Research Article

miRNA519a-3p and NKG2DL in Endometriosis

Ekspresi miRNA519a-3p dan NKG2DL pada Endometriosis

Cepi T. Pramayadi, Muharam Natadisastra, Kanadi Sumapradja, Aria Kekalih

Division of Reproductive Health
Department of Obstetrics and Gynecology
Faculty of Medicine Universitas Indonesia
Dr. Cipto Mangunkusumo General Hospital
Jakarta

Abstract

Objective: To investigate the correlation between miRNA-519a-3p expression with NKG2D ligands (MICA, MICB, ULBP 1-6) on endometriosis and non-endometriosis patients.

Methods: This was a cross-sectional study held in five centers: dr. Cipto Mangunkusumo General Hospital, Pelni Hospital, Bunda Hospital, YPK Mandiri Hospital, and Primaya Evasari Hospital from October 2020 to July 2021. miRNA and NKG2DL analysis were done in *Human Reproduction, Infertility and Family Planning* (HRIFP) cluster at IMERI FKUI.

Results: We obtained 19 patients in each study groups. NKG2D ligands and miRNA519a-3p relative expressions were not significantly different ($p > 0.05$). Increased miRNA519a-3p expression negatively affected NKG2D ligands expression. A decrease in ULBP1 and an increase in ULBP2 increased the probability for endometriosis. NKG2D ligands expression may be influenced by infection, pro-inflammatory cytokine production, dan polymorphism. NKG2D ligands expression level can be different depending on the origin of the sample. Lower expression of miRNA519a-3p indirectly inhibits tumor apoptosis by lowering NKG2D ligands, caspase, or mRNA.

Conclusion: We did not manage to establish a correlation between NKG2D ligands with miRNA519a-3p in endometriosis and non-endometriosis patients.

Keywords: apoptosis, endometriosis, immunologic cytotoxicity, miRNA519a-3p, NK cells, NKG2D ligands.

Abstrak

Tujuan: *Mengetahui korelasi antara ekspresi miRNA-519a-3p dengan ligan NKG2D (MICA, MICB, ULBP 1-6) pada pasien endometriosis dan non-endometriosis.*

Metode: *Studi ini merupakan studi potong lintang yang diadakan di lima pusat Kesehatan: Rumah Sakit (RS) dr. Ciptomangunkusumo, RS Pelni, RS Bunda, RS YPK mandiri, dan RS Primaya Evasari dari Oktober 2020 hingga Juli 2021. Analisis dattat mirNA dan NKG2DL dilakukan di cluster Human Reproduction, Infertility, and Family Planning (HRIFP) IMERI FKUI.*

Hasil: *Studi ini diikuti oleh 19 pasien di setiap kelompok. Ekspresi relatif ligan NKG2D dan miRNA519a-3p tidak bermakna secara signifikan ($p > 0.05$). Peningkatan ekspresi miRNA519a-3p menurunkan ekspresi ligan NKG2D. Penurunan ULBP1 dan peningkatan ULBP2 berhubungan dengan meningkatnya kemungkinan endometriosis. Ekspresi ligan NKG2D dapat dipengaruhi oleh infeksi, produksi sitokin pro-inflamasi, dan polimorfisme. Tingkat ekspresi ligan NKG2D dapat berbeda-beda berdasarkan asal sampel jaringan. Ekspresi miRNA519a-3p yang rendah secara tidak langsung meenghambat apoptosis tumor melalui penurunan ligan NKG2D, caspase, atau mRNA.*

Kesimpulan: *Tidak terdapat korelasi antara ekspresi miRNA519a-3p dengan ligan NKG2D pada pasien endometriosis dan non-endometriosis.*

Kata kunci: *apoptosis, sitotoksisitas imun, endometriosis, ligan NKG2D, miRNA519a-3p, sel NK.*

Correspondence author. Cepi T. Maryadi.Department of Obstetrics and Gynecology.
Faculty of Medicine Universitas Indonesia. Dr. Cipto Mangunkusumo General Hospital. Jakarta
Email:cteguh@yahoo.com

