THERANOSTICS OF UTERINE LEIOMYOMA: PRESENT AND FUTURE

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INTRODUCTION

Uterine myoma (UM) remains an actual problem of modern gynecology and reproductive medicine, as it is the most common benign tumor disease of the female genital organs. According to various literature data, the frequency of UM in women of reproductive age ranges from 20 to 50%, although the percentage of true morbidity is much higher, since this nosological form can have an asymptomatic course, and therefore, undiagnosed cases [1]. UM remains one of the main indications for hysterectomy, including in reproductive age (28%), which leads not only to the loss of reproductive and menstrual function, but also to pronounced vegetative-vascular and psychoemotional disorders [1, 2]. Therefore, the search for effective algorithms for predicting the course of UM for the selection of personalized treatment tactics based on epigenetic mechanisms of regulation and clinical and laboratory characteristics is an urgent scientific task in modern medicine and theranostics.

Today, the issue of predicting the course of uterine fibroids, and especially personalized prognosis, is extremely relevant, because this nosological form has a negative impact on both the state of health and the quality of life of a woman. However, the possibilities of predicting the course of UM are limited and require the introduction of new methods. The model for predicting the course of UM should include a variety of factors, both clinical and paraclinical, including epigenetic ones. Research in recent years has shown a significant contribution of epigenetic regulation disorders to the occurrence and course of UM [7]. There is more and more evidence about the role of epigenetic changes in reprogramming key signaling pathways associated with the development of LM, and it is shown that the study of the prognostic and diagnostic role of miRNA is one of the most promising directions [8]. In our work, we investigated the expression of miRNA-29b and miRNA-146a in tumor tissue, because the families of miRNA-29 and miRNA-146 are the most studied in the pathogenesis of UM [1, 9, 10].

In recent years, personalized (precision) medicine (PM) has turned from a theoretical concept into a powerful tool for improving medical care [11]. The basis of PM is a model that divides people into different groups, depending on the phenotype and features of the course of diseases. All types of treatment, doses and groups of drugs, invasive or non-invasive interventions are selected for each individual patient based on his predicted response or disease risk [12, 14]. Sometimes the authors use the definition "4P-medicine". This abbreviation contains the following determining definitions: predictive, preventive, personalized, participatory [11, 15]. Although the individualization of treatment depending on the patient's personality dates back at least to the time of Hippocrates – "I treat the patient and not the disease" [11, 16], in recent years this approach has gained more popularity due to the emergence of new diagnostic and informational approaches. In PM diagnostic testing is often used to select appropriate and optimal therapy based on the context of the patient's genetic makeup or other molecular or cellular analysis [17, 18]

One of the components of personalized medicine is theranostics, an approach to disease treatment using similar molecules for both imaging (diagnosis) and therapy. The term "theranostics" comes from the combination of the words "therapy" and "diagnosis" [19–21]. To a certain extent, theranostics is also the application of molecular genetic technologies for diagnosis and selection of the optimal treatment strategy. In particular, this concerns the determination of epigenetic changes in hyperproliferative diseases [11, 22]. The use of genetic information has played an important role in certain aspects of PM (e.g. pharmacogenomics). The term was first introduced in the context of genetics, although it has since expanded to encompass all types of personalization efforts, including the use of proteomics [19], image analysis, and nanoparticle-based theranostics [23].

If today theranostics is mainly used in oncology and radiation medicine, then in the future this principle can be applied to benign neoplasms, both from the point of view of differentiation of oncopathology and for the active management of patients at risk.

Theranostics deals with a tailor-made treatment plan based on the uniqueness of each individual, resulting in the right drug for the right patient at the right time. This enables the transition from traditional medicine to personalized medicine. Genetics plays a significant role in theranostics, as do pharmacogenetics, proteomics and biomarker profiling [19, 24, 25].

Research objectives: to develop an algorithm for predicting the growth of UM, taking into account the state of epigenetic regulation. In our research we investigated the expression of miRNA-29b and miRNA-146a in UM tumor tissue.

MATERIALS AND METHODS

The study was conducted on the basis of clinical divisions of the Department of Obstetrics and Gynecology in 2018–2021. 28 patients with uterine fibroids were examined. The scope of the examination was determined in accordance with current clinical protocols and the American College of Obstetricians and Gynecologists (ACOG) recommendations [3]. The changes in the size of the largest myomatous node during the year were evaluated in absolute and relative values.

In addition, miRNA expression in tumor tissue was determined in all women using real time PCR [4]. Total RNA was isolated using the commercial kit "RN-easy PFPE Kit" (QIAGEN, Germany) according to the manufacturer's protocol. The amount of isolated RNA was determined on a spectrophotometer "NanoDrop 2000c Spectrophotometer" (ThermoScientific, USA). The purity of the isolated RNA was monitored, using the ratio of optical absorption values at wavelengths of 260 and 280 nm. RNA was dissolved in Tris-EDTA buffer and stored at -20 °C until PCR.

