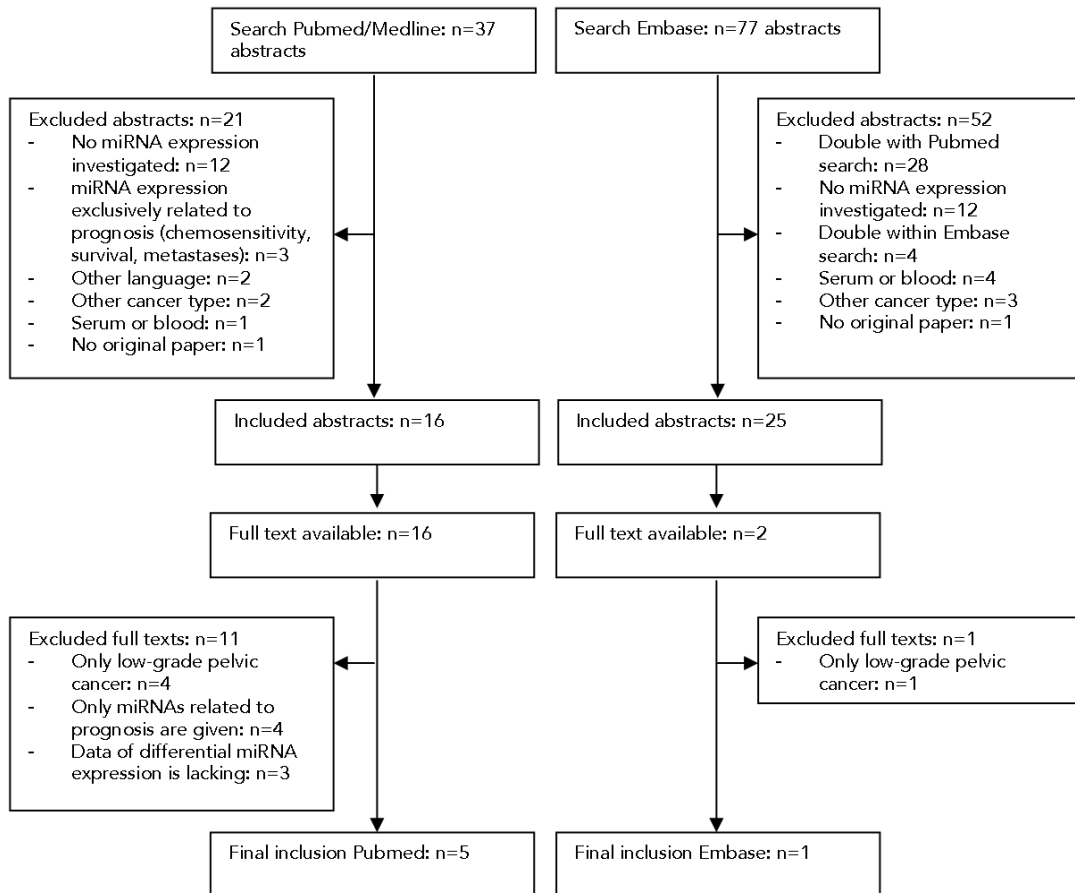
DATA EXTRACTION

Data of the tissue specimens and differential expressed miRNAs in PHGSC and, when available, benign ovarian or tubal tissue or ovarian or tubal surface epithelium cell line, were extracted independently by two investigators (WR and JtB) by means of a predefined registration form (Supplementary Table S1). Topics in this form were: first author, year of publication, journal, years of patient inclusion, method of case selection (retrospective or prospective), location/hospital of specimen collection, number of patients, age at time of diagnosis (mean, median, range), distribution of stage, tissue type of cancer and benign counterpart (pelvic, ovarian, tubal), method of fixation (fresh-frozen or formalin-fixed paraffin-embedded) or use of cell line (number), miRNA profiling method (microarray, deep-sequencing, polymerase chain reaction (PCR)), and number and name of upregulated and downregulated miRNAs in PHGSC.

ASSESSMENT OF STUDY QUALITY

Study quality was assessed independently by two investigators (WR and JtB) by means of a predefined quality form (Supplementary Table S2). As there are no generally accepted standards for measuring study quality, this form was designed following the work of Hayes et al. (1996) and McShane et al. (2005). In summary, the following criteria were investigated: whether (1) an unbiased sample was included (in- and exclusion criteria; prospective data collection); (2) a homogeneous sample was included (exclusively PHGSC); (3) the study population was properly described (control group included; ovarian or tubal tissue); (4) the baseline characteristics were properly described (age; cancer stage; primary cancer); (5) the methods were properly described (method of miRNA expression; whole genome); and (6) the data analysis was properly described (expression levels; multiple testing correction). Quality was measured on a scale from 0 (low quality) to 12 (high quality).

RESULTS

In total, 114 abstracts were reviewed, 41 abstracts were considered to meet the in- and exclusion criteria, and of 18 abstracts the full-text was available and was examined. Ultimately, six studies were included (Table 2 and 3; Flavin et al. 2009 (Mod Pathol); Flavin et al. 2009 (Int J Gyn Cancer); Gallagher et al. 2009; Kim et al. 2010; Lee et al. 2009; Liu et al. 2012). These were all retrospective studies. Five of six studies used a benign control group, of which two of them used normal ovaries and/or normal ovarian surface epithelium (NOSE) as a healthy control, one used a benign serous ovarian tumor, two one used normal fallopian tube samples and/or fallopian tube surface epithelium and/or NOSE. Four studies used formalin-fixed paraffin-embedded tissue (FFPE) and two used fresh-frozen tissue (FFT). The method of profiling was real-time(RT)-PCR in three studies and microarray in three studies. Four studies performed whole genome expression profiling and two studies only investigated the expression of specific miRNAs. The median quality score was 6.2 (range, 5^{24}-8^{18}) (Table 2).

