SHORT THESIS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY (PHD)

Nucleic acid and associated cell surface biomarker analyses in the diagnosis of ovarian cancer

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Head of the **Examination Committee**: Árpád Illés, PhD, DSc
Members of the Examination Committee: Katalin Koczok, PhD
Sándor Drávucz, PhD

The Examination takes place at 1st floor courtroom of Building B of Internal Medicine,
Faculty of Medicine, University of Debrecen
on 2nd October 2024. at 12.00 pm

Head of the **Defense Committee**: Árpád Illés, PhD, DSc
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The PhD Defense takes place at the Lecture Hall of Department of Obsterics and Gynecology,
Faculty of Medicine, University of Debrecen
on 2nd October 2024. at 2.00 pm

1 Introduction

Over the past decade, the survival rates for ovarian cancer have improved favourably due to the development of well-tailored chemotherapy regimens and biological therapies, as well as the effective use of supportive care. Nevertheless, malignant ovarian tumors are still the leading cause of death from gynaecological cancers, due to ovarian cancer being diagnosed at an advanced stage in most cases in developing countries [Mulisya *et al*., 2020].

The diagnostic tools include physical examination, ultrasound imaging, CT and MR imaging, if symptoms are present, and tumor markers such as Cancer Antigen 125 (CA125), and Human Epididymis Protein 4 (HE4) are tested, which is not highly sensitive and specific.

The investigation and subsequent use of new biomarkers are needed in the diagnosis of ovarian cancer. Nowadays, in other medical disciplines, the study of microRNAs, exosomes, and signalling proteins in tumors has shown promise. Biomarker studies started in other countries and supported by literature data are inspiring to rethink with new objectives to study in domestic populations. Biomarker studies may serve the future diagnosis and follow-up of ovarian cancer patients.

2 Literature review

2.1 Ovarian cancer

Ovarian cancer is one of the leading causes of death in women, due to non-specific symptoms, various risk factors, and genetic abnormalities underlying the disease [Mulisya *et al*., 2020

Ovarian tumors are morphologically and molecularly heterogeneous. Clinical variations are observed in clinical symptoms within the disease.

The majority of malignant ovarian tumors is epithelial tumors. High-grade serous ovarian cancer (HGSOC), with a poor prognosis, are aggressive, highly proliferous, and very often fatal, affecting women aged 65 years or older [Köbel and Kang, 2022].

Routine diagnostic procedures include ultrasound (UH), especially transvaginal ultrasound (TVS) and computed tomography (CT), although it is not possible to determine with certainty whether a tumor is benign or malignant. If the malignancy of the tumor is suspected, a precise staging is needed to develop a treatment plan, which is made more accurate by exploratory laparotomy and histological sampling and processing.

2.2 Liquid biopsy

Conventional biopsy is an invasive procedure to determine whether an abnormal cell proliferation is benign (good) or malignant (bad), although this can be dangerous for the patient, whereas liquid biopsy has many advantages, it is less invasive, easy to take and repeatable. Molecules obtained by liquid biopsy, including tumor cells, extracellular vesicles (apoptotic bodies, microvesicles, and exosomes), tumor-derived metabolites, proteins, and free-nucleic acids (cell-free DNA and RNA) can be important biomarkers for the study of tumors [Zhou *et al*., 2020]. Research into these molecules may make a major contribution to the future diagnosis of tumors.

2.3 MicroRNAs

For some years now, research has turned with great interest to microRNAs because of their importance in the development of many diseases, including tumors.

MicroRNAs are small (19-22 nucleotides), single-stranded, non-coding RNAs encoded by endogenous genes. Since they were first described, a large number of human microRNAs have been identified that may play a role in various biological processes, including cell differentiation, metabolism, immune response, inflammation and the post-transcriptional regulation of about 60% of human genes. These participate in the degradation of the target mRNA and also in the inhibition of translation [Lai, 2002; Lewis *et al*., 2003; Lengyel 2010; Contreras and Rao, 2012; Di Leva *et al*., 2014; Hayes *et al*., 2014].