Single-stranded DNA was synthesized from 100 ng of total RNA using a standard method for reverse transcription, for PCR with reverse transcriptase (RT-PCR) a ready-made kit "Reverta-L" (Amplisens, Russia) was used according to the manufacturer's instructions, using microRNAs specific to the studied primers hsa-mRNA-29b and hsa-mRNA-146a.

Primer sequences for RT-PCR and real-time PCR were determined using the Genomics tool (Hungary) [5] and synthesized by Metabion (Germany). RT-PCR was performed in the Tertsik amplifier (DNA-technology, Russia). According to the stem-loop primer sequences, the standard reverse primer 5'-GTGCAGGGTCCGAGGT-3' was used for real-time PCR for miRNA-29b and miRNA--146a, as well as forward primers, after the RT-PCR, a mixture of reagents was added to the reaction product: (table 1).

Table 1. Compound reaction mix for PCR

Component reaction mix	Amount on 20 µl reaction product
2X-universal mix Maxima SYBR Green/ROX qPCR Master Mix (2X), ThermoScientific, USA	10,00
purified water	4,00
20x mix of forward and reverse primers for miRNA	1,00
cDNA	5,00
Total	20,00

MicroRNA RNU48 was used as an endogenous control for the objectification of expression indicators, since it showed minimal dispersion in the periods of the threshold cycle (Ct) compared to other endogenous controls. This miRNA is one of the validated house-keeping small RNAs for research on human tumor material both *in vitro* and *ex vivo* [4].

Molecular and genetic studies were performed on the basis of LLC "Oncotheranostics" (Kyiv, Ukraine).

Linear regression analysis was performed to determine the influence of various factors on myoma growth. Individual correlations were tested using bivariate Pearson or Spearman correlations for variables that were not normally distributed and were not suitable for ranking on the ratio scale interval [6]. Linear and multivariate regression analyzes were performed using absolute size change and percent growth rate as dependent variables, with patient age, estradiol and progesterone levels, initial fibroid size, fibroid type, and miRNA expression as independent variables. Simple bivariate correlations were determined between age at first consultation and initial fibroid size, and between the presence of fibroid-related symptoms and age, initial fibroid size, fibroid growth rate, and fibroid location. Accordingly, the effects of patient age, it initial size and location on fibroid growth were investigated, and whether the presence of symptoms could be associated with the presence of epigenetic risk factors.

Data were evaluated using the Statistica 10.0 statistical software (StatSoft Inc., USA). The null hypothesis was accepted for $p < 0.05$ [6].

The research was conducted in accordance with modern bioethical standards. The research protocol was approved by the local ethics committee (protocol No. 16 dated May 18, 2020). All patients who participated in the study provided written informed consent.

RESULTS

The average age of the examined patients was 39.3 ± 1.0 years. 39.3% of patients had more than two myomatous nodes, the average number of nodes was 2.7 ± 0.4. The localization of the nodes varied, the most common was intramural-subserous (39.3%) and multiple hybrid localization (class XX according to the International Federation of Gynecology and Obstetrics (FIGO) classification) – 17.9%. In half of the cases, the size of myoma was more than 7 × 5 cm.

The hormonal profile of the examined women was characterized by moderate hyperestrogeny. The average content of estradiol in the I phase of the menstrual cycle was 222.2 ± 12.8 pg/ml, and in the II phase – 188.6 ± 11.4 pg/ml. The average progesterone content in phase I was 1.1 ± 0.1 ng/ml, in phase II – 11.3 ± 0.3 ng/ml.

Signs of myoma growth during the catamnesis period were determined in 12 women (42.8%) with an average increment in the largest diameter of 14.2 ± 0.4%. In 2 patients (7.1%), the tumor size decreased by 6 and 12%, respectively. The rest of the women either had no changes in the size of the nodule, or underwent surgical interventions of varying degrees of radicality (9 people). Further calculations were carried out for 19 persons in whom the observation plan was fully completed.

Since the cellular process, the growth of the extracellular matrix and apoptosis, the key processes of myoma growth, are under the control of gene products, and therefore miRNA, we conducted a study of miRNA expression. Based on the analysis of the literature and databases regarding the role of microRNAs in the growth of uterine fibroids, miRNA-29b and miRNA-146a were selected.

