Review

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Novel Approaches to Possible Targeted Therapies and Prophylaxis of the Uterine Fibroid

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Abstract: The uterine leiomyoma is the most common benign tumor in women of childbearing age. It may lead to problem of conception or complications during gestational period. The methods of treatment can be surgical (myomectomy and hysterectomy, embolization of arteries) and therapeutic treatment (Ulipristal acetate, Leuprolide acetate, Cetrorelix, Goserelin, Mifeprestone). Both approaches are efficient, but are incompatible with pregnancy planning. Therefore, there is a call for medical practice in developing therapeutical means of preventing leiomyoma onset in patients planning on pregnant. Based on the analysis of GWAS data on the search for mononucleotide polymorphisms associated with the risk of leiomyoma, meta-transcriptomic and meta-methylomic studies, target proteins have been proposed. Prospective therapeuticals of leiomyoma may be based on chemical compounds, humanized recombinant antibodies, vaccines based on markers of the uterine leiomyoma cells that are absent in the adult organism, DNA and RNA preparations. Three different nosological forms of the disease associated with driver mutations in the MED12, HMGA2 and FH genes should be considered when developing or prescribing drugs. *E.g.* synthetic inhibitors and vaccines based on matrix metalloproteinases MMP11 and MMP16 are expected to be effective only for the prevention of the occurrence of MED12-dependent nodules.

Keywords: uterine leiomyoma; fibroid; pregnancy; therapy; targets; inhibitors; vaccines; humanized antibodies; MED12; HMGA2; fumarate hydratase; IL17B; MMP11; MMP16

1. Introduction

Uterine fibroids are most common in women aged 35-50 years (frequency 30-35%), but there is a tendency for the first disease onset at an earlier age - 25 years and even younger [1]. In the structure of gynecological morbidity, uterine fibroids occupy the second place in frequency of occurrence, second only to inflammatory diseases of the female reproductive system, and remaining the most common benign tumor in gynecology [2].

According to data from various sources, among women of reproductive age, the incidence of the disease varies from 30 to 50%. The average age of detection of uterine fibroids is 32.8± 0.5 years, and indication for surgical treatment occur after 4.4± 0.3 years [3]. In 20-30% of women, fibroids are diagnosed at reproductive age, in 30-40% of women over 40 years old. The true frequency of the disease cannot be determined unambiguously due to the fact that >30% of patients have uterine fibroids without clinical manifestations. Leiomyoma is diagnosed in adolescents with uterine bleeding in 5-7%, in 4% – in the age group from 20 to 30 years, in 11-18% – in 30-40 years, in 33-40% – in 40-60 years. In recent years, there has been a tendency to decreasing the average age when the disease is detected for the first time. There is a category of women aged 20-25 years suffering from this pathology, which almost did not exist formerly [4]. This phenomenon is associated with a decrease in the average age of menarche achievement. There are no cases of fibroids in girls before puberty. There has been a decrease in cases of the disease in the menopausal period, which may be due to a gradual increase in the average age of menopause, which has increased from 40 to 50-52

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years over the past 200 years [3]. It is also known that the symptomatic leiomyoma requiring treatment is more often manifested in perimenopause, while after menopause its frequency decreases sharply [5]. Women who have given birth to 5 children are 4 times less at risk of the disease compared to those who have not given birth [6]. In primiparous women over 30 years of age, fibroids occur in 15-17% [3].

Among women diagnosed with uterine leiomyoma, 15-30% have symptoms of varying severity, including pain syndrome, infertility, dysfunction of adjacent organs, abnormal uterine bleeding, anemia, as well as a number of other severe complications [7]. Despite the benign nature, leiomyomas are able to metastasize, penetrating into various tissues of the body outside the uterus, in particular, into the lungs [8].

In gynecological hospitals in Russia, up to 50-70% of operations are performed specifically for symptomatic uterine leiomyoma, in addition, 40 to 60% of hysterectomies are performed specifically for this disease. In European countries, more than 300 thousand surgical interventions related to uterine fibroids are performed annually [9], and in the USA - approximately 200 thousand myomectomies and 30 thousand hysterectomies [10]. In the USA, approximately 600 thousand hysterectomies are performed every year, 200 thousand of which are due to fibroids, beyond 30 thousand myomectomies [11]. In the European Union, the yearly number of hysterectomies reaches more than 300 thousand, in China – 1 million [12]. In Canada, every 4th woman above 45 has been subjected to a hysterectomy, in the UK - every 5th, in the USA – every 3rd (>80% of women under 49 and 50% of them under 40) [13]. In [14] there is an estimate that uterine fibroids affect up to 77% of women during menopause, the annual health care costs for combating this disease in the United States amount to $ 34 billion.