One type of genetic mutation is the single nucleotide polymorphism (SNP), located in the sequence of genes encoding miRNAs, results in altered miRNA expression and affects the specificity and affinity of binding to the target molecule, thereby affecting disease susceptibility [Li *et al*., 2020].

SNPs in the microRNA genes and target genes of microRNAs change the level of miRNAs thereby affecting the risk of cancer development [Mullany *et al*., 2015].

In particular, miR-146 microRNA targets molecules that have a role in inflammatory processes and innate immunity [Taganov *et al*., 2006], and miR-146 has an important role in the development of ovarian cancer [Shen *et al*., 2008].

The oncogenic or tumor suppressor role of the miR-196 family has been reported in several manuscripts, including upregulation in gynaecological tumors (cervical and ovarian) and downregulation in breast cancer [Pourdavoud *et al*., 2020].

The miR-193 may slow down proliferation and cell cycle regulation in normal cells, and miR-193 also has tumorsuppressor function in tumors [Grossi *et al*., 2017].

2.4 CD24

The CD24 as a cell surface protein is found on haematopoietic cells, muscle cells, keratinocytes and epithelial cells, as well as on human carcinoma cells. [Aigner et al., 1997; Nagy et al., 2009; Fang et al., 2010]. Higher expression of CD24 was firstly measured in oncohematological cases and then in prostate cancer [Kristiansen et al., 2002, 2004]. Several publications have confirmed these data in many other types of tumours, such as B-cell lymphoma, bladder carcinoma, renal cell carcinoma, ovarian and breast cancer [Weichert et al, 2005; Runz et al., 2007; Fang et al., 2010]. CD24 expression is detectable in borderline ovarian tumours and high level of CD24 was determined in serous adenocarcinoma [Choi et al., 2005].

3 Objectives

Ovarian cancer is one of the leading causes of death among women because initially ovarian cancer cases are asymptomatic and are detected in advanced stage, when response to treatment and survival rates are reduced. This fact has led to a focus on the search for biomarkers that can be used in clinical diagnosis.

1. The primary aim of our studies was to search the allele and genotype frequencies of miR-146a, miR-196a, and miR-193b polymorphisms in blood samples of ovarian tumorous and non-tumorous cases to investigate the association with the development of ovarian cancer.
2. Investigation of the role of selected microRNAs through their target genes using bioinformatics methods.
3. In the other part of our research, we aim to investigate the presence of the cell surface marker CD24 between tissue samples of ovarian cancer and control subjects and to analyse the association between *CD24* expression and FIGO stages.
4. Investigation of the role of CD24 through its target genes using bioinformatic methods.

4 Materials and methods

4.1 Patients

Tissue and blood samples were collected at the Department of Obstetrics and Gynaecology, University of Debrecen. The molecular genetic studies were performed in collaboration with the Department of Human Genetics, University of Debrecen, under the ethical license number 30231-2/2016. Informed consent forms were obtained and signed by the participants to be included in the study.

Samples from 100 tumor patients (58.4±13.0 years) and 111 controls (58.0±12.0 years) were collected during our examinations. Patients with ovarian tumors were selected and sampled concerning gynaecological tumor surgery, and controls as healthy female volunteers were sampled at menopause clinic and during gynaecological surgery against uterine prolapse. Out of 100 ovarian tumor patients, the 84 cases were confirmed as high grade serous ovarian cancer; 7 cases were diagnosed as FIGO I/a, 15 cases as FIGO I/c, one case as FIGO II/b, two cases as FIGO II/c, two cases as FIGO III/a, 8 cases as FIGO III/b, 34 cases as FIGO III/c, 3 cases as FIGO IV/a and 12 cases as FIGO IV/b, and the remaining 16 cases were 3 cases as borderline and 13 cases as benign tumors.

The number of tumor and control cases included in each study was determined by the number of samples and histological results available at the time of each study.