Received: September,2021 Accepted: September,2022 Published: October,2022

INTRODUCTION

Endometriosis accounts for 15-25% cause of female infertility in Indonesia and is commonly found in reproductive age.[1,2] Undetected endometriosis are represented with persistent and progressive symptoms which negatively affect patients' wellbeing and comfort.[3] Literatures have shown that 70% of endometriosis patients may present with chronic pelvic pain and 15-20% are in risk of developing endometrioma. Infections are quite common in endometriosis patients, which can be found in 30-40% patients. Depression and anxiety disorders are not uncommon in endometriosis patients.[1,4]

miRNAs are specialized in controlling human innate and adaptive immune systems, mainly in autoimmune diseases and cancers.[5,6] Studies have shown several types of miRNAs effect on regulating natural killer (NK) cells activity. For instance, miRNA-150 and miRNA-180 may inhibit Nemo like kinase (NLK) to prevent NK cell maturation. Another type of miRNA, such as miRNA-203, miRNA494-3p- and miRNA-155 may affect PTEN-AKT-mTOR pathway to affect apoptosis.[7–9] miRNA-519 has been found to correlate with ovarian carcinoma and breast cancer. High expression of particular member of miRNA-519, miRNA-519a-3p, has been found to lower TNF-related apoptosis inducing ligand (TRAIL)-R2, caspase-7, and caspase-8 in breast cancer to avoid TRAIL or Fas ligand-mediated apoptosis. miRNA-519a-3p can bind to some ligands of NK cell receptor group 2 member D (NKG2D), namely ULBP2 and MICA to intercept tumour recognition, NK cell activation, and provides the tumour cell with resistance to granzyme B.[5,10]

Binding of NKG2D receptor in NK cells with its ligands maintains cell structure integrity as a response to DNA damage and promotes NK-cell mediated apoptosis.[11,12] However, overexpression of NKG2D ligands in ectopic endometriosis cells may signify a cytotoxic avoidance mechanism of NK cells.[13] Previous studies have shown various different results on the effect of NKG2D ligands and miRNA expression in neoplasms.[14,15]

METHODS

This was a cross-sectional study conducted from October 2020 through July 2021 in five different centers: Dr. Cipto Mangunkusuomo General Hospital, Pelni Hospital, Bunda Hospital, YPK Mandiri Hospital, and Primaya Evasari Hospital. This study has been approved by the Ethics Committee of the Faculty of Medicine, Universitas Indonesia – Dr. Cipto Mangunkusumo Hospital (KET-441/UN2.F1/ETIK/PPM.00.02/2021) in June 2020. Subjects included in this study were female with or without endometriosis aged 20-45 years old, not in pregnancy, confirmed not having other malignancies such as cervical cancer, severe cervical stenosis, nor other genital tract malignancies, and has agreed to informed consent. We targeted a minimum sample size of 19 samples using correlative analytic formula. Sampling was done through consecutive sampling method. Both endometriosis and endometrium samples were obtained through operative hysteroscopy and laparoscopy. miRNA and NKG2D ligands analysis were done through standard quantitative real-time PCR method. Data analysis were done through non-parametric analysis using IBM Statistical Package for the Social Sciences (SPSS) version 26.0.

RESULTS

We obtained a total of 29 patients with endometriosis and 26 patients without endometriosis. Average age of patients in endometriosis and control group were 33 years old and 36 years old, respectively. Patients in control group had higher body mass index (BMI) (25.63 + 4.27 kg/m^2) than those in endometriosis group (24.92 + 4.89 kg/m^2). Our endometriosis patients had menarche at slightly later age (12.52 + 0.99 years old) rather than those in control group (12.48 + 1.24 years old). Most samples were taken during the proliferative phase of endometrium. Subjects' characteristics can be seen on Table 1.