Fibroids are capable of rapid growth during pregnancy, which can lead to violations of the normal growth of the placenta and the development of defects in the fetus. Often such problem might lead to performance of urgent myomectomies in the early stages of pregnancy, or cesarean section before the delivery date. Fibroids are able to grow rapidly in size during pregnancy, and in the case of subserous localization, tumors form adhesions with internal organs, which during childbirth cause serious difficulties for uterine contractions during and after childbirth. Besides there are cases of postpartum bleeding due to uterine atony [15].

The development of postoperative relapses is a separate problem in the treatment of the disease, since the recurrence rate after myomectomy reaches 90%, while in almost every fourth case there is a need for repeated treatment until a new surgical intervention. The probability of recurrence depends on how thoroughly all the existing nodes were removed, however, in the presence of multiple tumors, the risk of recurrence is higher, since often small nodes are not visualized and, as a result, are not removed, continuing to grow in the postoperative period. According to review [16], the risk of repeated surgery for multiple uterine fibroids is 26%. With a single node, recurrence of fibroids occurs in 27%, and the risk of repeated surgery due to recurrence is 11% [17].

Hysterectomy is a radical way to prevent recurrence of leiomyoma, however, it is not acceptable for women planning pregnancy. Moreover, a significant problem arising as a result of myomectomy is the traumatization of the uterus, the occurrence of scars that lead to disruption of its normal functioning during pregnancy and the risk of ruptures during stretching and contraction before and in the course of the childbirth. In addition, the growth rate of fibroids accelerates during pregnancy, which is usually explained with a high level of the blood progesterone [18]. Thus, the most significant medical and social problem of fibroids therapy is the development of treatment methods that prevent the recurrence of the disease in patients planning pregnancy or in the process of gestation. The purpose of this review is to systematize existing methods for preventing the recurrence of fibroids or slowing the growth of existing nodes in patients planning childbirth or having a pregnancy.

2. Review structure and design

We suggest specifying the following principal directions of the uterine leiomyoma etiology study and development of approaches to treatment of this condition: (1) HMGA2 gene overexpression as a driver mutation in UL nodes; (2) the driver mutations in MED12 gene in UL

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nodes; (3) the driver null-mutations in FH gene in UL nodes; (4) using agonists and antagonists of steroid hormones as UL growth suppressors; (5) PI3K/Akt/mTOR signal pathway in UL nodes and its inhibitors as UL growth suppressors. The first three directions are important since UL nodes with different driver mutations differ substantially in the expression profile and consequently in the molecular targets of the candidate therapeutics. Direction 5 allows analyzing efficacy and side effects of commercially available and prospective medicines used for UL treatment. Directions 5 and particularly 6 allow identifying novel molecular targets for UL drug and vaccine design which are prerequisite for UL node formation and growth but absent in the normal myometrium. The key publications of each direction are summarized in Table 1.

Table 1. Principal directions of the uterine leiomyoma regulation study and development of approaches to treatment of this condition.

Year of publication, first author	Reference	Key results
Direction 1. HMGA2 gene overexpression and chromosomal rearrangements in UL nodes		
Nilbert et al, 1988	[19]	106 samples of leiomyoma biopsies were examined using classical cytogenetics methods. A normal parental karyotype was found in 57 samples (54%) capable of growing in culture, and chromosomal rearrangements were found in 20 samples (19%). In 10 cases (9%), they represented a translocation of the prethelomeric regions of chromosomes 12 and 14, and in 4 of them there were other chromosomal rearrangements. In 10 cases where there was no translocation of the thelomeric region of chromosomes 12 and 14, minor rearrangements in chromosomes were observed 1, 2, 3, 4, 6, 8, 9, 10, 11, 13 and 19. Most often – in five cases, they affected chromosome 1.
Nilbert et al, 1992	[20]	It is established that with a variety of different combinations, only rearrangements of type t(1;6)(q23;p21) and del(7)(q21.2q31.2) are systematically detected in independent samples and are sufficient for the formation of a leiomyoma node along with the most common rearrangements of type t(12;14) (q14-q15; q23-q24).
Nilbert et al, 1990	[21]	Fibroids with complete trisomies on chromosome 12, specific translocations, t(12;14)(q14-15;q23-24) were identified.
Hennig et al, 1997	[22]	It was discovered for the first time that the rearrangement involving the 12q14-15 prethelomeric region leads to an increase in the expression of the HMGIC gene, which later became known as HMGA2
Klotzbücher et al, 1999	[23]	The expression of HMGIC (HMGA2) and HMGIY (HMGA1) genes in leiomyomas was studied using immunohistochemical staining of tissue sections. These authors reported that their expression of the genes of these non-histone proteins controlling the chromatin structure was observed in 16 of 33 samples of biopsies of leiomyoma nodes. At the same time, expression has never been observed in normal myometrium, as well as vascular endothelium and fibroblasts of tumor nodes. In three of the 16 biopsy samples showing HMGIC (HMGA2) expression, a protein product of this gene with an abnormal molecular weight was observed.
Klemke et al, 2009	[24]	The level of expression of the HMGA2 gene was studied on a sufficiently panel of samples from 180 patients. The highest levels of HMGA2 expression were indeed observed in samples with rearrangements affecting the 12q14-15 region. But overexpression of HMGA2 was repeatedly found in leiomyoma samples without such aberrations, although at lower levels.
Klemke et al, 2010	[25]	It was found that uterine leiomyomas are characterized by chromosomal rearrangements in the 12q14-q15 region, leading to overexpression of the HMGA2 gene. Recent studies have identified