To investigate the miR-146 and miR-196a single nucleotide polymorphisms, 75 HGSOC (mean age: 58.6±13.7 years) and 75 control individuals (mean age: 60.4±12.4 years) were included in the study.

For the miR-193 single nucleotide polymorphism, 86 HGSOC (mean age: 58. 5 ± 13.6 years) and 102 control individuals (mean age: 59.4 ± 11.9 years) were included in the study.

For the CD24 cell surface marker and microRNA, 21 HGSOC patients (mean age 59.1 ± 8.8 years) and 8 control individuals (mean age 60.8 ± 11.7 years, $p = 0.72$) were included. Patients were also classified according to FIGO stages: 2 cases with FIGO I (66.5 ± 7.8 years; $p = 0.48$), 15 cases with FIGO III (57.5 ± 8.3 years; $p = 0.50$), 4 cases with FIGO IV (61.3 ± 10.6 years; $p = 0.94$). Plasma samples from the same cases were used for the related miR-146 expression assay.

4.2 Methods

Analysis of single nucleotide polymorphism

DNA was extracted from 200 µl of EDTA-coagulated blood samples by silica adsorption according to the manufacturer's instructions (High Pure PCR Template Preparation kit, Roche, Mannheim, Germany). DNA concentration and quality were determined using a NanoDrop spectrophotometer. LightSnip primers (TibMolbiol, Berlin, Germany) and LightCycler FastStart DNA Master Hybridization Probes mix (Roche, Penzdorf, Germany) designed for real-time PCR were used.

Plasma sample collection and storage

EDTA-coagulated blood samples from tumor and non-tumor subjects were processed as soon as possible after collection. They were centrifuged first at 2500 g and then at 16000 g in both cases for 10 min at 4°C. The supernatant was transferred to new tubes and stored in small volumes frozen at -80°C.

RNA isolation

RNA was extracted using the NucleoSpin RNA kit (Macharey Nagel, Düren, Germany) from 30 mg of ovarian tissue or 500 µl of plasma. Free RNAs including the miRNA fraction were extracted from plasma samples using a miRneasy serum/plasma kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. Total RNA concentration and purity were determined using NanoDropLite (Thermo Fisher Scientific, Waltham, MA, USA). The concentration of microRNAs was measured using a Qubit 2.0 Fluorometer (Invitrogen, Carlsbad, CA, USA).

cDNA synthesis

After RNA isolation, complementary DNA (cDNA) was synthesized by reverse transcription using the First Strand cDNA Synthesis kit for RT-PCR (Roche Diagnostics, Corporation, Indianapolis, IN, USA) according to the manufacturer's instructions for CD24 assays and miRCURY LNA RT Kit (Qiagen, Hilden, Germany) for microRNAs. The purity and concentration of cDNAs were measured using NanoDropLite and Qubit 2.0 Fluorometer, respectively.

Quantitative real-time polymerase chain reaction (qRT-PCR)

A primer-probe system was used to determine CD24. The sequences were designed and produced by TIBMOLBIOL (Berlin, Germany) and were as follows: for CD24, the sense strand: 5′-TgA AgA ACA TgT gAg Agg TTT gAC-3′, the antisense strand: 5′-gAA AAC TgA ATC TCC ATT CCA CAA-3′, and the probes were 5′-gAA AAC TgA ATC TCC ATT CCA CAA+3′-fluorescein and LC-640-AAC TCC AgC AgA TTT AAT ATT ggC ATT CAT CA-PH-3′. For gene expression analysis, the DNA Master Hybprobe kit (Roche, Mannheim, Germany) and the LightCycler 1.5 quantitative real-time capillary PCR instrument were used. To normalize our data, we used the beta-globin housekeeping gene (LightCycler® Control Kit DNA, Roche, Mannheim, Germany). For the microRNAs, we used miRCURY LNA SYBR Green Kit (Qiagen, Hilden, Germany), miR-146a primer assay, and miR-103 as housekeeping microRNA, also from Qiagen, we worked according to the manufacturer's instructions, and Roche Cobas Z 480 quantitative real-time PCR instrument was used for the analysis. The $2^{-\Delta Ct}$ method was used to determine the relative expression level.