TABLE 2. Studies included in the systematic review

Study	Year of publication	Journal	Inclusion period	Data collection	Specimen collection	Quality rating
[Flavin et al., 2009][24]	2009	Mod Pathol	1991-2006	Retrospective	St James's Hospital, Dublin	5
[Flavin et al., 2009][23]	2009	Int J Gynecol Cancer	1991-2006	Retrospective	St James's Hospital, Dublin	6
[Gallagher et al., 2009][22]	2009	J Ovarian Res	1991-2006	Retrospective	St James's Hospital, Dublin	6
[Kim et al., 2010][25]	2010	Histopathol	<2010	Retrospective	CHA Bundang Medical Center, Korea	6
[Lee et al., 2009][18]	2009	PLoS One	2003-2006	Retrospective	Vancouver General Hospital, Canada	8
[Liu et al., 2012][19]	2012	J Pathol	2007-2010	Retrospective	Northwestern Memorial Hospital, Chicago, USA	6

Abbreviations: PPC, primary peritoneal cancer; HGSOC, high-grade serous ovarian cancer; PHGSC, pelvic high-grade serous cancer; FFPE, formalin-fixed paraffin-embedded tissue; FFT, fresh frozen tissue; RT-PCR, real-time polymerase chain reaction; miR, microRNA; NS, not significant.

TABLE 3. Summary of the microRNAs expression profiling studies in PHGSC (relative to normal counterparts)

Study	Material	Age in years	Stage	Control	Methods	n	miRNAs altered in the study / The most significant expression alteration
[Flavin et al., 2009]	17 high-grade serous primary peritoneal cancer tissue samples versus 17 HGSOC tissue samples; FFPE.	Median, 68 PPC (range, 38-84) Median, 59 HGSOC (range, 40-86)	All; 4 st I/II (12%) 30 st III/IV (88%)	-	Quantitative RT-PCR (panel of 330 human miRNAs). Specific: miR-16, miR-195, miR-497.	3	Upregulated: none. Downregulated: miR-16, miR-195, miR-497.
[Flavin et al., 2009]	50 HGSOC tissue samples versus 22 normal whole ovaries containing NOSE; FFPE.	Unknown	St II-IV; 8 st II (16%) 42 st III/IV (84%)	Ovary (NOSE)	Quantitative RT-PCR. Specific: miR-29b.	1	Upregulated: none. Downregulated: miR-29b.
[Gallagher et al., 2009]	Training set: 6 HGSOC tissue samples versus 1 normal whole ovary; FFT. Cell culture: NTera2 and 2012Ep EC cell line. Validation set: 40 HGSOC tissue samples; FFPE.	Unknown	St II-IV.	Ovary	Quantitative RT-PCR (panel of 330 human miRNAs).	154	Upregulated (n=35): miR-7, miR-10a, miR-18a, miR-20b, miR-31, miR-34b,c, miR-93, miR-95, miR-130b, miR-135b, **miR-141, miR-182, miR-183**, miR-184, **miR-187, miR-200b,c**, miR-203, miR-213, miR-218, miR-223, miR-224, miR-301, miR-338, **miR-373**, miR-422a, **miR-429**, miR-483, miR-489, miR-499, miR-512-3p, miR-518a-2*, miR-519d, miR-522. Downregulated (n=119): miR-1, miR-9*, miR-10b, miR-17-3p, miR-18a*, miR-20a, miR-23b, miR-26a,b, miR-27b, miR-28, miR-29a,b, miR-30b,c, miR-30e-3p,5p, miR-32, miR-33, miR-34a, miR-92, miR-99a, miR-100, miR-101, miR-125a,b, miR-126*, miR-127, miR-130a, miR-132, miR-133a,b, miR-134, miR-136, miR-137, miR-140, miR-143, miR-145, miR-148a, miR-151, miR-152, **miR-153**, miR-154*, miR-186, **miR-188**, miR-189, miR-190, miR-192, miR-193a, **miR-195**, miR-199a, miR-202, **miR-202*, miR-204, miR-216**, miR-212, miR-214, miR-219, miR-222, miR-299-3p,5p, miR-323, miR-328, miR-329, miR-337, miR-339, miR-342, miR-345, miR-361, miR-362, miR-368, miR-369-5p, miR-374, miR-376a,b, miR-377, miR-379, miR-380-3p,5p, miR-381, miR-382, **miR-383**, miR-410, miR-424, miR-432, miR-433, miR-450, miR-451, miR-455, miR-485-5p, miR-486, miR-488, miR-489, miR-493, miR-494, miR-495, **miR-497**, miR-500, miR-501, miR-502, miR-503, miR-505, miR-506, miR-507, **miR-508, miR-509**, miR-517b, miR-520a*,c, miR-527, miR-UL112-1, let-7a,b,c,d,e,f,g,i. * The ten most up- and downregulated miRNAs in the validation set are bold.
[Kim et al., 2010]	Training set: 1 HGSOC tissue samples, 2 serous ovarian cancer cell lines (SKOV3 and OVCAR3) versus 1 benign serous ovarian tumor; FFT. Validation set: 29 HGSOC versus 5 benign serous ovarian tumors; FFT.	Median, 46.8 (range, 15-80)	All; 24 st I/II (44.4%) 30 st III/IV (55.6%)	Benign serous ovarian tumor	Microarray expression profiling with 739 human miRNAs, quantitative RT-PCR.	5	Upregulated: none. Downregulated: miR-153, miR-485-5p.