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		microRNAs of the let-7 family as post-transcriptional silencers of HMGA2 expression. Chromosomal rearrangements sometimes lead to the appearance of shortened or hybrid HMGA2 transcripts that lack 3'-UTR. The aim of the study was to use real-time RT-PCR to test how rearrangements of chromosomal region 12q14, leading to the appearance of shortened HMGA2 transcripts in leiomyoma nodes, affect the stability of mRNA. The presented results prove that chromosomal rearrangements involving the HMGA2 locus often lead to an increase in the mRNA lifetime, which contributes to overexpression.
Markowski 2010	[26]	Hormonal dependence of leiomyoma has long been known, and antitumor cytostatics are actively used in practice to suppress the growth of malignant neoplasms. The desire to use the PI3K/Akt/mTOR pathway as a therapeutic target is due to the fact that its shutdown does not cause immediate cell death, but leads to the so-called phenomenon of oncogen-induced aging, when cells under the action of proteins p16, p19, p53 and p21 gradually lose their division potential, weaken the antioxidant defense system, which weakens tumor aggressiveness with a relatively low level of side toxicity. Therefore a balance between HMGA2 and the p19Arf-TP53-CDKN1A axis was found to be essential for the growth of uterine leiomyoma
Schoenmakers 2013	[27]	It is reported that repeated genomic rearrangements: del(7)(q22), t(12;14)(q15;q24), t(1;2)(p36;p24), transpositions involving regions 6p21 and/or 10q22 occur in about 40% of leiomyoma nodes. These authors claim that in their previous works, they identified the genes HMGA1, HMGA2, RAD51L1, MORF and NCOA1 as the primary target of chromosomal rearrangements that cause the appearance of a benign tumor in each of the four variants of genome rearrangement using remote PCR methods.
Holzmann et al, 2014	[28]	The work reports that in foci of leiomyomas that do not have somatic mutations in the MED12 gene, chromotripsis phenomena were observed: numerous duplications and deletions of small segments grouped mainly in five chromosomal regions: 2p14-2pter, 2q33.1-2q37.3, 5q31.3-5qter, 11q14.1-11qter and 18p11.21-18q2.3. Due to the small size of the rearranged fragments of genomic DNA, such rearrangements can hardly be detected by methods of classical cytogenetics. Histologically, nodes with chromotrypsin, as a rule, represent a cellular leiomyoma with pronounced hyperproduction of hyaluronic acid. The results of the work show that leiomyomas with a normal karyotype and without somatic mutations in the MED12 gene are a heterogeneous group of diseases characterized by chromotripsis ("firestorm"), which does not affect the sites of chromosomal rearrangements characteristic of leiomyoma, such as 12q14-q15 and 6p21.
Pradhan et al, 2016	[29]	The results of using the remote reverse PCR method for the detection and screening of de novo DNA rearrangements in uterine leiomyomas are reported. The method used makes it possible to identify genome rearrangements in the leiomyome in comparison with the normal parental myometrium without putting forward an initial hypothesis about the location of recombination points. Screening of uterine leiomyoma samples for the presence of rearrangements in genomic locations allowed us to establish that the most susceptible to rearrangements of the genome in this type of tumor are located above the coding region of the HMGA2 gene and inside the RAD51B gene. In particular, a previously undescribed point of genomic rearrangement above the HMGA2 gene was identified, which went unnoticed in a previous study performed by

