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Biomarker potential of the *GRP78* cell-free RNA in endometrial cancer

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Abstract

Background: Circulating tumor cells represent an opportunity for the assessment of early recurrent disease or for real-time tracing of cancer. Glucose Regulated Protein 78 (*GRP78*) is known in the literature as a stress factor in endometrial cancer. We aimed to investigate the importance of the gene by targeting tumor traces circulating in the cell fluids of patients with Type 1 endometrial cancer, examining cell-free RNAs in patients' samples and performing ROC analysis.

Methodology: In this study, 32 endometrial cancer patients and 20 controls were included. This in vitro study evaluated, the *GRP78* cell-free mRNA expression levels in endometrial cancer patients, by quantitative real-time polymerase chain reaction qRT–PCR Light Cycler. Receiver operating characteristic (ROC) analysis is a tool used to identify the precision of a diagnostic test or prediction model. In our study, we investigated whether the expression levels of cell-free *GRP78* mRNA could be used as a diagnostic criterion.

Results: The ROC curve results for endometrial cancer diagnostic criterion of cfRNA *GRP78* mRNA indicated quite a significant value ($p < 0.001$).

Conclusion: Current findings show that cell-free mRNA *GRP78* is now a criterion that can be used together with smear mRNA *GRP78* without the need for invasive methods in endometrial cancer studies.

Keywords: Endometrium cancer, *GRP78*, Cell-free RNA

Background

Endometrial cancer (EC) is the fourth most common malignancy seen in females and its incidence is on the rise [1]. Around 320,000 females are recorded with EC every year. According to recent studies among these females, approximately 60.800 of them will severely suffer from a lethal version of the disease or a cause related to the disease in the next 5 years [2]. The survival rate is 95% if diagnosed in stage 1; but if the tumor has made a distant metastasis by the time of the diagnosis the number declines to 17% [3]. Cancer biopsies offer a lot of information on the planning of treatment. The benefits of biopsies include the ability to provide information about the type, aggressiveness, spreading path, immunological and molecular characterization of the samples taken in addition to the official diagnosis of cancer. However, biopsies have limitations. Firstly, as a cancer cell grows, it can spread to different areas that can change over time. Tumor biopsies taken when the disease was first diagnosed, may not reflect the later status of cancer. Secondly, repeating a biopsy to monitor cancer development can cause possible complications such as pain, infection, and bleeding. Thirdly, cancer cells that spread to different parts of the body may be different from cancer in the area where they started. For this reason, it is not possible for a tumor biopsy taken from a part of the body to adequately represent cancer in the body [4]. Liquid biopsies may be

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more suitable for observing cancer over time because the tumor shows heterogeneity, as well as biopsies taken from different parts, are likely to yield different results. In addition, chemotherapy and other radiological treatments taken during treatment do not provide accurate results about the patient's condition and the status of cancer. The disease recurs as a result of minimal residual tumor cells. On the other hand, liquid biopsies target different cancer cells that vary according to the changing characteristic structure of the tumor. The target therapeutic agent *GRP78* can send targets of small circulating cfRNAs. Because *GRP78* can target ever-changing cells, the information provided about cancer may be more informative [5]. There are cancer-related biomarkers in the blood. These include circulating tumor cells (CTCs) and tumor-derived cell-free DNA and RNA (cfDNA and cfRNA) that specifically target the tumor [6]. Extracellular circulating cell-free RNA (ccfRNA) are molecules that can be found in various liquids and can be measured. In studies conducted so far, it has been seen that ccfRNA is used in the investigation of disease causes such as diabetes, trauma, stroke, and myocardial infarction [7]. On the other hand, a relationship between the number of ccfRNA obtained from urine, blood, and other body fluids and cancer cell-derived RNAs in the plasma of cancer patients has been detected. In our study, we aimed to investigate the expression differences by investigating the *GRP78* gene, which is the ER stress gene, both in plasma and other fluids because it can give us more specific results for cancer detection.