Study	Samples	Age	Stage	Tissue	Method	N	miRNAs
[Lee et al., 2009]	33 HGSOC tissue samples versus 3 normal fallopian tube samples, FFT.	Mean, 62 (range, 39-85)	All; 7 st I/II (21%) 26 st III/IV (78%)	Fallopian tube	Microarray expression profiling with 668 miRNAs of which 328 known human miRNAs, quantitative RT-PCR. Specific: miR-34c, miR-143, miR-145, miR-29e, miR-29b.	28	Upregulated (n=19): miR-200c, miR-141, miR-106a,b, miR-17-5p, miR-107, miR-20a,b, miR-181b, miR-146b, miR-103, miR-205, miR-182, miR-203, miR15a, miR-422a,b, miR-21, miR-363. Downregulated (n=9): miR-145, miR-143, miR-34c, miR-195, miR-29c, miR-125b, let-7b,c, miR-126.
[Liu et al., 2012]	Training set: 5 HGSOC tissue samples. Validation set: 56 HGSOC tissue samples versus 21 fallopian tube samples; FFPE. Cell lines: NOSE T29 and T80, normal fallopian tube surface epithelial FTE187, ovarian cancer SKOV3, HEY and OVCAR3.	Unknown	Unknown	Fallopian tube, NOSE	Microarray expression profiling with 770 miRNAs, quantitative RT-PCR, miRNA in situ hybridization.	38	Upregulated (n=24): miR-182, miR-146b-5p, miR-345, miR-106a, miR-17, miR-663, miR-149, miR-183, miR-130e, miR-15b, miR-1291, miR-193b, miR-1259, miR-200e,c, miR-210, miR-197, miR-1301, miR-205, miR-714, miR-25, miR-320e,b, miR-425. Downregulated (n=14): miR-133a, miR-139-5p, miR-126, miR-140-3p, miR-10a,e, miR-143, miR-1180, miR-133b, miR-1247, miR-34b, miR-375, miR-34c-3p,5p.

Abbreviations: PHGSC, pelvic high-grade serous cancer; FFPE, formalin-fixed paraffin-embedded tissue; FFT, fresh-frozen tissue; NOSE, normal ovarian surface epithelium.

Twenty-nine miRNAs were consistently reported to be differentially expressed in PHGSC by ≥2 studies (Table 3). Upregulation of miR-182 and miR-200c was consistently reported in three independent studies,[18, 19, 22] and upregulation of miR-17[18, 19], miR-20b[18, 22], miR-106a[18, 19], miR-141[18, 22], miR-146b[18, 19], miR-183[19, 22], miR-200a[19, 22], miR-203[18, 22], miR-205[18, 19] and miR-422a[18, 22] was reported in two independent studies. Downregulation of miR-126[18, 19, 22], miR-143[17, 18, 21] and miR-195[17, 21, 22] was consistently reported in three independent studies, and downregulation of let-7b and c[18, 22], miR-10b[19, 22], miR-29b[22, 23], miR-34c[18, 19], miR-125b[18, 22], miR-133a and b[19, 22], miR-140[19, 22], miR-145[18, 22], miR-153[22, 25], miR-202, miR-485-5p[22, 25] and miR-497[22, 24] in two independent studies. A series of five miRNAs (miR-17, miR-20a, miR-34b and c, miR-130a and miR-345) are found to be upregulated in one study, but are described to be downregulated by another study.[18, 19, 22]

DISCUSSION

Despite enormous efforts that have been made in the field of cancer research, the knowledge concerning etiology and early development of PHGSC is still incomplete. In this systematic review, we present an overview of miRNAs that are aberrantly expressed in PHGSC when compared to benign ovarian or tubal tissue or epithelium and tried to relate this to the role and the function of these miRNAs. Our results show that miR-182 and miR-200c are upregulated in three independent studies, and miR-126, miR-143 and miR-195 are downregulated in three independent studies.

We have developed a quality score which is thought to provide a good estimation of study quality. High study quality was related to a high journal impact factor. In future studies, our quality score might serve as a further step towards the development of evidence-based quality assessment tools for systematic reviews.

To date, over 2000 human miRNAs have been sequenced and are detailed in the Sanger database (miRBase Release 19 – August 2012; http://www.mirbase.org/).[26] Most of the studies investigating the functions of miRNAs in gynecological malignancies focused on epithelial ovarian cancer. Zhang et al.[27] were the first to suggest that miRNAs fulfill an important role in epithelial ovarian cancer (2006) and showed that approximately 40% of the miRNA genes exhibit altered DNA copy numbers. Multiple studies have reported miRNA expression patterns and made a comparison between epithelial ovarian cancer and normal counterparts, focusing specifically on the serous subtype.[28-35] However, the majority of these studies did not make a distinction between low- and high-grade serous tumors, although both have a very different pathogenesis and mutation pattern. In our review of the literature, 29 miRNAs were consistently reported to be aberrantly expressed in PHGSC compared to normal tissue or cell lines which have been repeatedly reported in more than one study (Table 3). For some miRNA families or clusters, to know the miR-200 family, the miR-183 family, the miR-143/145 cluster, the let-7 family, and the miR-34 family, a role in PHGSC carcinogenesis might be expected.

MiR-200a, miR-200c and miR-141 were found to be upregulated in two[19, 22], two[18, 22] and three[18, 19, 22] independent studies, respectively. The miR-200 family contains five members, which are localized on two genomic clusters (miR-200a, miR-200b, miR-429 on chromosome 1; miR-200c, miR-141 on chromosome 12).[36] Members of the miR-200 family are highly enriched in epithelial tissues, and are thought to play an essential role in tumor suppression by inhibiting epithelial-mesenchymal transition (EMT), which is the initiating step of metastasis.[37, 38] Furthermore, it has been demonstrated that miR-141 and miR-200a modulate the oxidative stress response, and thereby regulating tumor growth.[39] Overexpression of all family members has been linked with serous ovarian cancer.[22, 28, 30, 31, 34] If EMT and the oxidative stress response are involved in the pathogenesis of PGHSC remains to be elucidated.