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		genome-wide sequencing, where 30 samples of uterine leiomyoma showed no rearrangements within 1107 bp and 1,996 bp analyzed in the RAD51B and HMGA2 rearrangement hotspots.
		Direction 2. Mutations in MED12
Mäkinen et al 2017	[30]	The authors of this work were the first to express the opinion that somatic mutations in the MED12 gene, biallelic inactivation of the fumarate hydratase gene and chromosomal aberrations leading to overexpression of the HMGA2 gene correspond to three mutually exclusive mechanisms of leiomyoma formation.
Wu et al, 2017	[31]	The work is devoted to elucidating the biological features of the two most common subtypes of uterine leiomyoma, mutant by MED12 (MED12-LM) and overexpressing HMGA2 (HMGA2-LM) uterine leiomyomas. Since each tumor carries only one genetic change, both subtypes are considered monoclonal. Approximately 90% of the cells in the HMGA2 uterine leiomyoma were smooth muscle cells with overexpression of HMGA2. In contrast, MED12-LM consisted of the same number of smooth muscle cells and tumor-associated fibroblasts (TAF). TAF did not carry mutations in MED12, which suggests an interaction between smooth muscle cells and fibroblasts having different origins during the formation and growth of the node.
Yin et al, 2015	[32]	Human uterine leiomyoma stem/progenitor cells expressing CD34 and CD49b initiate tumors in vivo
Mas et al, 2015	[33]	Stro-1/CD44 as putative human myometrial and fibroid stem cell markers
Mu et al, 2016	[34]	IGF-1 and VEGF can be used as prognostic indicators for patients with uterine fibroids treated with uterine artery embolization
Heikkinen et al, 2018	[35]	It was reported that there is a positive correlation between the increased expression of the COL3A1 gene in the nodes of leiomyomas and the expression of the HOXA13 gene, which is a regulator of the development of the organs of the female reproductive system, in particular, the cervix and vagina. According to these studies, a statistically significant increase in the expression of the HOXA13 gene above the level characteristic of normal myometrium was observed in both MED12-dependent and HMGA2-dependent nodes.
Reis et al, 2016	[36]	Overexpression of COL4A1 and COL4A2 collagens in MED12-positive leiomyoma nodes was detected
		Direction 3. Null-mutations in FH gene
Sulkowski 2018	[37]	Krebs-cycle-deficient hereditary cancer syndromes are defined by defects in homologous-recombination DNA repair
Mäkinen 2017	[30]	The authors of this work were the first to express the opinion that somatic mutations in the MED12 gene, biallelic inactivation of the fumarate hydratase gene and chromosomal aberrations leading to overexpression of the HMGA2 gene correspond to three mutually exclusive mechanisms of leiomyoma formation.
		Direction 4. Agonists and antagonists of steroid hormones
Gonzalez-Barcena et al, 1997	[38]	Treatment of uterine leiomyomas with luteinizing hormone-releasing hormone antagonist Cetrorelix
Nowicki et al, 2002	[39]	The influence of luteinizing hormone-releasing hormone analog on serum leptin and body composition in women with solitary uterine myoma
Engman et al, 2009	[40]	Mifepristone for treatment of uterine leiomyoma. A prospective randomized placebo controlled trial
Bouchard et al, 2011	[41]	Selective progesterone receptor modulators in reproductive medicine: pharmacology, clinical efficacy and safety
Malik et al, 2016	[42]	Gonadotropin-releasing hormone analogues inhibit leiomyoma extracellular matrix despite presence of gonadal hormones
Patel et al, 2016	[43]	Mifepristone inhibits extracellular matrix formation in uterine leiomyoma

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Islam et al, 2021	[44]	Extracellular matrix and Hippo signaling as therapeutic targets of antifibrotic compounds for uterine fibroids
Dababou et al, 2021	[45]	Linzagolix: a new GnRH-antagonist under investigation for the treatment of endometriosis and uterine myomas
Middelkoop et al, 2022	[46]	Evaluation of marketing authorization and clinical implementation of ulipristal acetate for uterine fibroids
Arjona et al, 2022	[47]	Development of relugolix combination therapy as a medical treatment option for women with uterine fibroids or endometriosis is described
Salas et al, 2022	[48]	New local ganirelix sustained release therapy for uterine leiomyoma. Evaluation in a preclinical organ model
Chwalisz 2023	[49]	Clinical development of the oral gonadotropin-releasing hormone antagonist elagolix
Direction 5. PI3K/Akt/mTOR and other intracellular signal pathway in UL nodes		
Hu et al, 2009	[50]	Blockade of Wnt signaling inhibits angiogenesis and tumor growth in hepatocellular carcinoma
Xu et al, 2014	[51]	It is hypothesized that the inhibition of AKT leads to the short-term triggering of specific mechanisms that ultimately lead cells to cellular aging or death by the mechanism of apoptosis. It was experimentally shown that inhibition of AKT leads to accelerated aging of culture cells. Treatment of MK-2206 cells with an allosteric AKT inhibitor increased the content of reactive oxygen species, the level of miR-182 microRNA production and transcripts of several genes that are considered as markers of ROS: p16, p53, p21 and β-galactosidase. The induction of ROS was associated with the hyperproduction of HMGA2, which was colocalized in the aging-related regions of heterochromatin.
Ye et al, 2014	[52]	Small molecule inhibitors targeting activator protein 1 (AP-1)
Galindo LJ et al, 2018	[53]	Comparative analysis of AKT and the related biomarkers in uterine leiomyomas with MED12, HMGA2, and FH mutations
Xie et al, 2018	[54]	The work is devoted to the study of the AKT signaling pathway and the mechanism of OIS in leiomyoma cells containing various driver mutations: MED12 mutations (n = 25), HMGA2 overexpression (n = 15) and biallelic inactivation of FH (n = 27). In each sample, the expression of genes involved in the response to sex steroids, the cell cycle and the AKT pathway was studied by immunohistochemical method. It was found that the ER and PR genes were well expressed in all types of leiomyoma except for the FH-dependent type, which showed low ER expression and increased PR expression. HMGA2-dependent type samples had significantly higher levels of AKT signaling and mitogenic activity than other types of leiomyoma nodes. HMGA2 activated AKT signaling by enhancing IGF2BP2 expression. Suppression of HER2 expression in leiomyoma cells led to a decrease in AKT activity and an increase in the expression of p16 and p21, which ultimately caused oncogen-induced cell aging.
Alzahrani et al, 2019	[55]	Application PI3K/Akt/mTOR inhibitors in cancer is described
Pilgrim et al, 2020	[56]	Characterization of the role of Activator Protein 1 signaling pathway on extracellular matrix deposition in uterine leiomyoma