Bioinformatics analysis (network research)

The TargetScan (www.targetscan.org), MirBase (www.microrna.sanger.ac.uk) and microRNA-Data-Integration-Portal (http://ophid.utoronto.ca/mirDIP) databases were used for analysing the target genes of miR-193b-5p, miR-146a-5p and miR-196a-2-5p. Biogrid (https://thebiogrid.org/) and miRTARGETLink (https://ccb-compute.cs.uni-saarland.de/mirtargetlink2) were used for analysing the connection among CD24, microRNAs and proteins.

Statistical analysis

When we examined microRNA polymorphisms, we compared the frequencies of categorical variables (allele and genotype) using a chi-square test (https://www.socscistatistics.com/tests/chisquare2/default2.aspx). To compare the expression of CD24 cell surface marker and microRNAs in the tumor and non-tumor groups, the Student's t-test was used. The significance level was set at $p<0.05$ in all cases.

5 Results

5.1 Identification of single nucleotide polymorphisms of miR-146a and miR-196a in ovarian tumor patients' blood samples

We used first time a melting peak analysis to determine the single nucleotide polymorphisms of miR-146a and miR-196a. The PCR conditions used were suitable for the reliable separation of each allele by melting point, and the melting points were read from the peaks of the melting curves. The melting Tm points were used to determine the alleles and hence the genotypes.

Our study included samples from 75 ovarian cancer and 75 non-tumor patients to determine the prevalence of the two miRNA SNPs. One of the inclusion criteria was that there should be no significant difference in mean age between the tumor and non-tumor groups. Based on our data, we calculated the allele and genotype frequencies of the single nucleotide polymorphisms. In the melting curve analysis, for miR-146a rs2910164, the G allele in 82.00% and C allele occurred in 18.00% in the patient group, and G allele in 72.67% and C allele occurred in 27.33% in the control group (p=0.053). In terms of genotype frequencies, GG occurred in 65.33%; GC in 33.33%, and CC in 1.33% of the patients, while in 53.33%, 38.67%, and 8.00% of the control group, respectively (p=0.0917). For miR-196a rs11614913, the prevalence of the C allele in 67.33% and T allele in 32.67% occurred in the patient group, and C allele in 59.33% and T allele in 40.67% occurred in the control group (p=0.15). The genotypes were found to be prevalent in CC 46.67%; CT 41.33% and TT 12.00% in the patient group, and 37.33%, 44.00%, and 18.67% in the control group (p=0.3815).

Using bioinformatics methods, four genes were identified as common target genes - Lamin B receptor (*LBR*), Rab4 interacting protein (*RUFY2*), Autophagy related 9a (*ATG9A*), and Methyl CpG binding domain 4 (*MBD4*).

5.2 Identification of miR-193b single nucleotide polymorphism in ovarian tumor samples

In our research, we used first time a melting peak analysis to determine the single nucleotide polymorphism of miRNA-193b in Hungarian population. We determined alleles, and thus genotypes, based on the melting Tm points.

The prevalence of the two miRNA SNPs was determined in samples from 102 control

individuals and 86 ovarian cancer patients, with no significant difference in mean age between the two groups. The T allele in 30.24%, C allele in 69.22% occurred in the patient group, and T allele in 35.78%, C allele in 64.22% occurred in the control group (p=0.2549). The genotypes were CC 45.35%, CT 48.84%, and TT 5.81% in the patient group, while in the control group CC 39.22%, CT 50.0%, and TT 10.78% (p=0.4096).