We found miR-182 and miR-183 to be upregulated in three[18, 19, 22] and two[19, 22] independent studies, respectively. The miR-183 family consists of three members (miR-96, miR-182, and miR-183), is located on chromosome 7, and members of this family have been identified as potential oncomiRs in several solitary malignancies,[19, 40-42] including ovarian

cancer.[49] Overexpression of miR-182 has been reported in STICs and early PHGSC, suggesting that miR-182 deregulation confers oncogenic potential in the early tumorigenesis of PGHCS.[19] The oncogenic properties of miR-182 are characterized by its regulation of cell differentiation (*FOXO1*),[43] invasion and metastasis (*FOXO3*, *MITF-M*, *MTSS1* and *HMGA2*),[19, 44] and DNA damage repair (*BRCA1, HMGA2*, and *FOXO3*).[45-47] Liu et al.[19] postulated that miR-182 overexpression is an early molecular event seen in STIC, and that miR-182 overexpression may impair the kinetics of DNA double-strand break repair in epithelial cells of the fallopian tube, likely through deregulation of several DNA damage repair related target genes (i.a. HMGA2 and MTSS1).

MiR-143 was reported upregulated by three independent studies,[17, 18, 21] and miR-145 was reported downregulated by two independent studies.[18, 22] Both miRNAs are from the same miRNA cluster located on chromosome 5. MiR-145 has shown to be downregulated in ovarian cancer or ovarian cancer cell lines.[18, 28, 30, 42, 48] A recent study of ovarian cancer tissues and cell lines showed that miR-145 downregulates HIF-1 and VEGF expression, leading to the inhibition of tumor growth and angiogenesis, and therefore serves as a tumor suppressor miRNA.[48] Others showed that loss of TP53 in ovarian cells can result in downregulation of miR-145 expression, which in turn can result in the activation of factors that promote oncogenesis and cellular pluripotency, leading to the development of ovarian cancer.[49] The protein UTP14c, which is expressed on ovaries, is hypothesized to disrupt protective signals that normally trigger TP53-mediated apoptosis and therefore predisposing to development of PHGSC.[49]

We found let-7b and c to be downregulated in two independent studies.[18, 22] The let-7 family, consisting of 10 mature miRNAs (let-7a-g/I, miR-98 and miR-202), has frequently been described to be downregulated in epithelial ovarian cancer and is thought to play a role in tumor suppression.[27, 28, 30, 31, 50] Furthermore, decrease in let-7 expression has been associated with higher histological grade, resistance to chemotherapy, and shorter progression-free survival.[50, 51, 30] However, the mechanisms leading to let-7 downregulation in cancer are still largely unclear. Recently, a positive correlation between the copy number of let-7b and mature let-7b expression in ovarian cancer was shown by Wang et al.,[52] which might be an important mechanism leading to the downregulation of expression of specific let-7 family members in PHGSC.

MiR-34c was found to be downregulated in two independent studies.[18, 19] The miR-34 family (miR-34b and c, chromosome 11) is frequently downregulated in epithelial ovarian cancer, and is even more reduced in advanced stage cancer.[53] The *TP53* gene is the most commonly mutated gene in epithelial ovarian cancer, and results in a truncated p53 protein. In mouse models it has been demonstrated that miR-34b and c are directly downregulated after p53 inactivation.[54] Also cell-line studies demonstrated that p53 directly transactivates miR-34b and c.[55, 56] Furthermore, miR-34b and c were shown to cooperate in suppressing cell proliferation and adhesion-independent colony formation of neoplastic epithelial ovarian cells.[57] Based on these findings, inactivation of mir-34b and c are proposed as one of the mechanisms by which p53 suppresses critical components of neoplastic growth in ovarian cancer, contributing to carcinogenesis and progression of PHGSC.[57, 58]

Much less is known in about the other miRNAs that were found up- or downregulated in two or more independent studies in this review, and their role in PHGSC carcinogenesis.

MiR-146b-5p (chromosome 10) has been found to be upregulated in epithelial ovarian cancer tissues and cell lines[29], especially in advanced stage ovarian cancers,[59] and in ovarian cancers with serous histology.[34] However, tumor grade was not specified in these studies. Ovarian cancers in *BRCA1* mutation carriers are mostly advanced stage high-grade serous cancers, and it has been shown that miR-146b-5p downregulates BRCA1 expression.[60] These results raise the possibility that miR-146b-5p could be involved in BRCA1 downregulation in sporadic ovarian cancer, but need to be investigated further.

Not much is known about miR-203 (chromosome 14) and miR-205 (chromosome 1), however they were found upregulated in two independent studies.[18, 22] It has been demonstrated that both miRNAs can be detected in exosomes (released by cancer into the peripheral circulation) in sera specimens of women with ovarian cancer and that levels are significantly higher compared to women with benign disease.[61] However, whether there is a relation with PHGSC carcinogenesis remains to be elucidated.

Downregulation of miR-125b was observed in two of the six papers studied in this review[18, 22] and in three other studies that investigated serous ovarian cancer (grade not specified).[18, 28, 30, 31] MiR-125b is transcribed from two loci on chromosome 11 and 21. Expression of miR-125b has been shown to be downregulated in cisplatin-resistant ovarian cancer cell lines, whereas it was upregulated in paclitaxel-resistent cell lines.[62] Furthermore, miR-125 as well as miR-143 have been associated with follicular development in the ovary of the mouse.[63]

MiR-126 (chromosome 9) was reported downregulated by three independent studies.[18, 19, 22] Although Hossain et al.[64] studied bovine ovary and found that miR-126 has a potential role in regulating diverse molecular and physiological pathways underlying the ovarian functionality, the role in PHGSC carcinogenesis remains to be elucidated.

