EJGO European Journal of Gynaecological Oncology

Determination of miR-193b rs30236 single nucleotide polymorphism in ovarian cancer patients

B. Soltész[1], J. Lukács[2], A. Penyige[1], R. Póka[2], B. Nagy[1]

[1]Department of Human Genetics, [2]Institute of Obstetrics and Gynecology, Faculty of Medicine, University of Debrecen, Debrecen (Hungary)

Summary

Background: Single nucleotide polymorphism (SNP) is the most common form of genetic variability. The miR-193b was connected to several types of tumors and also with occurrence of resistance to chemotherapy. This investigation aimed to determine miR-193b rs30236 polymorphism in ovarian cancer patients and controls. The authors performed bioinformatics analysis to discover the target genes of miR-193b. *Materials and Methods*: Eighty-six ovarian cancer patients and 102 matched controls were included in the study. Blood samples were drown into EDTA tubes and DNA was isolated by silica adsorption method. Mir-193b rs30236C/T polymorphism was determined by melting curve analysis. Chi-square test was used for statistical evaluation of the data. MiRTargetLink was used for determination of the has-miR-193b-3p microRNA target genes. *Results*: Based on melting curve analysis of miR-193b rs30236, the authors found 30.24% T allele frequency in the patients' group, and 35.78% in the control group ($p = 0.2549$). They detected CC genotype in 39.22%, CT genotype in 50.00%, and TT genotype in 10.78% of controls, while they occurred in 45.35%, 48.84%, and 5.81% of the patients ($p = 0.4096$), respectively. The most important target genes of miR-193b are cyclin D1 (CCND1), estrogen receptor (ESR1), plasminogen activator (PLAU), prolin rich acidic protein (PRAP1), and myeloid leukemia sequence (MCL1). *Conclusions*: The miR-193b has effect on the expression of several genes. The rs30236 polymorphism has not been studied in ovarian cancer patients, yet. The authors did not find significant difference in allele and genotype difference in patients and controls. To discover the exact role and suitability of this SNP for use as biomarker in the ovarian cancer prior to chemotherapy, more detailed studies are needed.

Key words: miRNA; miR-193b; SNP, Gene expression; Ovarian cancer; Melting curve analysis.

Introduction

MicroRNAs are short non-coding nucleic acids with the size of 18-25 bp that play an important role in the regulation of gene expression [1]. They participate in the posttranscriptional gene regulation [2]. Several study demonstrated their role in cellular, developmental, and pathological processes [3]. MiRNA-193b was connected to the development of several types of tumors, it is an oncogene in some, while tumor suppressor in the others [4]. Rauhala et al. studied it in prostate cancer, and proved that low expression plays role in the epigenetic modifications [5]. It is a main regulator in skin, colorectal, and in head and neck cancer [6]. Yong *et al.* performed their studies on samples of colorectal cancer patients; they discovered altered expression of miR-193a-3p, while they were not able to discover the exact controlling mechanism [7]. Recently it was studied in non-small cell lung cancer and there was a correlation with the survival; however in this type of cancer, also platinum treatment is the most commonly applied regimen [8]. The SNP rs30236 was associated with survival in non-small cell lung cancer [9]. MiR-193b down-regulates estrogen-receptor in breast cancer cell lines [10].

Ovarian cancer is the eighth most common tumor among women in the industrially developed countries according to the statistical data from 2014. The epithelial type accounts for about 95% of the cases. The diagnosis is difficult as the symptoms are not very specific. Besides the improvements in the operation techniques and chemotherapy treatments, the prognosis is still not very good, and the five-year survival rate is under 50% [11].

There is a need for biomarkers which could call the attention for the presence of ovarian cancer in the early stage. These could make for possible earlier detection, could be suitable for monitoring of treatments, and to predict which drug regimen is more advantageous for the given patient. MicroRNAs seem to be good candidates for that purpose. There is scientific data regarding miRNA polymorphism and ovarian cancer. The data is usually published from Chinese population as less information is available on Caucasian population [12]. The microRNA molecules are ideal from the laboratory view, they are stable, and resistant to freezing, thawing, and pH changes.