Tumor cells and *GRP78*

The binding immunoglobulin protein, known as *GRP78*, is a human protein that is encoded by the *HSPA5* gene [8]. It is located in the lumen of the ER and binds to newly synthesized proteins as they are translocated to the ER keeping them ready for folding and oligomerization. Under normal circumstances, *GRP78* is bound to three transducers, which are activating transcription factor 6 (*ATF6*), protein kinase RNA-like endoplasmic reticulum kinase (*PERK*), and Inositol-requiring enzyme 1 (*IRE1*) [9] keeping them inactive. When there is an imbalance between the ER's capacity to fold proteins and the demand for protein folding, it causes ER stress, caused by physiological or pathological imbalances. As a response to ER stress, most eukaryotic cells have evolved as a group of signal transduction pathways, called the unfolded protein response (UPR) [10]. When UPR is activated, *GRP78* separates itself from the other *ATF-6, IRE-1* and *PERK* chaperons. Activation of these chaperons results in upregulation of proteins involved in chaperoning misfolding proteins, protein folding, and *ER-*Associated Degradation (ERAD), including further

production of *GRP78.* As a result, ER's capacity to fold proteins increase. Meanwhile, the same response can lead a cell to apoptosis if intracellular hemostasis cannot be achieved [11]. The stress response is how a cell protects itself in situations when its protein production cannot keep up with the demand. This response allows the cell to survive through otherwise not survivable situations, such as glucose deprivation, hypoxia, and changes in calcium homeostasis [12]. Tumor cells are rapidly dividing much faster than normal cells, causing a higher need for energy to function, resulting in the consumption of enormous amounts of glucose. Solid tumors outgrow their blood supplies' ability to provide for them, thus creating situations that cause constant ER stress due to tumor cells being nearly always hypoxic and /or glucose deprived [13]. On the other hand, ER stress contributes to cytotoxic effects, inducing apoptosis while observing the molecule's potential usage as a biomarker only, there will be no consequence if the cell undergoes apoptosis or manages to proliferate, and the stress is equilibrated in both scenarios. In addition to the overexpression of *GRP78,* a positive association between *GRP78* and malignancy can be observed in the presence of cancer cells [14].

Methods

Study design and subjects

This in vitro study was carried out to evaluate the *GRP78* cell-free mRNA expression levels in endometrial cancer identified in female patients. It was a collaboration between Erciyes University, Department of Medical Genetics, and Erciyes University Department of Gynecology and Oncology conducted between January and December 2019.

Patient group

Volunteers aged 40–75; EC patients were included in the study. In total, according to power analysis, 32 EC patients were included in the study. These were selected from the patients who applied to the Erciyes University Department of Gynecology and were diagnosed with Type-1 EC according to Figo criteria. Patients with a history of other diseases and drug use were excluded. In addition, peripheral blood samples were taken from the patients to obtain leukocytes and cfRNA. *GRP78* mRNA expression was examined from all samples.

Control group

For this study, the control group consisting of 20 females aged between (40 and 65) years have been enrolled. All females were examined by the gynecologist and recruited based on not taking any drugs, and smoking so as not to affect their medical situation for at least 3 months before the study, as well as not being pregnant or having

a history of endometrial or any other cancer. This group was used to compare cell-free RNA, smear, and leukocyte parameters.

Cell-free RNA isolation from plasma

Equal amounts of blood (8 ml) were collected from both the patient and control groups. After centrifugation, (Zymo Research kit, USA) for cfRNA isolation blood and plasma was separated by centrifugation at 2000 rpm for 15 min and stored locally at a − 80 °C within 10 min after collection. Circulating cfRNA was extracted from plasma using Zymo's Quick-cfRNA Serum and Plasma Kit according to the manufacturer's protocol. Briefly, about 3 ml of plasma was mixed with $5 \times$ digestion buffer and assimilated by incubating with proteinase K solution at 55 °C over 30 min. After digestion, the RNA lysate was added with two volumes of DNA Binding Buffer and then transferred to a Zymo-Spin column. After centrifuging at 1000 g for 3 min, the flow-through was discarded. The column was washed with RNA Prep Buffer twice and followed with RNA Wash Buffer twice. The cfRNA was eluted with RNA Elution Buffer. 25 mg of tissue was digested by proteinase K at 56 °C overnight and then lysed at 70 °C for 10 min. After washing in the column and RNA was eluted. The quality of genomic RNA from tissue samples was evaluated by Nano Drop 2000 Thermo Scientific. cfRNA obtained from patients, 200 ng of cfDNA was used for all analyses.