Downregulation of the miR-195 and miR-497 cluster (chromosome 17) was found in three[17, 21, 22] and two[22, 24] studies. No data was found on the association with early PHGSC development. Further investigation is needed to examine the functional role of miR-195 and miR-497 as potential tumor-suppressor genes in primary peritoneal tumor development.

A series of five miRNAs were found to be upregulated in one study but downregulated by another.[18, 19, 22] Lee et al.[18] observed upregulation of miR-17 and miR-20a, whereas downregulation of those particular miRNAs was found by Gallagher et al.[22] Upregulation of miR-17, miR-130a and miR-345 was present in the Liu et al.[19] study, however Gallagher et al.[22] found downregulation of these miRNAs. Gallagher et al.[22] observed upregulation of miR-34b and c, whereas downregulation was found by Lee et al.[18] and Liu et al.[19] miR-34 has shown to be downregulated in advanced stage PHGSC and this might explain the differences in miR-34 expression between studies.

A complicating factor is the choice and availability of the appropriate controls. There is emerging evidence that fallopian tube epithelium is the tissue of origin for many if not all PHGSCs. Therefore it is likely that comparison to tubal epithelium will reveal a more representative and accurate set of deregulated miRNAs for PHGSC. Ovarian tissue containing

epithelium al well as stromal tissue, which are not considered fair control tissues for PHGSC, were used in some of the studies, whereas a number of authors utilized NOSE cell lines. For this systematic review, a stratified analysis, based on the type of control tissue (ovarian or tubal), would give more insight in miRNA patterns involved in PHGSC. However, the number of included studies is too low to perform sub analyses for studies using normal ovarian tissue[22, 23] compared to normal tubal tissue[18, 65] as a control.

Benign ovarian or tubal tissue only contains a minor amount of epithelial cells, together with stromal tissue, if not obtained by laser capture microdissection. Since isolation of epithelium from FFPE material is technically limiting even with laser-capture microdissection, some authors used whole ovarian[22, 23] or tubal[18, 19] tissue samples as a control, including both epithelium and stromal tissue. Nevertheless, based on the current knowledge on the development of PHGSC, it makes more sense to opt for a tubal epithelium, or whole tubal tissue, as a control than an ovarian control. Despite methodological differences, the existence of significant discrepancies in expression patterns of certain miRNAs observed by various authors indicated the need of further and more in-depth research that would elucidate those equivocal results.

Strengths of this systematic review are the clear inclusion of papers assessing miRNA expression in PHGSC and the independent revision of abstracts and full-texts by two investigators. The study is limited by the small number of articles that were included.

Concluding, the results of this systematic review associate deregulation of the several miRNA families or clusters with the repression of tumor suppressor genes or the upregulation of oncogenes involved in PHGSC. There is reason to believe that the miR-200 family, the miR-183 family, and the miR-143/145 cluster, that are frequently upregulated in PHGSC, play an essential role as tumor suppressor miRNAs. The frequently downregulated let-7 family and miR-34 family might to be linked to enhanced levels of oncogenes. The expression of these miRNAs was considerably different compared to its normal counterparts, and it is possible that they are involved in early development of PHGSC, although this has to be investigated in future studies.

SUPPLEMENTARY TABLE S1. Predefined registration form that was used to include papers

Title	
First author	
Journal	
Year of publication	
Country	
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Benign control	☐ No ☐ Yes
Tissue type benign control	☐ Not applicable ☐ FFT ☐ FFPE ☐ Cell-line, namely……………………………………………
Type of cancer	☐ Pelvic ☐ Ovarian ☐ Tubal
Tissue type cancer	☐ FFT ☐ FFPE ☐ Cell-line, namely……………………………………………
Number of patients	☐ Cancer: N = … ☐ Benign: N = … ☐ Other, namely……………………………………………
Year of patient inclusion	……..
Age at time of diagnosis	☐ Mean:……………… ☐ Median:…………….. ☐ Range: ………………
Distribution of stage	☐ Stage I………… N = ……, % = ……… ☐ Stage II………… N = ……, % = ……… ☐ Stage III…………N = ……, % = ……… ☐ Stage IV…………N = ……, % = ………
Method of case selection	☐ Retrospective cohort ☐ Prospective cohort ☐ Different, ……………………………………………
Serous	☐ Yes ☐ No → exclusion
High-grade	☐ Yes ☐ No → exclusion ☐ Unclear, …………………………………………....
Primary tumor	☐ Primary ☐ Recurrent → exclusion ☐ Metastases → exclusion
Chemotherapy prior to surgery	☐ Yes → exclusion ☐ No
miRNA investigation	☐ Expression profiling ☐ Specific miRNAs, namely……………………………
miRNA profiling method	☐ Microarray ☐ Deep-sequencing ☐ PCR
Number and name of upregulated miRNAs in cancer*	N = … Names (i.e. miR-19):……………………………………
Number and name of downregulated miRNAs in cancer*	N = … Names (i.e. miR-19):……………………………………
Number and name of upregulated miRNAs in benign tissue*	N = … Names (i.e. miR-19):……………………………………
Number and name of downregulated miRNAs in benign tissue*	N = … Names (i.e. miR-19):……………………………………

SUPPLEMENTARY TABLE S2. Criteria for quality assessment (predefined quality form)