The present authors' aim was to determine the miRNA-193b rs30236 polymorphism, and discover the allele and genotype frequencies in the collected samples

Revised manuscript accepted for publication May 15, 2018

Eur. J. Gynaecol. Oncol. - ISSN: 0392-2936
XL, n. 4, 2019
doi: 10.12892/ejgo4514.2019

7847050 Canada Inc.
www.irog.net

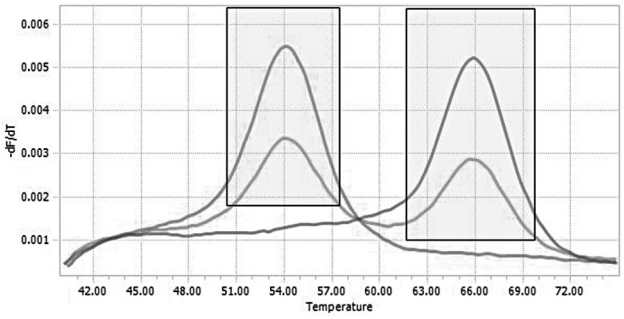

Figure 1. — Melting curve analysis of miR-193b rs30236 C/T detection.The figure shows the melting curves converted to melting peaks by plotting the negative derivative of fluorescence against temperature. The melting points (Tm) are T allele 54ºC and for the C allele 66ºC. The TT genotype is shown by the red curve, the CT genotype with the green curve and the CC genotype with the grey curve.

Table 1. — *Allele and genotype frequencies of miR-193b rs30236.*

Alleles			
Study group	*C*	*T*	
Controls (n=102)	131 (64.22%)	73 (35.78%)*	
Patients (n=86)	120 (69.76%)	52 (30.24%)	
χ^2. p = 0.2549, OR: 0.7776.			
Genotypes			
Study group	*CC*	*CT*	*TT*
Controls (n=102)	40 (39.22%)	51 (50.00%)	11 (10.78%)*
Patients (n=86)	39 (45.35%)	42 (48.84%)	5 (5.81%)
χ^2. p = 0.4096, OR: 1.9582.			

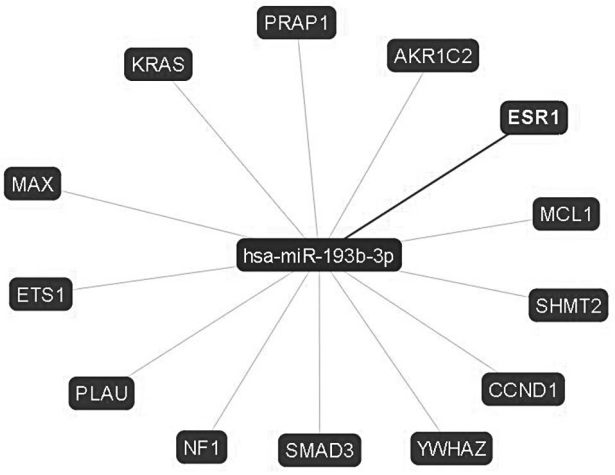

Figure 2. — The main target genes of miR-193b-3p. The figure shows the main target genes of miR-193b-3p in a network system, it was made by the use of miRTargetLink database.

from healthy controls and ovarian cancer patients in a Caucasian population. The authors also planned a network analysis to assess the main target genes.

Materials and Methods

Patients were enrolled and examined at the Institute of Obstetrics and Gynecology, University of Debrecen in a period of 2016-2017. This study was approved by the Medical Research Council (Budapest, Hungary), and all subjects gave written informed consent to participate. This pilot study enrolled 102 controls (mean age: 59.44 ± 11.85 years) and 86 ovarian cancer patients (mean age: 58.45 ± 13.62 years). The patients FIGO classification was the following: borderline 2; Ia borderline 2; Ia6, Ic 12, IIb 1, IIc2, IIIa 1; IIIb7, IIIc 26, IV 4; IVb 4, and in 15 cases it was not available. The occurrence of the tumor was sporadic in these cases.