Analysis of the expression levels of miRNA-29b and miRNA-146a in the tumor tissue of patients with uterine fibroids

revealed significant variability of their indicators. It was found that the characteristic feature of the majority of the examined tissue samples were miRNA-29b expression levels in the range of 2–7 units and microRNA-146a 30–67 units, which were determined in 71.42% ($p < 0.05$) and 75.0% ($p < 0.05$) of cases. Since a relatively uniform distribution of the samples in terms of the expression of the investigated miRNAs was found within the studied sample, we conducted an analysis of the adjacent expression of miRNA-29b and conditional miRNA-146a. However, in the total sample, we failed to identify cohorts with specific expression of these miRNAs, as there was no linear relationship between the expression of miRNA-29b and miRNA-146a. There were also no significant differences in miRNA-29b and miRNA-146a indicators depending on age, the number and size of the fibromatous node. However, a clear correlation of the levels of both studied microRNAs with the body mass index (BMI) of the patients was observed: for miRNA-29b $r = 0.42$ ($p < 0.05$), for miRNA-146a $r = 0.45$ ($p < 0.05$) (Table 2).

Table 2. Indicators of miRNA-29b and miRNA-146a expression in tumor tissue depending on BMI and age

	miRNA-29b		miRNA-146a	
Age, years	≤ 40	> 40	≤ 40	> 40
M	4.24	5.15	48.86	56.92
m	2.90	2.74	28.28	20.86
r	0.27		0.45	
BMI, kg/m²	< 24	> 24	< 24	> 24
M	3.91	5.59	44.89	62.22
m	1.01	1.21	23.06	25.43
r	0.42*		0.45*	

* $p < 0,05$.

Table 3. Relative expression values of miRNA-29b and miRNA-146a

Index	Indices of miRNA expression vs internal control RNU48	
1–2 fibroids	miRNA-29b	< 4 / > 5
	miRNA-146a	< 40 / > 50
> 2 fibroids	miRNA-29b	2–6
	miRNA-146a	30–75
Size of the largest fibroid < 6 × 6 cm	miRNA-29b	< 4 / > 6
	miRNA-146a	< 40 / > 60
Size of the largest fibroid > 7 × 5 cm	miRNA-29b	2–6
	miRNA-146a	20–75

Table 3 shows the relative expression values of miRNA-29b and miRNA-146a, depending on the number and size of myomatous nodes.

The study of the association of the levels of miRNA-29b and miRNA-146a with the number of nodes and the size of the largest node did not reveal any significant differences, however, the analysis of the adjacent expression of these miRNAs made it possible to form cohorts of patients for whom these indicators will have prognostic value (Table 3). Thus, in 90% of samples with indicators of microRNA-29b 2–6 units and microRNA-146a 30–75 units 3 or more myomatous nodes were detected, in 75% of patients with miRNA-29b expression 2–6 units and microRNA-146a 20–75 units the size of the largest node exceeded 7 × 5 cm.

Further analysis showed that the expression of microRNA-29b has the greatest significance for growth prognosis (Wald statistic). In general, the logistic regression equation looks like this:

$X(\Delta) = -0.39A + 0.52E_2(1) - 0.90E_2(2) - 0.30PG(1) + 0.35PG(2) - 0.27S - 0.44miRNA\text{-}29b - 0.24miRNA\text{-}146a,$

where A is age (years), $E_2(1)$ is the level of estradiol in phase I of menstrual cycle (pg/ml), $E_2(2)$ is the level of estradiol in phase II of cycle (pg/ml), PG(1) is the level of progesterone in I phase of cycle (ng/ml), PG(2) – progesterone level in II phase of cycle (ng/ml), S-diameter of the largest node, miRNA-29b – miRNA-29b expression, miRNA146a – miRNA-146a expression (Table 4).

It can be assumed that the identified main catalysts of myomatous node growth are the mediated effect of microRNAs on collagen synthesis in uterine tissues, as well as their effect on the production of sex hormones and their reception by myometrial tissue. The conducted study shows that the use of dynamic analysis of miRNA expression profile as an additional marker in the diagnosis and treatment of UM is promising and requires more detailed research from the perspective of theranostics.

CONCLUSIONS

1. The future in the diagnosis and treatment of gynecological pathology, in particular UM, belongs to theranostics, which represents a holistic transition from trial-and-error medicine to prognostic, preventive and personalized medicine that is leads to an improvement in the quality of life of patients. Further study of the correlation of clinical and paraclinical parameters

Table 4. The contribution of various risk factors in the prognostic model

Indicators	b* in	Partial - Cor.	Semipart - Cor.	Tolerance	R-square	t(10)	p
Age	-0.385386	-0.515495	-0.298627	0.600435	0.399565	-1.90238	0.086283
$E_2(1)$	0.523476	0.669599	0.447527	0.730879	0.269121	2.85094	0.017222
$E_2(2)$	-0.896065	-0.789961	-0.639535	0.509389	0.490611	-4.07411	0.002235
PG(1)	-0.303440	-0.477695	-0.269915	0.791242	0.208758	-1.71948	0.116271
PG(2)	0.348822	0.494001	0.282039	0.653748	0.346252	1.79671	0.102601
Size	-0.268280	-0.427847	-0.234976	0.767132	0.232868	-1.49690	0.165300
miRNA-29b	-0.435790	-0.599865	-0.372168	0.729330	0.270670	-2.37087	0.039219
miRNA-146a	-0.237977	-0.362780	-0.193249	0.659425	0.340575	-1.23108	0.246461

can make it possible to predict the course of UM, and therefore to apply an effective personalized treatment plan.