Using the cited publications, the following sections are formed in the review: Mechanisms and risk factors for the development of recurrent uterine leiomyoma, Existing methods of medicinal treatment of the recurrent uterine leiomyomata, Prediction of genetic risk and identification of potential therapeutic targets in leiomyoma cells using metagenomic, metatranscriptomic and metamethylomic methods of analysis of complete genomes, Approaches to development of new candidate therapeutics for the prevention and treatment of relapses of leiomyoma, including patients preparing for pregnancy or in the process of gestation, and Conclusion.

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3. Mechanisms and risk factors for the development of recurrent uterine leiomyoma

Currently, the molecular mechanism of the occurrence of leiomyoma nodes remains unclear [57]. It is assumed that the initiator of the transformation of myometrial cells or their stem progenitors into fibroids cells is local hypoxia and the effect of hormones, the concentration of which varies significantly depending on the phase of the estrous cycle [58]. There is no doubt about the dependence of the occurrence and growth of fibroids on sex steroids - estrogens, progesterone and, and pituitary hormones – gonadotropin, follicle-stimulating, luteinizing and anti-Mullerian hormones, prolactin. An essential role is played by the individual genotype of the patient (heredity), ethnic origin and a high body mass index.

Since 2017, the concept of a driver mutation leading to the appearance of a leiomyoma node has been established in the literature [30]. Noteworthy, each node of the leiomyoma is monoclonal, originating from a single progenitor cell [59]. This leads to the fact that the genotype of the so-called "driver mutation" in different nodes of one patient with multiple fibroids usually does not match. The most common driver mutations are somatic missense mutation in exon 2 of the MED12 gene (codons 130 and 131 as well as in frame deletions) encoding the regulatory subunit of RNA polymerase 2, and rearrangements leading to overexpression of the HMGA2 protein, a non-histone chromatin-binding protein involved in the regulation of the chromatic condensation and regulation of gene expression. These driver mutations embrace for more than 95% of cases of leiomyoma. In addition, biallelic inactivation of the fumarate hydratase gene (Krebs cycle enzyme) and rare chromosomal rearrangements, in particular, the loss of the Xq22.3 region, leading to the loss of COL4A5-COL4A6 collagen genes, can act as a driver mutation [60].

Conflicting opinions have been expressed in the literature regarding the possibility of two driver mutations co-existing in one cell. Most authors suggest that two mutations in one cell are incompatible. In the works [57,61–63] it was shown by methods of genome-wide meta-transcriptomic and meta-methylomic analysis that the global profiles of gene expression and methylation in the case of MED12-dependent and HMGA2-dependent nodes differ significantly. A feature of MED12-dependent nodes is a high proportion of fibroblasts (which do not have a driver mutation and correspond to the normal genotype of the patient), a high content of extracellular matrix (ECM) and a low degree of vascularization. Nodes of this type have a relatively small size, but show a tendency of multiplicity. Due to the high content of fibroblasts, the growth of MED12-dependent nodes is stimulated not only by progesterone, but also by estrogens, which is not typical for other types of nodes.