RNA isolation from smear, leukocytes, blood

RNA extracted from samples (smear, leukocytes, blood) by using TRIZOL and Red Cell Lysis Reagent (Thermo Fisher Scientific, USA). Trizol ensures RNA integrity while lysing cells and dissolving cell components during homogenization or lysis. RNA concentration was determined by Nanodrop 2000 Thermo Scientific.

cDNA synthesis and gene expressions

By using the kit of EvoScript Universal cDNA Master System for general laboratory use from (Roche), complementary DNA (cDNA) was synthesized using reverse transcriptase (RT). The expression level of the *GRP78* gene was examined by a quantitative real-time RT-PCR Light Cycler 480 kit from (Roche) using the cycling conditions recommended and a specific primer *GRP78* (Assay ID:110,805).

Statistical analyses

Histogram and *q–q* plots were used to examine and assess the data normality. A Mann–Whitney *U* test was conducted to compare the differences between continuous variables. A Wilcoxon Signed Rank test was used to compare two repeated measure variables. Receiver

operating characteristic (ROC) curves were used to identify the discriminative effect of cfRNA on endometrial cancer. The area under the ROC curves was calculated with 95% confidence intervals. The index was applied to determine the optimal cut-off value. Sensitivity, specificity, positive and negative predictive values were calculated with 95% confidence intervals (Fig. 1). Analyses were conducted using R 3.2.0 (http://www.r-project.org), MVN [15], easy ROC [16] software and TURCOSA (Turcosa Analytics Ltd. Co., Turkey) statistical software. (https://turcosa.com.tr/).

Results

GRP78 mRNA expression results of endometrial cancer patients compared to control are given in Table 1. When we compared the *GRP78* mRNA expression results in smear samples taken from endometrial cancer patients with the control, a highly significant result was obtained ($p < 0.001$). The median value of the smear control group is 0.55 and the 25p–75p values are as follows (0.41–0.77) The median value in the patient group is 2.09 and the 25p–75p values are as follows (0.97–4.37), respectively Table 1.

We looked at the preoperative cell-free *GRP78* mRNA expression results of the same person on a time basis in preoperative and postoperative patients. It was observed that there was no significant change in the individuals whose tumor was removed after the operation when compared to the individuals before the operation ($p = 0.534$). The median values in the patient and control groups are; 2.19 and 1.54. The values at 25 and 75p in the patient and control groups are given in Table 2. When we look at the *GRP78* mRNA expression values from the leukocyte values obtained from the patients before and after operation, significant difference could not observed ($p = 0.718$). The median values of mRNA *GRP78* expression results acquired from leukocytes obtained from patients before and after surgery are as follows. Post-operation leukocyte *GRP78* mRNA median is 1.15 and pre-operation leukocyte *GRP78* mRNA median is 0.88. The values at 25 and 75p in the patient and control groups are given in Table 2.

The level of effectiveness of cell-free *GRP78* mRNAs in diagnosis was determined to be above. Considering our results, a high significance was found ($p < 0.001$). While the sensitivity value of cell-free RNAs in the diagnosis

Table 1 Clinical and Laboratory characteristics of population according to Control and Patients

Variable	Control	Patients	p
	Median (25p–75p)	Median (25p–75p)	
Smear *GRP78* mRNA (ng/ul)	0.55 (0.41–0.77)	2.09 (0.97–4.37)	< 0.001

Table 2 Comparison results acording to patient and control groups pre and post operation

Variable	Pre-operation median(25p–75p)	Post-operation median (25p–75p)	p
cfRNA *GRP78* mRNA (ng/µl)	1.54 (0.67–4.68)	2.19 (0.37–6.07)	0.534
Leukocyte *GRP78* mRNA (ng/µl)	0.88 (0.47–8.73)	1.15 (0.69–5.26)	0.718

Comparison of the preoperative and postoperative cfRNA *GRP78* mRNA expression results of the patients

of the disease is 0.806, the specificity value is 0.700. The positive and negative predictive values are 0.806 and 0.700 respectively Table 3.