Six milliliters of peripheral blood was drawn from each patient into a potassium diamine tetra-acetic acid (EDTA) tube and stored at 4ºC. DNA was extracted from 0.2 ml of samples using high pure PCR template preparation kit according to the manufacturer's instructions [13].

Allele determination was performed using polymerase chain reaction (PCR) followed by melting curve analysis. PCR was performed by measuring one microliter of isolated DNA, one microliter of LightSnip mix, 3.0 mM MgCl2, and one microliter Light Cycler FastStart DNA master hybridization probes mix into a 10 µl final volume. The authors used miR-193b rs30236 SNP LightSnip primers for the analysis.

The authors used miRTargetLink (https://ccb-web.cs.uni-saarland.de/mirtargetlink/?id=www/www-ccb/html/mirtargetlink) for the determination of the has-miR-193b-3p microRNA target genes. They applied the MirBase (www.microrna.sanger.ac.uk) and microRNA-Data-Integration-Portal (http://ophid.utoronto.ca/mirDIP) prediction database and algorithms. These softwares used for the exact targets pointing beside the miRNA "seed" sequences and complementary mRNA sequences the environment of the seed and evolutionary conservation.

The SPSS software package (version 11.5; SPSS) was used for all statistical analyses. Hardy-Weinberg equilibrium was initially performed and followed by χ^2 test. The odds ratio (OR) and 95% confidence interval were calculated using unconditional logistic regression model. For statistical significant difference $p < 0.05$ was considered.

Results

The authors introduced a new melting curve determination based system for the accurate detection of the SNP polymorphism of miR-193b rs30236. The applied PCR conditions were suitable for the exact Tm assignments and Figure 1 shows the typical melting curves.

Based on the melting curve analysis of miR-193b rs30236 the authors found 30.24% T allele frequency in the patients' group, and 35.78% in the control group (p = 0.2549). They detected CC genotype in 39.22%, CT genotype in 50.00%, and TT genotype in 10.78% in controls, while they occurred in 45.35%, 48.84%, and 5.81% in the patients, respectively (p = 0.4096) (Table 1). Statistical analysis of the data did not show significant difference.

Figure 2 shows the most important target genes in a networking form. The most important target genes regulated by miR-193b are cyclinD1 (CCND1), estrogen receptor (ESR1), plasminogen activator (PLAU), prolin rich acidic protein (PRAP1) and myeloid cell leukemia sequence (MCL1).

Discussion

The growing number of oncological cases requires new diagnostic, monitoring, and therapeutic possibilities protocols. Recently microRNAs became in the focus of the studies. They have important role in the regulation of cell cycle, cell proliferation, apoptosis, and other important physiological processes [13]. They could behave as tumor suppressors or as oncogenes. MiR-17-92 is overexpressed in B-cell lymphoma and in lung cancer, while miR-21 in colon cancer [14-16]. Members of the let-7 cluster downregulated in several tumors, RAS is their target molecule [17].

Ovarian cancer is the second most common tumor in the female genital system and the fifth in the mortality statistics. In about 70% of the cases, it is diagnosed at a later stage. Beside the modern treatments the survival rate does not change [18]. The five-year survival rate could reach 90% in the case of early diagnosis. Patients having Stage III or IV have worse prognosis besides the recently developed operation techniques and modern chemotherapeutic protocols [10].

Introduction of new biomarkers helps in the early diagnosis, for that purpose miRNAs are promising aspirants. There are several studies on gene expression and determination of SNP polymorphisms is also in the focus of the research. A few reports were published on miR-193b and correlation was found with the development of resistance to chemotherapy [5, 11]. There is no published information on miR-193b rs30236 polymorphism in ovarian cancer patients.

The present authors determined the rs30236 alleles and genotypes in the population of Hungarian (Caucasian) ovarian cancer patients and healthy controls. The authors introduced a melting curve analysis for the accurate determination of the alleles. They counted allele and genotype frequencies in the studied groups. They also found information only on allele frequencies in Reference SNP Cluster Report (https://www.ncbi.nlm.hih.gov/projects/SNP). The database shows that in the European population the C allele present in 56.76%, and the T allele 43.24% in 1,008 studied persons. Results of the present study in the healthy control group are slightly different 64.22% and 35.78%. In the database European term is used and does not refer to a Caucasian population, so the authors do not know the exact source of collection [19]. The present study population was Caucasian. Based on the literature the T allele is the rarest in the Afro-American population (30%) (https://www.ncbi.nlm.hih.gov/projects/SNP). In the present work the authors did not find statistical difference in allele and genotype frequencies among the ovarian cancer patients and the healthy subjects.