Criterion:	Points
Is there an unbiased sample included?	**2**
1. Is the population under study defined with in- and exclusion criteria?	1
2. Were tissue samples of patients prospectively collected for the study purpose?	1
Is there a homogeneous sample included?	**1**
3. Are only patients with high-grade serous tumor histology included?	1
Study population:	**2**
4. Was a control group included of benign counterpart tissue?	1
5. If a control group was included, did it concern fallopian tube tissue/epithelium? (instead of ovarian tissue/surface epithelium)	1
Baseline characteristics:	**3**
6. Is the (median/mean) age of the included patients reported?	1
7: Is data on cancer stage reported?	1
8. Is explicitly reported that it is a primary carcinoma (no chemotherapy before surgery)?	1
Methods:	**2**
9. Is the method used for determination of miRNA expression specified? (i.e. microarray, RT-PCR or deep sequencing)	1
10. was a whole genome miRNA expression profile performed? (instead of a few selected miRNAs)	1
Data analysis:	**2**
11. Are the expression levels of the differentially expressed miRNAs presented in the text, a table or as supplementary data?	1
12. In case multiple statistical analyses were performed, has multiple testing correction (i.e. Bonferroni correction) been applied?	1

Max. 12 points

REFERENCES

1. Jemal A, Siegel R, Xu J, Ward E. Cancer statistics, 2010. CA Cancer J Clin. 2010; **60**(5): 277-300.

2. Singer G, Oldt R, 3rd, Cohen Y, Wang BG, Sidransky D, Kurman RJ, et al. Mutations in BRAF and KRAS characterize the development of low-grade ovarian serous carcinoma. J Natl Cancer Inst. 2003; **95**(6): 484-6.

3. Shih Ie M, Kurman RJ. Ovarian tumorigenesis: a proposed model based on morphological and molecular genetic analysis. Am J Pathol. 2004; **164**(5): 1511-8.

4. Singer G, Stohr R, Cope L, Dehari R, Hartmann A, Cao DF, et al. Patterns of p53 mutations separate ovarian serous borderline tumors and low- and high-grade carcinomas and provide support for a new model of ovarian carcinogenesis: a mutational analysis with immunohistochemical correlation. Am J Surg Pathol. 2005; **29**(2): 218-24.

5. Crum CP, Drapkin R, Kindelberger D, Medeiros F, Miron A, Lee Y. Lessons from BRCA: the tubal fimbria emerges as an origin for pelvic serous cancer. Clin Med Res. 2007; **5**(1): 35-44.

6. Kim J, Coffey DM, Creighton CJ, Yu Z, Hawkins SM, Matzuk MM. High-grade serous ovarian cancer arises from fallopian tube in a mouse model. Proc Natl Acad Sci U S A. 2012; **109**(10): 3921-6.

7. Kobel M, Kalloger SE, Huntsman DG, Santos JL, Swenerton KD, Seidman JD, et al. Differences in tumor type in low-stage versus high-stage ovarian carcinomas. Int J Gynecol Pathol. 2010; **29**(3): 203-11.

8. Antoniou A, Pharoah PD, Narod S, Risch HA, Eyfjord JE, Hopper JL, et al. Average risks of breast and ovarian cancer associated with BRCA1 or BRCA2 mutations detected in case Series unselected for family history: a combined analysis of 22 studies. Am J Hum Genet. 2003; **72**(5): 1117-30.

9. Chen S, Parmigiani G. Meta-analysis of BRCA1 and BRCA2 penetrance. J Clin Oncol. 2007; **25**(11): 1329-33.

10. Gregory RI, Shiekhattar R. MicroRNA biogenesis and cancer. Cancer Res. 2005; **65**(9): 3509-12.

11. Zhang W, Dahlberg JE, Tam W. MicroRNAs in tumorigenesis: a primer. Am J Pathol. 2007; **171**(3): 728-38.

12. Valencia-Sanchez MA, Liu J, Hannon GJ, Parker R. Control of translation and mRNA degradation by miRNAs and siRNAs. Genes Dev. 2006; **20**(5): 515-24.

13. Croce CM, Calin GA. miRNAs, cancer, and stem cell division. Cell. 2005; **122**(1): 6-7.

14. Iorio MV, Croce CM. MicroRNAs in cancer: small molecules with a huge impact. J Clin Oncol. 2009; **27**(34): 5848-56.

15. Esquela-Kerscher A, Slack FJ. Oncomirs - microRNAs with a role in cancer. Nat Rev Cancer. 2006; **6**(4): 259-69.

16. Shen J, Ambrosone CB, DiCioccio RA, Odunsi K, Lele SB, Zhao H. A functional polymorphism in the miR-146a gene and age of familial breast/ovarian cancer diagnosis. Carcinogenesis. 2008; **29**(10): 1963-6.

17. Cho WC. OncomiRs: the discovery and progress of microRNAs in cancers. Mol Cancer. 2007; **6**: 60.

18. Lee CH, Subramanian S, Beck AH, Espinosa I, Senz J, Zhu SX, et al. MicroRNA profiling of BRCA1/2 mutation-carrying and non-mutation-carrying high-grade serous carcinomas of ovary. PLoS One. 2009; **4**(10): e7314.

19. Liu Z, Liu J, Segura MF, Shao C, Lee P, Gong Y, et al. MiR-182 overexpression in tumourigenesis of high-grade serous ovarian carcinoma. J Pathol. 2012; **228**(2): 204-15.

20. Sampson VB, Rong NH, Han J, Yang Q, Aris V, Soteropoulos P, et al. MicroRNA let-7a down-regulates MYC and reverts MYC-induced growth in Burkitt lymphoma cells. Cancer Res. 2007; **67**(20): 9762-70.