Table 1. Subjects Characteristics

Variables	Endometriosis (n = 29)	Control (n = 26)
Age (years old)	33.89 + 5.33	36.27 + 6.79
Weight (kg)	61.78 + 12.22	63.90 + 10.18
Height (m)	1.57 + 0.05	1.58 + 0.06
BMI	24.92 + 4.89	25.63 + 4.27
Menarche age (years old)	12.52 + 0.99	12.48 + 1.24
Endometrium phase		
Proliferative	13	9
Secretory	10	7
Unknown	6	10

Relative expression and fold changes for each NKG2D ligands and miRNA-519a-3p was assessed through Livak's formula. We excluded

10 subjects from endometriosis group and 7 from control group due to extreme cycle threshold (CT) value which may produce inaccurate results. We divided the samples into two groups: tumour compared to control (TC) and endometriosis endometrium compared to control (EC). MICA expression in endometriosis tissue was found to be 2.4 times lower (0.42 + 0.79) than in control group. We found that MICB expression in endometriosis tissue was 100 times lower (0.01 + 0.02) than control group. ULBP1, ULBP2, ULBP3, and ULBP6 were found to be 3.7 times (0.27 + 0.46), 33.3 times (0.03 + 0.06), 5 times (0.20 + 0.52), and 12,5 times (0.08 + 0.11) lower than control group. Higher expression of MICA (1.09 times; 1.09 + 3.12), ULBP1 (5.64 times; 5.64 + 13.51), ULBP4 (7.49 times; 7.49 + 12.91), and ULBP5 (5.75 times; 5.75 + 8.90) were found in endometrium tissue of endometriosis patients. Relative expression of miRNA-519a-3p were 11.1 times lower in tumour tissue (0.09 + 0.24) and 5.55 times lower in endometriosis patients' endometrium (0.18 + 0.40).

Table 2. Relative Expression of NKG2D Ligands and miRNA519a-3p Compared to Control

Variables	TC	EC
MICA	0.42 + 0.79	1.09 + 3.12
MICB	0.01 + 0.02	0.02 + 0.05
ULBP1	0.27 + 0.46	5.64 + 13.51
ULBP2	0.03 + 0.06	0.04 + 0.05
ULBP3	0.20 + 0.52	0.25 + 0.42
ULBP4	1.65 + 2.40	7.49 + 12.91
ULBP5	2.99 + 5.05	5.75 + 8.90
ULBP6	0.08 + 0.11	0.44 + 0.80
miRNA-519a-3p	0.09 + 0.24	0.18 + 0.40

There was no significant difference of NKG2D ligands and miRNA-519a-3p expression between TC and EC (p value > 0.05). Lower median value of ULBP1, ULBP3, ULBP4, ULBP5, and miRNA-519a-3p were observed in tumour tissue compared to control group.

Table 3. Comparative Analysis of NKG2D Ligands and miRNA519a-3p

NKG2D ligands	TC	EC	*P- value*
MICA	0.17 (0.01-3.53)	0.17 (0.02-13.74)	0.804
MICB	0.02 (0.0003-0.11)	0.01 (0.00-0.19)	0.589
ULBP1	0.11 (0.01-1.89)	0.19 (0.002-50.21)	0.397
ULBP2	0.01 (0.0008-0.22)	0.01 (0.0004-0.17)	0.579
ULBP3	0.05 (0.005-2.31)	0.07 (0.001-1.84)	0.226
ULBP4	0.65 (0.00-8.82)	0.82 (0.02-50.21)	0.274
ULBP5	1.36 (0.03-21.56)	1.43 (0.01-31.34)	0.358
ULBP6	0.04 (0.0007-0.42)	0.03 (0.001-3.12)	0.422
miRNA-519a-3p	0.01 (0.001-1.01)	0.03 (0.0002-1.72)	0.405

Correlative analysis in this study was carried using Spearman's analysis. This research found that miRNA-519a-3p correlates negatively with MICA (r = -0.305), MICB (r = -0.009), ULBP1 (r = -0.107), ULBP2 (r = -0.128), ULBP3 (r = -0.128), and ULBP5 (r = - 0.126) expression in endometrium tissue of endometriosis patients. Higher miRNA519a-3p expression may depress most of NKG2D ligands expression save for MICB (r = 0.088) and ULBP 1 (r = 0.126) in endometriosis tumour tissue.