Discussion

The first cfRNA discovered was found in the plasma of nasopharyngeal carcinoma patients in 1999 [17]. This discovery was followed by identifying it in serum cells of melanoma patients [17, 18]. cfRNAs are also very unstable due to their susceptibility to degradation by RNAs present in the blood and therefore difficult to detect [17, 19, 20]. cfRNA presents important signatures that characterize oncological patients with high levels in plasma, suggesting an essential role in the diagnosis and monitoring cancer [21]. Circulating RNAs are increasingly important for determining both tumor characterization as well as in monitoring personalized treatments in liquid biopsies of cancer patients. Analysis of cfRNAs acts as a biomarker that can provide complementary information regarding gene expression profiles or epigenetic changes compared to tumor tissue [22]. Although cfRNAs have a long history, they have not been translated into clinical practice today [23]. While cfDNA can be released in body fluids via apoptosis and/or necrosis, the specific mechanism of cfRNAs release is still unknown [17, 22, 24]. The altered balance between circRNAs and their linear

Table 3 ROC curve results and statistical diagnostic measures for cfRNA ≥ 1 for determining Endometrial Cancer

Statistics	Estimate	Confidence interval (95%)
ROC curve statistics		
Area under curve	0.749	0.608–0.860
p value	< 0.001	
Diagnostic measures		
Sensitivity	0.806	0.637–0.908
Specificity	0.700	0.481–0.854
Positive predictive value	0.806	0.637–0.908
Negative predictive value	0.700	0.481–0.854
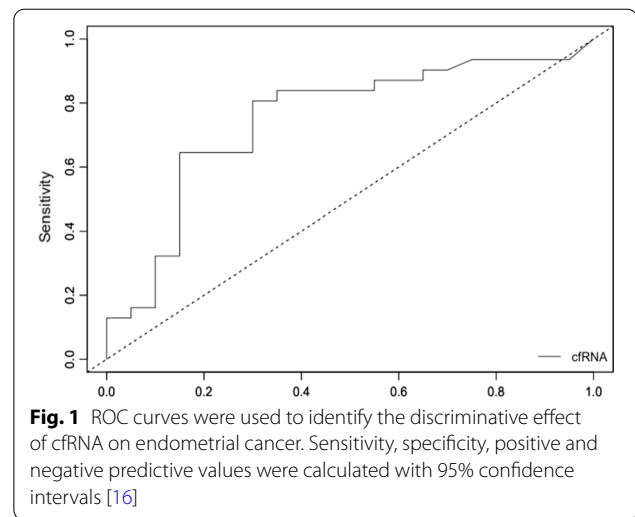
Fig. 1 ROC curves were used to identify the discriminative effect of cfRNA on endometrial cancer. Sensitivity, specificity, positive and negative predictive values were calculated with 95% confidence intervals [16]

mRNAs results in aberrant expression of oncogenes and tumor suppressor genes [17, 25]. The first study to analyze circulating RNAs in liquid biopsy of endometrial cancer was described by Torres et al. where they investigated RT-qPCR showing high expression levels of miR-99a, miR-100 and miR-199b in plasma samples [26, 27]. Most of the miRNA identified in these studies were reported to interact with numerous genes involved in the PTEN-PI3K-AKT-mTOR pathway, which is known to play an important role in the development of EC [28, 29].