To map the miR-193b microRNA target genes, a network search by bioinformatic tools was performed. We identified the key genes regulated by miR-193b and their amplification assays using the miRTarbase, TargetScan, MirBase, and microRNA-DataIntegration-Portal prediction databases and the algorithms they offer. The effect of cyclinD1 (*CCND1*), estrogen receptor (*ESR1*), plasminogen activator (*PLAU*), proline-rich acidic protein (*PRAP1*), and myeloid cell leukemia sequence (*MCL1*) genes was confirmed by several methods.

5.3 Determination of CD24 cell surface marker in ovarian cancer and non-tumor individuals

The expression of *CD24* was investigated in ovarian tissue samples using a primer-probe detection system with quantitative real-time polymerase chain reaction. A statistically significant difference in *CD24* expression was found between tissue samples from ovarian cancer and non-tumor subjects (44.97 ± 68.06; 0.16 ± 0.32; $p < 0.01$). Following histological examination, patients were classified into different FIGO stages. *CD24* expression was found to be higher in FIGO I, III, and IV stages (FIGO I, 72.22 ± 88.54; FIGO III, 17.34 ± 32.09; FIGO IV, 134.90 ± 91.42) compared to controls, thus suggesting that higher stages are associated with higher *CD24* expression but the result was not significant.

After taking anamnesis of patients and controls, we took samples from individuals who had not received treatment before sampling and followed up with the subjects (21 individuals), 2 patients died during the study period (one month after sampling) (FIGO stage III and FIGO stage IV patients) due to other health problems, showing a survival rate of 90.48% (3-29 months) in the study group.

The relationship between CD24 cell surface protein and other proteins was determined using the Biogrid database. Four proteins were identified that are related to CD24: LYN, SELP, FGR, and NPM1. Several microRNAs have been described to affect CD24 expression, and the microRNA-CD24 relationship was determined using the miRTargetLink database. In the

network analysis, 31 microRNAs were found to be associated with CD24, of which hsa-miR34a and hsa-miR373-3p are the most specific.

According to the literature data we tried to identify a possible link between miR-146a microRNA and CD24, as CD24 has been identified as a novel functional target of miR-146a.We examined miR-146a expression in ovarian tumor patients described in this chapter and found that it was more but not significantly expressed compared to the control group (0.30±0.15; 0.19±0.16; p=0.183) and also showed stage dependence but not significant from lower to higher stage (0.25±0.13; 0.28±0.16; 0.37±0.15; p=0.647, p=0.255, p=0.117).

6 Discussion

Ovarian tumors are the leading cause of death among women. Non-invasive sampling procedures such as liquid biopsy may contribute to the early detection of various diseases, including ovarian cancer. In the development of non-invasive techniques, attention has focused on microRNAs, as small non-coding RNAs earlier predicting tumors [Sharma *et al*., 2017].

In our work, we investigated three microRNA polymorphisms thought to be biomarkers for the diagnosis of ovarian cancer. The miR-193b polymorphism was shown to be associated with platinum treatment efficacy [Rauhala *et al*., 2010; Ziliak *et al*., 2012].

In a previous study examining miR-146 polymorphisms in ovarian cancer patients and healthy controls, the GG genotype was 3.73 times more frequent than the CC genotype, the CG + GG genotype was 1.68 times more frequent than the CC genotype, and the GG genotype was 3.02 times more frequent than the CG + CC genotype, with a statistically significant difference in all three comparisons [Sun *et al*., 2016]. In our present study, the same comparisons resulted in the following ratios 7.35 (GG genotype to CC genotype), 6.4 (CG + GG genotypes to CC genotype), 1.65 (GG genotype to CC + CG genotypes), but none were statistically significant. Based on our results, we hypothesize that the miR146a polymorphism is not exclusive in the development of ovarian cancer.

Based on the analysis of miR-146a rs2910164 melting curve, we found that 72.67% of G allele frequency was detected in the control group and 82.00% in the patient group ($p = 0.053$). GG, GC and CC genotypes were detected at 53.33%, 38.67% and 8.00% frequency in the controls, while in the patients at 65.33%, 33.33% and 1.33% frequency, respectively ($p = 0.0917$).