Table 4. Correlation of miRNA-519a-3p and NKG2D Ligands Expression

Variables	TC		TC	
	r	*p-value*	r	*p-value*
MICA	-0.125	0.611	-0.305	0.204
MICB	0.088	0.721	-0.009	0.972
ULBP1	0.126	0.606	-0.107	0.663
ULBP2	-0.082	0.737	-0.128	0.601
ULBP3	-0.244	0.314	-0.128	0.601
ULBP4	-0.047	0.847	0.074	0.764
ULBP5	-0.040	0.870	-0.126	0.606
ULBP6	-0.144	0.557	0.100	0.684

DISCUSSION

This study reported the correlation and differences in expression between miRNA519a-3p and the NKG2D ligands in endometriosis and non-endometriosis patients. Ectopic endometrial tumour tissue had higher expression of NKG2D and miRNA519a-3p ligands than endometrial eutopic tissue in similar patients. ULBP4 and ULBP5 are NKG2D ligands were elevated in tumour and eutopic endometrial tissue of endometriosis patients compared to control group. Enhanced expression of ULBP4 and ULBP5 did not increase the likelihood of endometriosis, except for ULBP1 and ULBP2. Difference in relative expression of NKG2D ligands and miRNA519a-3p between ectopic endometriosis and endometrial tissue of endometriosis patients was not statistically significant. Relative expression of miRNA519a-3p tends to negatively correlated with most of NKG2D ligands, although this correlation is statistically insignificant. The expression of NKG2D ligands and miRNA519a-3p plays a major role in determining the diagnosis of endometriosis.

We found an increase in relative expression of MICA in endometrium tissue (1.09 + 3.12) and tumour tissue of endometriosis patients (0.42 + 0.79) compared to control. Further analysis showed that the difference in MICA expression was not statistically significant (p = 0.804). This finding is reciprocal with a study in 2012 [16] which found lower expression of MICA and ULBP2 in breast cancer cells due to the influence of miRNA-519a-3p could attenuate the activation and cytotoxicity of NK cells. Our findings are in contrary with study by Xu and colleagues[15] which found no change in the expression of MICA, MICB, and ULBP-1 in endometriosis patients. The discrepancy between the results of this research and other studies may be due to histological factors and differences in the ELISA method used in the previous study.[17]

MICB expression was found to be lower in the endometrium in the endometriosis group (0.02 + 0.05) and in the endometrial tumour tissue (0.01 + 0.02) compared to the control and not statistically significant (p = 0.589). Similar findings were found in a study [15] which found that MICA/MICB protein expression did not show any difference/ increase, compared to normal endometrial tissue. This finding is in contrast [18] who stated that soluble MICB levels were found to be elevated in serum in many patients with gastric, colon, and rectal carcinomas. Any difference in soluble MICA and MICB levels might be due to differential degradation in human serum.[19]

MICA, ULBP 1, 2, and 3 can be increased in infection or tumour transformation, Increased MICA, ULBP1, 2, and 3 may promote activation of NK cells.[20] Our results found that endometrial tissue of endometriosis patients had higher ULBP1 expression (5.64 + 13.51) than tumour tissue of the same patient (0.27 + 0.46) and control patients. The difference between these ULBP1 relative expression was not statistically significant (p = 0.397). DNA damage may induce ULBP transcription.[21]