HMGA2-dependent nodes do not contain a clearly determined *sensu stricto* driver mutation, but their common and fundamental feature is the overexpression of the HMGA2 gene, and sometimes also of the HMGA1 and PLAG1 genes, which determine overall expression profile and metabolism of the cell. The most common cause of HMGA2 overexpression may be the translocation of the 12q14.3 region containing the HMGA2 gene to chromosome 14 or to other parts of the genome where the gene falls under the control of promoters of other genes (most often RAD51L1), or its 3'-terminal region is deleted, contributing to the destabilization of the transcript. The second common cause of HMGA2 overexpression is deletion of the long arm of chromosome 7(q21.2q31.2), which contains the regulatory RNA genes miR-21, miR-23b, miR-29b and miR-197 [64] and the CUX1 homeobox gene [27]. These micro-RNAs, primarily miR-29b, are repressors of HMGA2 expression, so their loss leads to overexpression of the regulated gene. More than half of the leiomyoma nodes with HMGA2 overexpression do not have detected chromosomal rearrangements, and HMGA2 overexpression is usually suggested to be achieved there by changing the methylation status [65]. The presence of several independent mechanisms of HMGA2 overexpression significantly complicates the identification of such nodes at the level of laboratory diagnostics: the most reliable way to solve this problem is to detect the HMGA2 protein using immunohistochemical staining or its transcript with RT-qPCR. The accuracy of diagnosis is facilitated by the absence or extremely low level of HMGA2 expression in normal myometrium, although there are reports of the possibility of moderate HMGA2 expression in MED12-dependent uterine leiomyoma nodes.

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Biallelic inactivation of the fumarate hydratase gene is characteristic mainly for a rare type of leiomyoma with bizarre nuclei [66]. According to [62], cells of this type have a special type of metabolism: overexpression of genes of the pentose phosphate pathway of sugar oxidation and glucose-6-phosphate dehydrogenase, suppression of expression of Krebs cycle enzymes and pyruvate dehydrogenase, hyperproduction of enzymes responsible for the synthesis of reduced glutathione.

Other driver mutations occurring in the nodes of leiomyomas are so rare that their systematic study on the basis of statistically reliable data is practically difficult. Information about these mutations is present in the works of [60,62]. It was suggested that the driver of deletions in the COL4A5 and COL4A6 genes encoding basal membrane collagens is the IRS4 gene, the product of which is an intracellular messenger phosphorylated by the insulin receptor or IGF1 after binding to them by an extracellular ligand [65]. In this regard, one should note that the IRS4 gene is located in the Xq22.3 chromosome region nearby to the COL4A5 and COL4A6 genes. Holzmann et al, 2014 [28] reported that leiomyoma foci having nor somatic mutations in the MED12 gene neither HMGA2 overproducing, are prone to so called chromotripsis (multiple duplications and deletions of small segments grouped mainly in five chromosomal regions 2p14-2pter, 2q33.1-2q37.3, 5q31.3-5qter, 11q14.1-11qter and 18p11.21-18q2.3 were found). Due to the small size of the rearranged fragments of genomic DNA, such rearrangements are difficult to be detected by methods of classical cytogenetics.

4. Existing methods of medicinal treatment of the recurrent uterine leiomyomata

Historically, the first methods of therapy for leiomyomas were the use of hormone antagonists or antitumor cytostatics acting at the level of the PI3K (phosphatidylinosylkinase 3) / Akt / mTOR signaling pathway (target of the antibiotic rapamycin inhibiting autophagy) [55]. This is due to the fact that the hormonal dependence of leiomyoma has long been known, and antitumor cytostatics are actively used in practice to suppress the growth of malignant neoplasms. The desire to use the PI3K/Akt/mTOR pathway as a therapeutic target is due to the fact that its shutdown does not cause immediate cell death, but leads to the so-called phenomenon of oncogene-induced senescence (OIS), when cells under the action of proteins p16, p19, p53 and p21 gradually lose their division potential, weaken the antioxidant protection system, which weakens the tumor aggressiveness. Induction of this pathway is the normal mechanism of excessive myometrium, degeneration after the childbirth and its launching in leiomyoma cells theoretically can be highly specific and cause a relatively low level of side toxicity.

A consistent increasing production of pACT and GF2BP2 was observed in the cells of leiomyoma nodes with overexpression of HMGA2 [54]. Specific suppression of GF2BP2 expression by RNA interference causes a decrease in the level of pACT.

Suppression of the pACT expression with miR-182 micro-RNA or with a chemical inhibitor MK-2206 was reported to lead to an increase in the production of proteins p16 (Ink4a), p53 and p21, which ultimately causes the phenomenon of premature senescence in leiomyoma cells [51]. However, [26] reported that basic expression level of p16(Ink4a) and p19 (Arf) in the leiomyoma cells was many times higher compared to the myometrium, but this fact does not lead to signs of oncogen-induced senescence of the fibroid nodes.