Here, we looked at the expression levels of *GRP78* mRNA in plasma, smear and leukocytes from patients before and after operation. Smear was the gold standard for us. Because it would determine our path in cfRNAs by giving information about the patient and the control. We looked at the smear *GRP78* mRNA expression level and found highly significant results were found ($p < 0.001$).

We performed ROC analysis to look at the importance of cell-free *GRP78* mRNA expression level in clinical diagnosis. We found it highly significant ($p < 0.001$). The fact that our cell-free *GRP78* mRNA expression levels were ≥ 1 indicated that it helps us to detect the disease with a minimally invasive diagnostic method. The region under the ROC curve results in 0.749. The limit here is 0.70 and exceeding this limit means that we are now able to easily distinguish these cells. The sensitivity of this study was 0.806 and was within the confidence interval (0.637–0.908). The specificity of this study results in 0.700 and falls within the confidence interval (0.481–0.854). The positive predictive value is 0.806 and confidence interval values are given in Table 3. The negative confidence interval is 0.700 and is in the confidence interval (0.481–0.854). ROC curves were used to identify the discriminative effect of cfRNA on endometrial cancer. The area under the ROC curves was calculated with 95% confidence intervals.

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Cell-free *GRP78* mRNA levels were compared in preoperative and postoperative patients and could not observe any significant results were found. As mentioned above, since cfRNAs are sensitive and difficult to obtain as biological materials and the *GRP78* which investigating is a stress factor, it is possible that the real result could not be reached with implicating factors, such as surgery, can increase the stress factor. In addition, it was concluded that it was appropriate to start the research after expanding the patient group and making sure that the stress factors in the patient's body were completely eliminated after operation.

We investigated cfRNA *GRP78* mRNA expression in patients with endometrial cancer. Extracellular circular RNAs (cf-RNA) are molecules that can be found and measured in a variety of human-derived fluids. In studies carried out so far, it was clear that cf-RNA had been used to investigate the causes of diseases such as diabetes, trauma, stroke, and myocardial infarction [30, 31]. In addition, relationship was determined between the number of cfRNA obtained from urine, blood, and other body fluids, and the presence of cancer cell-derived RNAs in the plasma of cancer patients was determined [32].

The role and function of *GRP78* and cancer have become more and more clear, and it has been further linked to several other types of cancers [33–36]. Of interest, some other studies have even shown a link between EC and *GRP78,* when a biopsy was acquired from different tissues in the body, such as adipose tissues [33].

Conclusions

The significant increase of cell-free mRNA *GRP78* expression obtained from endometrial cancer patients is promising as it can be used as a diagnostic criterion with smear mRNA *GRP78* without the need for invasive methods.

Limitations of the study

- Biomarker studies should be carried out by considering the larger patient group, and the larger patient group of this current study should be considered.
- It should be determined whether the *GRP78* gene is a biomarker in different cancer groups.
- It is not enough to obtain a meaningful result as expression. Protein studies need to be done.

Abbreviations
AGC: Atypical-glandular-cells; *ATF6*: Activating transcription factor 6; cfRNA: Cell-free RNA; cfDNA: Cell-free DNA; cDNA: Complementary DNA; ccfRNA: Circulating cell-free RNA; CTCs: Circulating tumor cells; EC: Endometrial cancer; ER: Endoplasmic reticulum; ERAD: *ER*-Associated degradation; *GRP78*: Glucose regulated protein 78; *HSPA5*: Heat shock protein A5; *IRE1*: Inositol-requiring enzyme 1; mRNA: Messenger RNA; *PERK*: Protein kinase RNA-like endoplasmic reticulum kinase; RT: Reverse transcriptase; ROC: Receiver operating characteristic; UPR: Unfolded protein response.

Availability of data and materials
Data is available upon request.

Declarations

Ethics approval and consent to participate
The study was approved by Erciyes University Hospitals, Medical Genetics Department, Kayseri, Turkey. Reference number TDK-2019–8765. All the patients in the study had signed for written consent before the procedure.

Consent for publication
Not Applicable.

Competing interests
The authors did not report any competing interests.