The genetic abnormalities of miR-196a-1 have been linked to the development of ovarian cancer [Eccles *et al*., 1990]. In addition, when investigating genes affected by miR-196a, the researchers identified the high mobility AT-hook 2 (*HMGA2*) gene as a primary target, whose cause DNA double-strand breaks [Chen *et al*., 2011]. The miR-196a may act as a tumor promoter where the target gene (*HOXA 10*) is located downstream [Yang *et al*., 2016], thus increased expression of miR-196a-1 is associated with poor prognosis in ovarian cancer patients [Fan *et al*., 2015].

In the analysis of miR-196a rs11614913, the C allele occurred in 59.33% of controls and 67.33% of patients ($p = 0.15$). CC, CT, and TT genotypes occurred in 37.33%, 44.00%, and

18.67% of controls, respectively, compared to 46.67%; 41.33%, and 12.00% of patients, respectively (p = 0.3815).

Previous studies have found that CC genotypes are 1.34 times more likely to occur in ovarian cancer patients than in healthy controls compared to wild-type (TT) or heterozygous (CT) genotypes [Song *et al*., 2016]. The odds ratio in our study was 1.5, which was not statistically significant. Our research concluded that polymorphisms of miR-146a rs2910164 and miR-196a rs11614913 in ovarian cancer do not show statistically significant differences compared to healthy controls and a published paper came to the same conclusion [Ni and Huang, 2016].

Two miRNAs, miR-146a and miR-196a, play an essential role in tumor development and chemoresistance, and by mapping the genes they affect, we can better understand the mechanism of action of these microRNAs. Our bioinformatics network analysis identified four genes that are jointly affected by the two miRNAs under investigation: *LBR, RUFY2, ATG9A* and *MBD4*, their altered expression levels were determined.

Nine genes have been found to contribute to the development of platinum-resistant ovarian cancer, one of these genes, *LBR* [Helleman *et al*., 2006].

In ovarian cancer patients, the rs30236 SNP polymorphism in the miR-193b gene has not yet been investigated, but the Ensembl database has information on the prevalence of alleles, with 43.2% of the T allele and 56.8% of the C allele in the European population, based on 503 individuals. These allele frequencies are slightly different from our data (control group: T allele 35.78%, C allele 64.22%, patient group T allele 30.24%, C allele 69.76%), but we do not have precise information on the origin of the samples in the database [https://www.ensembl.org]. Chi-square testing of the results was not significant (p=0.2549). Genotype frequencies are also available in the Ensembl database for different ethnic groups, but for the European population the following data are available: TT genotype 20.1%, CC genotype 33.6% and CT genotype 46.3% prevalence, our results from our studies (Caucasian population) are slightly different, in the control group TT genotype 10.78%, CC genotype 39, 22% and CT genotype 50.0%, and in the patient group TT genotype 5.81%, CC genotype 45.35% and CT genotype 48.84%, but using chi-square test our results were not statistically significant (p=0.4096).

Our results from bioinformatics network analysis show that miR-193b is regulated by different genes that are key determinants of many physiological processes. Some of the most important genes are *CCND1, ESR1, PLAU, PRAP1* which the elements of several regulating processes.

Among other things, miR-193b is strongly associated with the Myeloid Cell Leukemia Sequence 1 Apoptosis Regulator (MCL1; BCL2 Family Member) molecule, a member of the BCL-2 anti-apoptotic protein family, which plays an important role in the development of platinum resistance during chemotherapy [Sugio *et al*., 2014].

The use of CD24 as an independent biomarker appears promising in some tumors, including ovarian cancer. CD24 expression has been associated with tumor progression, FIGO stages, and survival.

We determined the expression of CD24 in ovarian tissue from ovarian tumor and non-tumor individuals' tissue samples by quantitative real-time PCR. The β-globin gene was used as a housekeeping gene to normalize the values [Nagy *et al*., 2008, 2009]. We identified a significant difference between CD24 expression measured in tissue samples from control and ovarian cancer patients ($p < 0.01$), which was also associated not significantly with more advanced FIGO stages. This was in correlation with previous scientific reports that CD24 expression was higher in malignant, higher-grade ovarian cancers than in lower-grade cancers [Kristiansen *et al*., 2002; Moulla *et al*., 2013].