A compound T-5224, an inhibitor of transcription factor AP1 (activating protein 1) involved in the activation of ECM synthesis and the formation of fibrosis foci was described by [52]. Pilgrim et al, 2020 [56] reported that after stimulation of the leiomyoma cells culture with TGFß3 for 24 hours, the content of fibronectin increased by 2.16 ± 0.14 times, and versican (specific hyaline cartilage hyalurone-binding glycoprotein) by 4.71 ± 0.15 times. The content of collagen 1A after 6 hours increased by 1.32 ± 0.01 times compared to the baseline level, and after 24 hours – by 6.49 ± 0.02 times. 4 hours after treatment with an AP1 inhibitor (SR11302), a decrease in the content of collagen 1A was observed by 0.59 ± 0.03 times, and after 6 hours - by 0.42± 0.05 times. The decrease in the content of versican under the action of SR11302 decreased by 0.84± 0.04 times 6 hours after treatment. The inhibitor significantly reduced the fibronectin content in 8 hours of treatment (0.68± 0.05 times).

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The use of gonadotropin-releasing hormone (GnRH) agonist trials (Leuprolide acetate) and GnRH antagonists (Cetrorelix acetate) as a candidate therapy for leiomyomas is described by [42]. It is reported that 3D cultures of leiomyoma cells exposed to estrogen E2 for 24 hours showed increased expression of collagen 1, fibronectin and versican, which persisted for 72 hours. Progesterone treatment increased the level of collagen 1 within 24 hours after exposure. Simultaneous application of estrogen and progesterone caused a significant increase in all ECM proteins. When treating the culture of Leuprolide acetate and Cetrorelix acetate for 24 hours, a significant decrease in the production of ECM proteins was observed. Both compounds reduced the production of ECM proteins both in the absence and in the presence of one or both sex steroids. There are some other communications about GnRH agonist or antagonist trials for the uterine fibroid therapy *e.g* Linzagolix (OBE 2109, KLH 2109) [45], Relugolix as a separet substance or in combination with estradiol and norethindrone acetate (NETA) [47], Elagolix [49], Ganirelix [48].

The use of selective progesterone receptor modulators (Ulipristal acetate and Asoprisnil), antiprogestin (Mifepristone - RU486) is described in [43]. Treatment of immortalized two-dimensional (2D) and three-dimensional (3D) human leiomyoma and myometrial cells with progesterone agonist progestin stimulated the production of COL1A1, fibronectin, versican and dermatopontin. Treatment with Mifepristone, an approved agent for the treatment of progesterone-dependent types of breast cancer, suppressed the synthesis of ECM components, especially versican. The combined treatment of cultures with an agonist and an antagonist of progesterone caused suppression of the synthesis of ECM components. In the countries of the European Union and Canada, Ulipristal acetate (SPRM) is allowed for medical use as a contraceptive and in the treatment of uterine fibroids and endometriosis in doses from 5 to 10 mg. Asoprisnil (J867), a compound of the same group as Ulipristal acetate, is also a selective modulator of progesterone receptors (SPRM), exhibiting the properties of both an agonist and an antagonist, depending on the type of a target tissue.

The effects of Goserelin, an agonist of luteinizing hormone releasing hormone, on serum leptin levels and body composition in women with uterine leiomyoma were reported by [39]. Fifteen women with a normal course of the sexual cycle participated in the trials. Serum concentrations of leptin, insulin, testosterone, progesterone and estradiol were measured in all subjects, as well as body mass index and waist-hip ratio before and after 4, 8 and 12 weeks of treatment with Goserelin, which was given at a dose of 3.6 mg once every 4 weeks. Body fat mass and muscle mass were measured by two-energy radiographic densitometry at the beginning and after 12 weeks of therapy. Treatment led to a significant regression of fibroids. Body weight, fat and muscle mass have not changed. During the treatment, there were no changes in the level of leptin in blood plasma. The level of estradiol in the blood plasma decreased below the level typical for postmenopause. Progesterone in plasma decreased significantly, and testosterone tended to decrease throughout the study.

Results of clinical trials for the treatment of uterine leiomyoma with *Clostridium histolyticum* collagenase (CCH), which selectively hydrolyzes collagens of types I and III and changes the stiffness of the ECM, in combination with verteporfin, an inhibitor of the YAP factor (Hippo/YAP pathway) and the antifibrosis drug Nintedanib were reported in [44]. The introduction of CCH in doses of 0.1-0.2 mg/cm^3 into fibroids after 60 days resulted in a 46% decrease in stiffness compared to the control. The level of PCNA, a marker of cell proliferation of nuclear antigen of proliferative cells, was reduced 60 days after injection of high doses of CCH. The content of key intracellular growth signaling factors Hippo and the phosphorylated form of YAP (p-YAP), as a result of the use of CCH, were increased, which contributed to maintaining a high rate of fibroid growth. Inhibition of YAP by verteporfin reduced cell proliferation, gene expression and proteins of key factors contributing to fibrosis and mechanotransduction in fibrous cells. The antifibrotic drug Nintedanib additionally reduced the activity of YAP and showed an antifibrotic effect.