Decreased ULBP2 expression was found in endometrial tissue (0.04 + 0.05) and ectopic tumour tissue of endometriosis tumour (0.03 + 0.06) compared to control with no statistical significance in difference (p = 0.579). This finding is in line who found that the expression of ULBP2 in endometriosis patients was much lower than in patients without endometriosis.[15] Decreased expression of ULBP2 and ULBP3 may help patients' eutopic/ectopic endometrial cells escape immune surveillance, and may also play a role in the reduction of NKG2D-mediated NK cell cytotoxicity. Contradicting evidence was found in a study by Gonzalez-Foruria et al., (2015)[17] who found that ULBP2 levels were found to be significantly higher in 27 endometriosis patients compared to controls (p = 0.012). Disparity between RNA and protein expression occurs in the process of translation. This implies that protein molecules are separated from the cell membrane, and therefore, cannot be detected in the endometrium.[15]

Endometrial ectopic tissue was found to have lower ULBP3 expression than endometrial tissue in endometriosis and non-endometriosis patients.[15] Our study produced a similar result, in which we observed a decreased ULBP3 expression in endometrial tissue (0.25 + 0.42) and in endometriosis tumour tissue of patients with endometriosis (0.20 + 0.52) compared to control. The disparity of ULBP3 expression held no statistical significance (p = 0.226). Contrary to our finding, found high ULBP3 expression in ovarian carcinoma tissue. Elevated ULBP3 expression was positively and significantly associated with ovarian carcinoma recurrence and lower survival rates compared to patients with low ULBP3.[22] Differences in ULBP3 expression in this study with previous studies could be influenced by histological factors. NK cells mediated immune system may act differently in certain epithelial

tissues.

We managed to found higher average ULBP4 expression in endometrial tissue of patients with endometriosis (7.49 + 12.91) and in subsequent tumour tissue endometriosis (1.65 + 2.40) compared to control. This difference was not statistically significant (p = 0.274). ULBP4 expression is generally only found in human monocyte cells and cannot be found in large numbers in NK cells. ULBP4 expression was increased in cervical cancer tissue and squamous carcinoma of the larynx, renal pelvis, colon, thyroid, and ovaries[23]. ULBP4 expression in malignant cells is affected by levels of TGFβ. Decreased TGFβ expression enhances ULBP4 and other NKG2D ligands such as MICA and ULBP2. TGFβ produced by malignant cells suppresses NKG2D receptors expression on NK cells through paracrine pathways and decrease NKG2D ligands on cancer cells surface through autocrine pathways. These two pathways provide invisibility from NK cells.[24]

The average of ULBP5 expression was found higher in endometrial tissue of patients with endometriosis (5.75 + 8.90) and in tumour tissue of patients with endometriosis (2.99 + 5.05) than in control, but was not statistically significant (p = 0.358). The expression of ULBP5 in patients without malignancy is generally low in healthy tissues.[25] The increase in ULBP5 can be caused by increased succinate, inhibition of fumarate hydratase activity, and decreased glutathione activity. Fumarate accumulation causes oxidative stress that triggers the expression of NKG2D ligands such as ULBP2 and ULBP5.[26]

Suppressed ULBP6 expression was found in endometrial tissue of patients with endometriosis (0.44 + 0.80) and in tumour tissue of patients with endometriosis (0.08 + 0.11). We found no statistical significance in difference between these two samples (p = 0.422). Our result contradicted another study which showed a 5-fold increase in ULBP6 on patients with haematological malignancy compared to ULBP6 in those without.[27] Variations in ULBP6 expression are regulated by ULBP6 gene polymorphisms. Single nucleotide mutation on V52F gene was found to reduce the production and cytotoxic activity of ULBP6.[28] Increased NKG2D ligand expression is widely known to reinforce NK cell activity in suppressing tumour growth. However, overly alleviated expression of ULBP6 may cripple NK cell action.