21. Corney DC, Nikitin AY. MicroRNA and ovarian cancer. Histol Histopathol. 2008; **23**(9): 1161-9.

22. Gallagher MF, Flavin RJ, Elbaruni SA, McInerney JK, Smyth PC, Salley YM, et al. Regulation of microRNA biosynthesis and expression in 2102Ep embryonal carcinoma stem cells is mirrored in ovarian serous adenocarcinoma patients. J Ovarian Res. 2009; **2**: 19.

23. Flavin R, Smyth P, Barrett C, Russell S, Wen H, Wei J, et al. miR-29b expression is associated with disease-free survival in patients with ovarian serous carcinoma. Int J Gynecol Cancer. 2009; **19**(4): 641-7.

24. Flavin RJ, Smyth PC, Laios A, O'Toole SA, Barrett C, Finn SP, et al. Potentially important microRNA cluster on chromosome 17p13.1 in primary peritoneal carcinoma. Mod Pathol. 2009; **22**(2): 197-205.

25. Kim TH, Kim YK, Kwon Y, Heo JH, Kang H, Kim G, et al. Deregulation of miR-519a, 153, and 485-5p and its clinicopathological relevance in ovarian epithelial tumours. Histopathology. 2010; **57**(5): 734-43.

26. Griffiths-Jones S, Saini HK, van Dongen S, Enright AJ. miRBase: tools for microRNA genomics. Nucleic Acids Res. 2008; **36**(Database issue): D154-8.

27. Zhang L, Huang J, Yang N, Greshock J, Megraw MS, Giannakakis A, et al. microRNAs exhibit high frequency genomic alterations in human cancer. Proc Natl Acad Sci U S A. 2006; **103**(24): 9136-41.

28. Iorio MV, Visone R, Di Leva G, Donati V, Petrocca F, Casalini P, et al. MicroRNA signatures in human ovarian cancer. Cancer Res. 2007; **67**(18): 8699-707.

29. Dahiya N, Sherman-Baust CA, Wang TL, Davidson B, Shih Ie M, Zhang Y, et al. MicroRNA expression and identification of putative miRNA targets in ovarian cancer. PLoS One. 2008; **3**(6): e2436.

30. Nam EJ, Yoon H, Kim SW, Kim H, Kim YT, Kim JH, et al. MicroRNA expression profiles in serous ovarian carcinoma. Clin Cancer Res. 2008; **14**(9): 2690-5.

31. Yang H, Kong W, He L, Zhao JJ, O'Donnell JD, Wang J, et al. MicroRNA expression profiling in human ovarian cancer: miR-214 induces cell survival and cisplatin resistance by targeting PTEN. Cancer Res. 2008; **68**(2): 425-33.

32. Zhang L, Volinia S, Bonome T, Calin GA, Greshock J, Yang N, et al. Genomic and epigenetic alterations deregulate microRNA expression in human epithelial ovarian cancer. Proc Natl Acad Sci U S A. 2008; **105**(19): 7004-9.

33. Resnick KE, Alder H, Hagan JP, Richardson DL, Croce CM, Cohn DE. The detection of differentially expressed microRNAs from the serum of ovarian cancer patients using a novel real-time PCR platform. Gynecol Oncol. 2009; **112**(1): 55-9.

34. Wyman SK, Parkin RK, Mitchell PS, Fritz BR, O'Briant K, Godwin AK, et al. Repertoire of microRNAs in epithelial ovarian cancer as determined by next generation sequencing of small RNA cDNA libraries. PLoS One. 2009; **4**(4): e5311.

35. Miles GD, Seiler M, Rodriguez L, Rajagopal G, Bhanot G. Identifying microRNA/mRNA dysregulations in ovarian cancer. BMC Res Notes. 2012; **5**: 164.

36. Korpal M, Kang Y. The emerging role of miR-200 family of microRNAs in epithelial-mesenchymal transition and cancer metastasis. RNA Biol. 2008; **5**(3): 115-9.

37. Lu J, Getz G, Miska EA, Alvarez-Saavedra E, Lamb J, Peck D, et al. MicroRNA expression profiles classify human cancers. Nature. 2005; **435**(7043): 834-8.

38. Bendoraite A, Knouf EC, Garg KS, Parkin RK, Kroh EM, O'Briant KC, et al. Regulation of miR-200 family microRNAs and ZEB transcription factors in ovarian cancer: evidence supporting a mesothelial-to-epithelial transition. Gynecol Oncol. 2010; **116**(1): 117-25.

39. Mateescu B, Batista L, Cardon M, Gruosso T, de Feraudy Y, Mariani O, et al. miR-141 and miR-200a act on ovarian tumorigenesis by controlling oxidative stress response. Nat Med. 2011; **17**(12): 1627-35.

40. Guttilla IK, White BA. Coordinate regulation of FOXO1 by miR-27a, miR-96, and miR-182 in breast cancer cells. J Biol Chem. 2009; **284**(35): 23204-16.

41. Cekaite L, Rantala JK, Bruun J, Guriby M, Agesen TH, Danielsen SA, et al. MiR-9, -31, and -182 deregulation promote proliferation and tumor cell survival in colon cancer. Neoplasia. 2012; **14**(9): 868-79.

42. Vaksman O, Stavnes HT, Kaern J, Trope CG, Davidson B, Reich R. miRNA profiling along tumour progression in ovarian carcinoma. J Cell Mol Med. 2011; **15**(7): 1593-602.

43. Segura MF, Hanniford D, Menendez S, Reavie L, Zou X, Alvarez-Diaz S, et al. Aberrant miR-182 expression promotes melanoma metastasis by repressing FOXO3 and microphthalmia-associated transcription factor. Proc Natl Acad Sci U S A. 2009; **106**(6): 1814-9.