The bioinformatics analysis shows from the gene network of miR-193b, that CCND1 is the most important target, regulating CDK (cyclindependent kinase). Mutations in this gene could results in overexpression, and tumor development. Dai et al. observed, that cisplatin could inhibit the expression of CCND1 in human epithelial cells [20]. Estrogen-receptor (ESR1) regulates the growth and differentiation of the ovarian epithelium, certain researchers consider it as a prognostic biomarker for the ovarian cancer [21]. Havrilesky et al. were able to divide the suitable markers into three groups, hormonal regulators of the cell division extracellular mucins; proteolitic, coagulation, and fibrinolytic pathway regulators [22]. The PRAP1 was connected with the growth of cancer cells, and a recent study considered it as an important factor in the tumor cell survival by activating of p53 [23]. MCL1 belongs to the BCL-2 antiapoptotic proteins, miRNAs have a strong regulation on them, and they have effect on the development of resistance to chemotherapy [24]. Other genes from the miR-193b network also have important role in different physiological processes.

MiR-193b has on effect on several genes participating in the development of platina resistance. Further miRNA SNP determinations could make possible to introduce diagnostic panels making possible to select patients before the beginning of chemotherapy to decide which therapy would be the most advantageous.

References

[1] Yates L.A., Norbury C.J., Gilbert R.J.: "The long and short of microRNA". *Cell,* 2013, *153*, 516.

[2] Davidson B., Tropé G.C., Reich R.: "The clinical and diagnostic role of microRNAs in ovarian carcinoma". *Gynecol. Oncol.,* 2014, *133*, 640.

[3] Weng L., Ziliak D., Im H.K., Gamazon E.R., Philips S., Nguyen A.T., *et al*.: "Genome-wide discovery of genetic variants affecting tamoxifen sensitivity and their clinical and functional validation". *Ann. Oncol.,* 2013, *24*, 1867.

[4] Unno K., Zhou Y., Zimmerman T., Platanias L.C, Wickrema A.: "Identification of a novel microRNA cluster miR-193b-365 in multiple myeloma". *Leuk. Lymphoma,* 2009, *50*, 1865.

[5] Rauhala H.E., Jalava S.E., Isotalo J., Bracken H., Lehmusvaara S., Tammela T.L., *et al*.: "miR-193b is an epigenetically regulated putatitive tumor supressor in prostate cancer". *Int. J. Cancer*, 2010, *127*, 1363.

[6] Lujambio A., Ropero S., Ballestar E., Fraga M.F., Cerrato C., Setién F., *et al*.: "Genetic unmasking of an epigenetically silenced microRNA in human cancer cells". *Cancer Res.,* 2007, *67*, 1424.

[7] Yong L.F., Law W.C., Wang W.C.: "Potentiality of triple microRNA classifier: miR-193a-3p, miR-23a and miR-339-5p for early detection of colorectal cancer". *BMC Cancer,* 2013, *13*, 280.

[8] Fang C., Li X.P., Gong W.J., Wu N.Y., Tang J., Yin J.Y., *et al*.: "Age-related common miRNA polymorphism associated with severe toxicity in lung cancer patients treated with platinum-based chemotherapy". *Clin. Exp. Pharmacol. Physiol.,* 2016, *44*, 21.

[9] Hu Z., Shu Y., Chen Y., Dong J., Liu Y., Pan S., *et al.*: "Genetic polymorphism in precursor microRNA flanking region and non-small cell lung cancer survival". *Am. J. Respir. Crit. Care Med.,* 2011, *183*, 641.

[10] Leivonen S.K., Makela R., Ostling P., Kohonen P., Haapa-Paananen S., Kleivi K., *et al.*: "Protein lysate microarray analysis to identify microRNAs regulating estogen receptor signaling in breast cancer cell lines". *Oncogene,* 2009, *28*, 3926.