We did not find a correlation between high CD24 expression and low patient survival, but other studies have previously shown a link between the two factors [Kristiansen *et al*., 2002].

According to an earlier study, CD24 cell surface protein can be used as a specific marker in the differential diagnosis of serous ovarian carcinoma and malignant pleural mesothelioma, and some scientific publications suggest that it may be a specific and sensitive biomarker in the diagnosis of ovarian cancer [Ozols, 2005; Davidson, 2016; Nakamura *et al*., 2017].

In addition to gene expression studies, we considered it is important to analyse the association of CD24 with microRNAs and proteins. We performed network searches to explore these relationships using the Biogrid, miRTargetLink, miRDIP databases, which may be connected to the regulating processes of cancer development.

The network search revealed possible links between microRNAs and target molecules, but in our experience, the use of a single database was not sufficient, so we searched several databases and published scientific papers. Based on the literature data, miR-146a post-transcriptionally modifies CD24 and has therefore been identified as a novel functional target of it [Ghuwalewala *et al*., 2021], our research team found higher, not significant miR-146a expression in ovarian

tumor patients compared to the control group, and also a not significant stage dependence from lower to higher stage.

7 New scientific findings

1. To the best of our knowledge, we are the first to investigate the polymorphisms of miR-146a, miR-196a-2 and miR-193b in Hungarian patients with high-grade serous ovarian cancer using PCR and melting curve based detection systems. We found that our method is efficient and rapid in detecting these polymorphisms. The GG genotype was found to occur at a higher but not significant frequency (p=0.0917) in the miR-146a rs2910164 polymorphism analysis in the ovarian cancer patient group compared to controls. The CC genotype was found to occur at a higher but not significant frequency in the patient group compared to the control group (p=0.3815; p=0.4096) when testing the miR-196a-2 rs11614913 and miR-193b rs30236 SNP polymorphisms. The extension of these studies to a larger number of cases may raise the possibility of a combined study of miR-146a, miR-196a-2 and miR-193b polymorphisms in connection with ovarian cancer susceptibility.
2. The determination of CD24 expression in tissue samples from Hungarian female patients and healthy controls was performed for the first time. Based on our results, significantly higher CD24 expression may indicate the presence of ovarian cancer, and CD24 expression was associated with FIGO stages, but not significantly.

8 Summary

Ovarian tumor is the leading cause of death among women. The high mortality rate is due to non-specific symptoms and late diagnosis. This disease is characterized by a poor survival rate despite modern treatment strategies, so the development of new diagnostic, therapeutic, and monitoring options has become necessary. During our work, we searched for biomarkers that could be promising candidates for the detection of ovarian tumors and the coming out of future diagnostic tests.

Using non-invasive procedures, attention was primarily directed towards microRNAs, so in the first part of our research we examined microRNA polymorphisms that were thought to be biomarkers in the diagnosis of ovarian tumors. Although there was no significant difference between the examined polymorphisms (miR-146a rs2910164, miR-196a rs11614913, and miR-193b rs30236) between the examined ovarian cancer patients and healthy controls, it does not rule out their applicability if they are tested in combination with other polymorphisms, however, it is important to emphasize that we examined these polymorphisms for the first time in a Hungarian patient group. The expression of CD24 was determined on tissue samples, also for the first time examining Hungarian cases. We identified a significant difference between the CD24 expression measured in tissue samples from control and ovarian tumor patients, which also showed a correlation but not significantly with more advanced FIGO stages.

The significance of our work is that we were the first to investigate miR-146a rs2910164, miR-196a-2 rs11614913 and miR-193b rs30236 microRNA polymorphisms, and the expression of CD24 in Hungarian women.