2. Theranostics is a new approach to the diagnosis and treatment of the patient, based on the uniqueness of each person in order to choose the optimal treatment strategy against the background of the molecular genetic technologies, in particular to determine epigenetic changes in hyperproliferative diseases. The study of miRNA expression may find its place in the theranostics of uterine fibroids in the future.

Conflict of interest

The authors declare no conflict of interest.

ТЕРАНОСТИКА ЛЕЙОМІОМИ МАТКИ: СЬОГОДЕННЯ ТА МАЙБУТНЄ

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Мета дослідження: розробка алгоритму прогнозування росту міоми матки з урахуванням стану епігенетичної регуляції.
Матеріали та методи. Дослідження проведене на базі клінічних підрозділів кафедри акушерства та гінекології у 2018–2021 рр. Обстежено 28 пацієнток із фіброміомою матки. У всіх жінок визначали експресію мікроРНК-29b та мікроРНК-146а у пухлинній тканині фіброміоми матки за допомогою полімеразної ланцюгової реакції в режимі реального часу. Дані оцінювали за допомогою статистичної програми Statistica 10.0 (StatSoft Inc., США).
Результати. Середній вік обстежених пацієнток становив 39,3 ± 1,0 року. Середня кількість вузлів – 2,7 ± 0,4. У більшості досліджених зразків тканини найчастіше визначалися рівні експресії мікроРНК-29b в межах 2–7 умовних одиниць та мікроРНК-146а у межах 30–67 умовних одиниць. Найбільше значення для прогнозу росту має експресія мікроРНК-29b (метод Wald). Одержано рівняння логістичної регресії, відповідно до якого прогностичним чинниками є вік пацієнтки, рівень естрадіолу та прогестерону в I та II фазі менструального циклу, діаметр найбільшого вузла, експресія мікроРНК-29b і мікроРНК-146а.
Висновки. Результати проведеного дослідження свідчать, що застосування профілю експресії мікроРНК як додаткового маркера в діагностиці й лікуванні фіброміоми матки є перспективним і потребує більш детального дослідження. Подальше вивчення кореляції клінічних та параклінічних параметрів може дати змогу прогнозувати перебіг міоми матки, а отже, застосовувати ефективний персоніфікований план лікування. Тераностика є новим підхідом до діагностики та лікування пацієнтів, що ґрунтується на унікальності кожної людини, з метою вибору оптимальної лікувальної стратегії на тлі використання молекулярно-генетичних технологій, зокрема для визначення епігенетичних змін при гіперпроліферативних захворюваннях. Дослідження експресії мікроРНК надалі може знайти своє місце в тераностиці міоми матки.

Ключові слова: міома матки, епігенетика, тераностика, прогнозування.

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Research objectives: to develop an algorithm for predicting the growth of uterine fibrodis, taking into account the state of epigenetic regulation.
Materials and methods. The study was conducted on the basis of clinical divisions of the Department of Obstetrics and Gynecology in 2018–2021. 28 patients with uterine fibroids were examined. Expression of miRNA-29b and miRNA-146a in tumor tissue was determined in all women using real-time PCR. Obtained data were analyzed using the statistical program Statistica 10.0 (StatSoft Inc., USA).
Results. The average age of the patients was 39.3 ± 1.0 years. The average number of nodes was 2.7 ± 0.4. The expression levels of microRNA-29b were most frequently determined in the range of 2–7 units and microRNA-146a in the range of 30–67 units in most of the examined tissue samples. The expression of miRNA-29b has the greatest significance for the growth forecast (Wald statistic). According to the logistic regression equation the prognostic factors are patient's age, estradiol and progesterone level in the I and II phases of the menstrual cycle, diameter of the largest node, expression of miRNA-29b and miRNA-146a.
Conclusions. This study shows that the use of miRNA expression profile as an additional marker in the diagnosis and treatment of uterine fibroids is promising and requires more detailed research. Further study of the correlation of clinical and paraclinical parameters can make it possible to predict the course of uterine fibroids, and therefore to apply an effective personalized treatment plan. Theranostics is a new approach to the diagnosis and treatment of patients, based on the uniqueness of each person in order to choose the optimal treatment strategy against the background of the molecular genetic technologies, in particular to determine epigenetic changes in hyperproliferative diseases. The study of miRNA expression may find its place in the theranostics of uterine fibroids in the future.

Keywords: uterine myoma, epigenetics, theranostics, prognosis.