[11] Nakamura K., Sawada K., Yoshimura A., Kinose Y., Nakatsuka E.,

Kimura T.: "Clinical relevance of circulating cell-free microRNAs in ovarian cancer". *Mol. Cancer,* 2016 ,*15*, 48.
[12] Ziliak D., Gamazon R.E., Lacroix B., Kyung I. H., Wen Y., Huang R.S.: "Genetic variation that predicts platinum sensitivity reveals the role of miR-193b in chemotherapeutic susceptibility". *Mol. Cancer Ther.,* 2012, *11*, 2054.
[13] Nagy B., Bán Z., Papp Z.: "The DNA isolation method has effect on allele drop out and on the results of fluorescent PCR and DNA fragment analysis". *Clin. Chim. Acta,* 2005, *360*, 128.
[14] Huang R.S., Johnatty E.S., Gamazon R.E., Im H.K., Ziliak D., Duan S., *et al*.: "Platinum sensitivity-related germline polymorphism discovered via a cell-based approach and analysis of its association with outcome in ovarian cancer patients". *Clin. Cancer Res.,* 2011, *17*, 5490.
[15] Torres A., Torres K., Maciejewski R., Harwey M.H.: "MicroRNAs and their role in gynecological tumors". *Med. Res. Rev.,* 2011, *31*, 895.
[16] He L., Thompson J.M., Hemann M.T., Hernando-Monge E, Mu D, Goodson S., *et al.:* "A microRNA polycistron as a potential human oncogene". *Nature,* 2005, *435*, 828.
[17] Schetter A.J., Leung S.Y., Sohn J.J., Zanetti K.A., Bowman E.D., Yanaihara N., *et al.:* "MicroRNA expression profiles associated with prognosis and therapeutic outcome in colon adenocarcinoma". *J. Am. Med. Assoc.,* 2008, *299*, 425.
[18] Johnson S.M., Grosshans H., Shinghara J., Byrom M., Jarvis R., Cheng A., *et al*.: "RAS is regulated by the *let-7* microRNA family. *Cell,* 2005, *120*, 13944.
[19] Cannistra S.A.: "Cancer of the ovary". *N. Engl. J. Med.,* 2004, *51*, 2519.
[20] Dai J., Wei R-J, Li R., Feng J.B., Yu Y.L., Liu P.S.: "A study of CCND1 with epithelial ovarian cancer cell proliferation and apoptosis". *Eur. Rev. Med. Pharmacol. Sci.,* 2016, *20*, 4230.
[21] Zamagni C., Wirtz M..R.., De Iaco P., Rosati M., Veltrup E., Rosati F., *et al.:* "Oestrogen receptor a mRNA is a prognostic factor in ovarian cancer patients treated with neo-adjuvant chemotherapy: determination by array and kinetic PCR in fresh tissue biopsies. *Endocr. Relat. Cancer*, 2009, *16,* 1241.
[22] Havrilesky J.L., Whitehead M.C., Rubatt M.J., Cheek R.L., Groelke J., He Q., *et al*.: "Evaluation of biomarker panels for early stage ovarian cancer detection and monitoring for disease recurrence". *Gynecol. Oncol.*, 2008, *110,* 374.
[23] Huang H.B., Zhuo L.J., Leung W.H.C., Lu G.D., Liu J.J., Yap C.T., Hooi S.C.: "PRAP1 is a novel executor of p53-dependent mechanisms in cell survival after DNA damage". *Cell Death Dis.*, 2012, *3*, e442.
[24] Sugio A., Iwasaki M., Habata S.. Mariya T., Suzuki M., Osogami H., *et al*.: "BAG3 upregulates MCL-1 through downregulation of miR-29b to induce anticancer drug resistance in ovarian cancer". *Gynecol. Oncol.,* 2014, *134*, 615.

Corresponding Author:
BALINT NAGY, Ph.D., D.Sc.
Egyetem tér 1
4032 Debrecen (Hungary)
e-mail: nagy.balint@med.unideb.hu