MiRNA519a-3p expression decreased the production of mRNA, caspase, and NKG2D ligand in breast cancer cells. This activity inhibited cancer cell apoptosis and NK cell cytotoxicity.[5] Our study found diminished miRNA519a-3p expression in endometrial tissue (0.18 + 0.40) and tumour tissue endometriosis patients compared to the control group. Healthy patients were found with higher miRNA519a-3p expression than tumour tissue (0.09 + 0.24). Similar results were expressed who found a decrease in miRNA-519a-3p expression in breast cancer patients.[29] Different result was presented on liver cancer cells. Increased miRNA519a was associated with a larger cancer size, metastases, higher grading, and worse outcome.[30]

Polymorphisms or other gene mutations related to NKG2D ligand expression may be the discrepancy source of this study's results with those of previous studies. We did not conduct an investigation at gene level, which might be one of several flaws of this study. We were only able to obtain samples from patients with stage 2 to stage 4 endometriosis, making this study not able to profile the expression of NKG2D ligand and miRNA519a-3p in patients with stage 1 endometriosis. Another shortcoming of this study was due to coronavirus disease 2019 (COVID-19) pandemic, which hampered the sampling process due to the limited use of general anaesthesia in order to prevent the spread of the virus. Results from this study require further study with larger sample to provide more valid results.

CONCLUSION

Increased miRNA-519a-3p may be followed by low NKG2D ligands expression. We did not find any significant difference between relative expressions of NKG2D ligands and miRNA-519a-3p in endometrium and tumour tissue on endometriosis patients. miRNA-519a-3p and NKG2D ligands assay may be a plausible future non-invasive diagnostic method for endometriosis.

ACKNOWLEDGEMENTS

The authors declared no conflicts of interest/ competing interest.