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References
1. Kiraz A, Açmaz G, Uysal G, Unal D, Dönmez-Altuntas H (2016) Micronucleus testing as a cancer detector: endometrial hyperplasia to carcinoma. Arch Gynecol Obstet 293(5):1065–1071
2. Acmaz G, Aksoy H, Albayrak E, Baser M, Ozyurt S, Aksoy U, et al. (2014) Evaluation of endometrial precancerous lesions in postmenopausal obese women: A high risk group?. Asian Pacific J Cancer Prev
3. Morice P, Leary A, Creutzberg C, Abu-Rustum N, Darai E (2016) Endometrial cancer. Lancet 387(10023):1094–1108
4. Crowley E, Di Nicolantonio F, Loupakis F, Bardelli A (2013) Liquid biopsy: monitoring cancer-genetics in the blood. Nat Rev Clin Oncol 10(8):472–484. https://doi.org/10.1038/nrclinonc.2013.110
5. Domínguez-Vigil IG, Moreno-Martínez AK, Wang JY, Roehrl MHA, Barrera-Saldaña HA (2018) The dawn of the liquid biopsy in the fight against cancer. Oncotarget 9(2):2912–2922
6. Alix-Panabières Pantel CK (2013) Circulating tumor cells: Liquid biopsy of cancer. Clin Chem 59(1):110–118

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7. Butt AN, Shalchi Z, Hamaoui K, Samadhan A, Powrie J, Smith S et al (2006) Circulating nucleic acids and diabetic complications. Ann N Y Acad Sci 1075:258–270

8. Ting J, Lee AS (1988) Human gene encoding the 78,000-dalton glucose-regulated protein and its pseudogene: structure, conservation, and regulation. DNA 7(4):275–286

9. Localization of the Gene Encoding Human BiP/GRP78, the endoplasmic reticulum cognate of the HSP70 family, to chromosome 9q34 - ScienceDirect [Internet]. [cited 2020 Dec 13]. Available from: https://www.sciencedirect.com/science/article/abs/pii/S0888754384711669?via%3Dihub.

10. Lin JH, Walter P, Yen TSB (2008) Endoplasmic reticulum stress in disease pathogenesis. Ann Rev Pathol Mech Dis 3:399

11. Pfaffenbach KT, Lee AS (2011) The critical role of GRP78 in physiologic and pathologic stress. Curr Opin Cell Biol 23(2):153–156

12. Cell Surface GRP78, a new paradigm in signal transduction biology | ScienceDirect [Internet]. [cited 2020 Dec 13]. Available from: https://www.sciencedirect.com/book/9780128123515/cell-surface-grp78-a-new-paradigm-in-signal-transduction-biology.

13. Martinon F (2012) Targeting endoplasmic reticulum signaling pathways in cancer. Acta Oncol 51(7):822–830

14. (No Title) [Internet]. [cited 2020 Dec 13]. Available from: https://khub.nthu.edu.tw/document/courses/10310TIGP718900.pdf.

15. Korkmaz S, Goksuluk D, Zararsiz G (2014) MVN: an R package for assessing multivariate normality. R J 6(2):151–162

16. Goksuluk D, Korkmaz S, Zararsiz G, Karaagaoglu AE (2016) EasyROC: an interactive web-tool for roc curve analysis using r language environment. R J 8(2):213–230

17. Pardini B, Sabo AA, Birolo G, Calin GA (2019) Noncoding rnas in extracellular fluids as cancer biomarkers: the new frontier of liquid biopsies. Cancers (Basel) 11(8):1–52

18. Kopreski MS, Benko FA, Kwak LW, Gocke CD (1999) Detection of tumor messenger RNA in the serum of patients with malignant melanoma. Clin Cancer Res 5(8):1961–1965

19. Holford NC, Sandhu HS, Thakkar H, Butt AN, Swaminathan R (2008) Stability of β-actin mRNA in plasma. Ann N Y Acad Sci 1137:108–111