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9.2 Certified list of references on which the thesis is based and further publications

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Candidate: János Lukács
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List of publications related to the dissertation

1. Soltész, B., **Lukács, J.**, Penyige, A., Póka, R., Nagy, B.: Determination of miR-193b rs30236 single nucleotide polymorphism in ovarian cancer patients.
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4. Márton, É., Beke-Varga, A. E., Soltész, B., Penyige, A., **Lukács, J.**, Póka, R., Nagy, B., Szilágyi, M.: Comparative Analysis of Cell-Free miR-205-5p, let-7f-5p, and miR-483-5p Expression in Ovarian Cell Cultures and Plasma Samples of Patients with Ovarian Cancer.
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10 April, 2024

10 Conference presentations

10.1 Lectures in Hungarian

Lukács J, Krasznai Z., Póka R.: Treatment of endometrial carcinoma cases with rapid progression despite favourable histological results. *Scientific Meeting of the Northeastern Hungarian Section of the Hungarian Society of Gynecology, Debrecen- Hungary , 1-2 April 2016.*

Lukács J., Soltész B., Keserű J., Szentesiné Sz.K., Hadáné B.Zs., Nagy B., Póka R.: Determination of miR-196a polymorphism by melting curve analysis in a group of ovarian cancer patients. *MHGT XI. Congress Pécs-Hungary, 13-15 October 2016.*

Lukács J., Soltész B., Keserű J., Szentesiné Sziráki K., Hadáné Birkó Zs., Nagy B., Póka R.: Identification of microRNA polymorphisms by melting curve analysis in a group of ovarian cancer patients. *MNT Cervical Pathology Section XXII Scientific and Training Meeting, Hajdúszoboszló-Hungary, 24-25 March 2017.*

Lukács J., Soltész B., Keserű J., Szentesiné Sz K., Hadáné B.Zs., Nagy B., Póka R.: Identification of microRNA polymorphisms by melting curve analysis in a group of ovarian cancer patients. *Scientific Meeting of the Northeastern Hungary Section of MNT, Hernádvécse -Hungary, 8. April 2017.*

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Szilágyi-Bónizs M., Márton É., Lukács J., Soltész B., Janka E., Penyige A., Póka R., Nagy B.: The potential of miR200a, miR200b and miR200c as biomarkers in ovarian cancer diagnostics. *XII Congress of the Hungarian Human Genetics and Genomics Society Debrecen- Hungary, 6-8 September 2018.*

Márton É., Lukács J., Szabó R., Soltész B., Janka E., Penyige A., Póka R., Nagy B., Szilágyi-Bónizs M.: Investigating the applicability of miR141 and miR429 in ovarian cancer diagnostics. *XII Congress of the Hungarian Human Genetics and Genomics Society Debrecen- Hungary, 6-8 September 2018.*

Keserű J., Soltész B., Lukács J., Póka R., Nagy B.: Detection of mitochondrial DNA copy number in plasma of ovarian cancer patients. *XII Congress of the Hungarian Human Genetics and Genomics Society Debrecen- Hungary, 6-8 September 2018.*

10.2 Posters in English

Lukács J, Soltész B, Nagy B., Póka R.: Analysis of miR-196A, miR-146A and two miR193B single nucleotide polymorphism in ovarian cancer patients. *ESGO Congress-2017. 6 November 2017, Vienna-Austria.*
Lukács J, Soltész B, Nagy B., Póka R.: Expression of CD24 in ovarian cancer. *ESGO Congress-2019, 2-5 November 2019 Athens-Greece*
Póka R., Molnár Sz., Baráth L., Lukács J., Lampé R., Krasznai Z., Hernádi Z., Méhes G.: Negative PARP immunohistochemistry as a predictor of platinum sensitivity in ovarian cancer. *ESGO Congress-2017. 6 November 2017, Vienna-Austria.*

11 Key words

microRNA; CD24; Single nucleotide polymorphism (SNP); Gynecological oncology; Ovarian cancer; Free nucleic acids

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