REFERENCES

1. Mukti P. Faktor Risiko Kejadian Endometriosis. *Unnes J Public Heal.* 2014;3(3):1–10.
2. Fuldeore MJ, Soliman AM. Prevalence and Symptomatic Burden of Diagnosed Endometriosis in the United States: National Estimates from a Cross-Sectional Survey of 59,411 Women. *Gynecol Obstet Invest.* 2016;82(5):453–61.
3. Agarwal SK, Chapron C, Giudice LC, et al. Clinical diagnosis of endometriosis: a call to action. *Am J Obstet Gynecol.* 2019;220(4):1–12.
4. Sourial S, Tempest N, Hapangama DK. Theories on the Pathogenesis of Endometriosis. *Endometr Sci Pract.* 2014;2014:453–61.
5. Breunig C, Pahl J, Küblbeck M, et al. MicroRNA-519a-3p mediates apoptosis resistance in breast cancer cells and their escape from recognition by natural killer cells. *Cell Death Dis.* 2017;8(8):1–12.
6. Momen-Heravi F, Bala S. miRNA regulation of innate immunity. *J Leukoc Biol.* 2018;103(6):1205–17.
7. Pesce S, Greppi M, Ferretti E, et al. miRNAs in NK Cell-Based Immune Responses and Cancer Immunotherapy. *Front Cell Dev Biol.* 2020;8:1–13.
8. Pesce S, Squillario M, Greppi M, et al. New miRNA signature heralds human NK cell subsets at different maturation steps: Involvement of miR-146a-5p in the regulation of KIR expression. *Front Immunol.* 2018;9:1–18.
9. Beaulieu AM, Bezman NA, Lee JE, et al. MicroRNA function in NK cell biology. *Immunol Rev.* 2013;253(1):40–52.
10. Bassani B, Baci D, Gallazzi M, et al. Natural killer cells as key players of tumor progression and angiogenesis: Old and novel tools to divert their pro-tumor activities into potent anti-tumor effects. *Cancers (Basel).* 2019;11(4):1–29.
11. Zingoni A, Molfetta R, Fionda C, et al. NKG2D and its ligands: "One for all, all for one." *Front Immunol.* 2018;9:1–12.
12. Duan S, Guo W, Xu Z, et al. Natural killer group 2D receptor and its ligands in cancer immune escape. *Mol Cancer.* 2019;18(1):1–14.
13. Yang S, Wang H, Li D, et al. Role of endometrial autophagy in physiological and pathophysiological processes. *J Cancer.* 2019;10(15):3459–71.
14. Kim TH, Kim YK, Kwon Y, et al. Deregulation of miR-519a, 153, and 485-5p and its clinicopathological relevance in ovarian epithelial tumours. *Histopathol.* 2010;57(5):734–43.
15. Xu H. Expressions of natural cytotoxicity receptor, NKG2D and NKG2D ligands in endometriosis. *J Reprod Immunol.* 2019;136(February):1–7.
16. de Kruijf EM, Sajet A, van Nes JGH, et al. NKG2D ligand tumor expression and association with clinical outcome in early breast cancer patients: An observational study. *BMC Cancer.* 2012;12(24):1–20.
17. González-Foruria I, Santulli P, Chouzenoux S, et al. Soluble ligands for the NKG2D receptor are released during endometriosis and correlate with disease severity. *PLoS One.* 2015;10(3):1–15.
18. Salih HR, Rammensee H-G, Steinle A. Cutting Edge: Down-Regulation of MICA on Human Tumors by Proteolytic Shedding. *J Immunol.* 2002;169(8):4098–102.
19. Salih HR, Goehlsdorf D, Steinle A. Release of MICB Molecules by Tumor Cells: Mechanism and Soluble MICB in Sera of Cancer Patients. *Hum Immunol.* 2006;67(3):188–95.
20. Eleme K, Taner SB, Önfelt B, et al. Cell Surface Organization of Stress-inducible Proteins ULBP and MICA That Stimulate Human NK Cells and T Cells via NKG2D. *J Exp Med.* 2004;199(7):1005–10.
21. Jones S, Zhang X, Parsons DW, et al. Core signaling pathways in human pancreatic cancers revealed by global genomic analyses. *Science (80-).* 2008;321(5897):1801–6.
22. Lee GH, An HJ, Kim TH, et al. Clinical impact of natural killer group 2d receptor expression and that of its ligand in ovarian carcinomas: A retrospective study. *Yonsei Med J.* 2021;62(4):288–97.
23. Lazarova M, Steinle A. Impairment of NKG2D-Mediated Tumor Immunity by TGF-β. *Front Immunol.* 2019;10:10–3.
24. Fujita H, Hatanaka Y, Sutoh Y, et al. Immunohistochemical Validation and Expression Profiling of NKG2D Ligands in a Wide Spectrum of Human Epithelial Neoplasms. *J Histochem Cytochem.* 2015;63(3):217–27.
25. Ruan GT, Wang S, Zhu LC, et al. Investigation and verification of the clinical significance and perspective of natural killer group 2 member D ligands in colon adenocarcinoma. *Aging (Albany NY).* 2021;13(9):12565–86.
26. Høgh RI, Droujinine A, Møller SH, et al. Fumarate Upregulates Surface Expression of ULBP2/ULBP5 by Scavenging Glutathione Antioxidant Capacity. *J Immunol.* 2020;204(7):1746–59.
27. Zuo J, Willcox CR, Mohammed F, et al. A disease-linked ULBP6 polymorphism inhibits NKG2D-mediated target cell killing by enhancing the stability of NKG2D ligand binding. *Sci Signal.* 2017;10(481):1–15.
28. Soremekun OS, Soliman MES. From genomic variation to protein aberration: Mutational analysis of single nucleotide polymorphism present in ULBP6 gene and implication in immune response. *Comput Biol Med.* 2019;11:103354. https://doi.org/10.1016/j.compbiomed.2019.103354
29. Ren L, Li Y, Zhao Q, et al. MiR-519 regulates the proliferation of breast cancer cells via targeting human antigen R. *Oncol Lett.* 2020;19(2):1567–76.
30. Tu K, Liu Z, Yao B, et al. MicroRNA-519a promotes tumor growth by targeting PTEN/PI3K/AKT signaling in hepatocellular carcinoma. *Int J Oncol.* 2016;48(3):965–74.