20. Fernando MR, Norton SE, Luna KK, Lechner JM, Qin J (2012) Stabilization of cell-free RNA in blood samples using a new collection device. Clin Biochem 45(16–17):1497–1502. https://doi.org/10.1016/j.clinbiochem.2012.07.090

21. Pös O, Biró O, Szemes T, Nagy B (2018) Circulating cell-free nucleic acids: characteristics and applications. Eur J Hum Genet 26(7):937–945

22. Geeurickx E, Hendrix A (2019) Targets pitfalls and reference materials for liquid biopsy tests in cancer diagnostics. Mol Aspects Med 2020(72):100828. https://doi.org/10.1016/j.mam.2019.10.005

23. Fernandez-Mercado M, Manterola L, Larrea E, Goicoechea I, Arestin M, Armesto M et al (2015) The circulating transcriptome as a source of non-invasive cancer biomarkers: Concepts and controversies of non-coding and coding RNA in body fluids. J Cell Mol Med 19(10):2307–2323

24. Stroun M, Lyautey J, Lederrey C, Olson-Sand A, Anker P (2001) About the possible origin and mechanism of circulating DNA: apoptosis and active DNA release. Clin Chim Acta 313(1–2):139–142

25. Dragomir M, Calin GA (2018) Circular RNAs in cancer—lessons learned from microRNAs. Front Oncol 8:179

26. Muinelo-Romay L, Casas-Arozamena C, Abal M (2018) Liquid biopsy in endometrial cancer: new opportunities for personalized oncology. Int J Mol Sci 19(8):2311

27. Torres A, Torres K, Pesci A, Ceccaroni M, Paszkowski T, Cassandrini P et al (2012) Deregulation of miR-100, miR-99a and miR-199b in tissues and plasma coexists with increased expression of mTOR kinase in endometrioid endometrial carcinoma. BMC Cancer 12(1):1–13

28. Torres A, Torres K, Pesci A, Ceccaroni M, Paszkowski T, Cassandrini P et al (2013) Diagnostic and prognostic significance of miRNA signatures in tissues and plasma of endometrioid endometrial carcinoma patients. Int J Cancer 132(7):1633–1645

29. Dong P, Konno Y, Watari H, Hosaka M, Noguchi M, Sakuragi N (2014) The impact of microRNA-mediated PI3K/AKT signaling on epithelial-mesenchymal transition and cancer stemness in endometrial cancer. J Transl Med 12(1):1–9

30. Swaminathan R, Butt AN (2006) Circulating nucleic acids in plasma and serum: recent developments. Ann N Y Acad Sci 1075:1–9

31. Tzimagiorgis G, Michailidou EZ, Kritis A, Markopoulos AK, Kouidou S (2011) Recovering circulating extracellular or cell-free RNA from bodily fluids. Cancer Epidemiol 35(6):580–589

32. Rahat B, Ali T, Sapehia D, Mahajan A, Kaur J (2020) Circulating cell-free nucleic acids as epigenetic biomarkers in precision medicine. Front Genet 11(August):1–20

33. Matsuo K, Gray MJ, Yang DY, Srivastava SA, Tripathi PB, Sonoda LA et al (2013) The endoplasmic reticulum stress marker, glucose-regulated protein-78 (GRP78) in visceral adipocytes predicts endometrial cancer progression and patient survival. Gynecol Oncol 128(3):552–559

34. Uramoto H, Sugio K, Oyama T, Nakata S, Ono K, Yoshimastu T et al (2005) Expression of endoplasmic reticulum molecular chaperone Grp78 in human lung cancer and its clinical significance. Lung Cancer 49(1):55–62

35. Daneshmand S, Quek ML, Lin E, Lee C, Cote RJ, Hawes D et al (2007) Glucose-regulated protein GRP78 is up-regulated in prostate cancer and correlates with recurrence and survival. Hum Pathol 38(10):1547–1552

36. Lee AS (2007) GRP78 induction in cancer: therapeutic and prognostic implications. Cancer Res 67(8):3496–3499

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