44. Moskwa P, Buffa FM, Pan Y, Panchakshari R, Gottipati P, Muschel RJ, et al. miR-182-mediated downregulation of BRCA1 impacts DNA repair and sensitivity to PARP inhibitors. Mol Cell. 2011; **41**(2): 210-20.

45. Li AY, Boo LM, Wang SY, Lin HH, Wang CC, Yen Y, et al. Suppression of nonhomologous end joining repair by overexpression of HMGA2. Cancer Res. 2009; **69**(14): 5699-706.

46. Tran H, Brunet A, Grenier JM, Datta SR, Fornace AJ, Jr., DiStefano PS, et al. DNA repair pathway stimulated by the forkhead transcription factor FOXO3a through the Gadd45 protein. Science. 2002; **296**(5567): 530-4.

47. Wei JJ, Wu J, Luan C, Yeldandi A, Lee P, Keh P, et al. HMGA2: a potential biomarker complement to P53 for detection of early-stage high-grade papillary serous carcinoma in fallopian tubes. Am J Surg Pathol. 2010; **34**(1): 18-26.

48. Xu Q, Liu LZ, Qian X, Chen Q, Jiang Y, Li D, et al. MiR-145 directly targets p70S6K1 in cancer cells to inhibit tumor growth and angiogenesis. Nucleic Acids Res. 2012; **40**(2): 761-74.

49. Rohozinski J, Edwards CL, Anderson ML. Does expression of the retrogene UTP14c in the ovary pre-dispose women to ovarian cancer? Med Hypotheses. 2012; **78**(4): 446-9.

50. Yang N, Kaur S, Volinia S, Greshock J, Lassus H, Hasegawa K, et al. MicroRNA microarray identifies Let-7i as a novel biomarker and therapeutic target in human epithelial ovarian cancer. Cancer Res. 2008; **68**(24): 10307-14.

51. Shell S, Park SM, Radjabi AR, Schickel R, Kistner EO, Jewell DA, et al. Let-7 expression defines two differentiation stages of cancer. Proc Natl Acad Sci U S A. 2007; **104**(27): 11400-5.

52. Wang Y, Hu X, Greshock J, Shen L, Yang X, Shao Z, et al. Genomic DNA copy-number alterations of the let-7 family in human cancers. PLoS One. 2012; **7**(9): e44399.

53. Corney DC, Hwang CI, Matoso A, Vogt M, Flesken-Nikitin A, Godwin AK, et al. Frequent downregulation of miR-34 family in human ovarian cancers. Clin Cancer Res. 2010; **16**(4): 1119-28.

54. Flesken-Nikitin A, Choi KC, Eng JP, Shmidt EN, Nikitin AY. Induction of carcinogenesis by concurrent inactivation of p53 and Rb1 in the mouse ovarian surface epithelium. Cancer Res. 2003; **63**(13): 3459-63.

55. Bommer GT, Gerin I, Feng Y, Kaczorowski AJ, Kuick R, Love RE, et al. p53-mediated activation of miRNA34 candidate tumor-suppressor genes. Curr Biol. 2007; **17**(15): 1298-307.

56. He L, He X, Lim LP, de Stanchina E, Xuan Z, Liang Y, et al. A microRNA component of the p53 tumour suppressor network. Nature. 2007; **447**(7148): 1130-4.

57. Corney DC, Flesken-Nikitin A, Godwin AK, Wang W, Nikitin AY. MicroRNA-34b and MicroRNA-34c are targets of p53 and cooperate in control of cell proliferation and adhesion-independent growth. Cancer Res. 2007; **67**(18): 8433-8.

58. Zhang Q, He XJ, Ma LP, Li N, Yang J, Cheng YX, et al. [Expression and significance of microRNAs in the p53 pathway in ovarian cancer cells and serous ovarian cancer tissues]. Zhonghua Zhong Liu Za Zhi. 2011; **33**(12): 885-90.

59. Eitan R, Kushnir M, Lithwick-Yanai G, David MB, Hoshen M, Glezerman M, et al. Tumor microRNA expression patterns associated with resistance to platinum based chemotherapy and survival in ovarian cancer patients. Gynecol Oncol. 2009; **114**(2): 253-9.

60. Lakhani SR, Manek S, Penault-Llorca F, Flanagan A, Arnout L, Merrett S, et al. Pathology of ovarian

cancers in BRCA1 and BRCA2 carriers. Clin Cancer Res. 2004; **10**(7): 2473-81.

61. Taylor DD, Gercel-Taylor C. MicroRNA signatures of tumor-derived exosomes as diagnostic biomarkers of ovarian cancer. Gynecol Oncol. 2008; **110**(1): 13-21.

62. Sorrentino A, Liu CG, Addario A, Peschle C, Scambia G, Ferlini C. Role of microRNAs in drug-resistant ovarian cancer cells. Gynecol Oncol. 2008; **111**(3): 478-86.

63. Yao N, Lu CL, Zhao JJ, Xia HF, Sun DG, Shi XQ, et al. A network of miRNAs expressed in the ovary are regulated by FSH. Front Biosci. 2009; **14**: 3239-45.

64. Hossain MM, Ghanem N, Hoelker M, Rings F, Phatsara C, Tholen E, et al. Identification and characterization of miRNAs expressed in the bovine ovary. BMC Genomics. 2009; **10**: 443.

65. Ambros RA, Sherman ME, Zahn CM, Bitterman P, Kurman RJ. Endometrial intraepithelial carcinoma: a distinctive lesion specifically associated with tumors displaying serous differentiation. Hum Pathol. 1995; **26**(11): 1260-7.