Trials of the plant-derived polyphenolic compound Resveratrol for the treatment of leiomyoma are described in [67]. The author reported a clear inhibitory effect of resveratrol on the proliferation of primary cultures of human uterus leiomyoma cells. Resveratrol stopped cell proliferation via avß3 integrin signaling since expression of this protein was suppressed by the drug. At the same time,

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Resveratrol inhibited constitutive phosphorylation of AKT in fibrous cells. Resveratrol treatment induced the expression of pro-apoptotic genes: cyclooxygenase (COX)-2, p21 and CDKN2. On the contrary, the expression of proliferative (anti-apoptotic) genes was either suppressed (BCL2), or unchanged (cyclin D1 and PCNA). There was a decrease in the production of insulin-like growth factor receptor (IGF-1R). Resveratrol treatment suppressed IGF1-induced proliferation of the uterine leiomyoma cells. The authors suggested that the arrest of the leiomyoma cell growth by Resveratrol occurs as a result of cross-interaction between the integrin avß3 and IGF-1R. This message can be called promising, but it should be borne in mind that currently resveratrol is not available for pharmaceutical use, since its chemical synthesis is not established, and its content in plant raw materials is not sufficient for practical use. Moreover, clinical trials of this drug have not yet begun, which does not allow expecting its rapid introduction into practical use.

A method of therapy of leiomyomata with a driver mutation in the fumarate hydratase gene was proposed in [37]. They claimed that fumarate and succinate inhibit the DNA repair mechanism that involves the homologous recombination mechanism and is necessary to eliminate double-stranded breaks in chromatin. This circumstance makes tumor cells, unlike normal ones, vulnerable to Olaparib and Niraparib, synthetic inhibitors of poly-ADP-ribose polymerase (PARP1, also known as NAD+-ADP-ribosyltransferase 1 and PARP2) binding at the NAD+-binding site of the target enzyme. PARP inhibitors are approved for pharmaceutical use and should be suggested as available potential medicines for atypical variants of leiomyoma containing a mutation of biallelic inactivation of the fumarate hydratase gene.

5. Prediction of genetic risk and identification of potential therapeutic targets in leiomyoma cells using metagenomic, metatranscriptomic and metamethylomic methods of analysis of complete genomes

To date, a number of works has been carried out worldwide aimed at identifying factors of hereditary predisposition to the occurrence of leiomyomas [14, 68, 69, 70, 71, 72, and 73]. The main directions of the studies (1) genome-wide associated studies pursuing mapping polymorphisms associated with predisposition to UL onset; (2) genome-wide transcriptome and DNA-methylome studies of UL nodes pursuing discovery of the gene transcription alterations leading to transformation of the normal myometrium to the fibroid tissue; (3) candidate medicines for UL treatment based on small molecules, vaccines and RNA are summarized in Table 2. As a result, more than 100 single nucleotide polymorphisms (SNP) were identified, the genotype of which affects the risk of developing leiomyoma. However, the practical use of the results of these works is not organized so far. Moreover, although many of these studies used highly representative groups of several tens of thousands of patients and up to 523 thousand control patients, in none of them authors used data on the presence of a burdened family history of patients. Moreover, the tumor samples themselves were not classified depending on the type of driver mutation. Meanwhile, it can be assumed that the hereditary factors of predisposition to the occurrence of somatic mutations in the MED12 gene, HGMA2 overexpression and biallelic inactivation of the fumarate hydratase gene are unlikely to completely coincide. Therefore, now there is no possibility to predict the risk of each type of leiomyoma based on the analysis of the patient's genotype.

Table 2. Principal directions of the uterine leiomyoma regulation study by GWAS, metatranscipromic and metamethylomic approaches and using their data for development of candidate medicines for UL treatment based on small molecules, vaccines and RNA.

Direction 1. GWAS for mapping polymorphisms associated with predisposition to UL onset		
Cha et al, 2011	[68]	GWAS identifies three loci associated with susceptibility to uterine fibroids
Eggert et al 2012	[69]	Genome-wide linkage and association analyses implicate FASN in predisposition to Uterine Leiomyomata
Hellwege et al 2017	[70]	A multi-stage genome-wide association study of uterine fibroids in African Americans

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Välimäki et al, 2018	[71]	These authors used genome-wide association analysis (GWAS) to identify genetic variants that are more common in people with fibroids. Using data from the British Biobank, the genomes of more than 15,000 women with fibroids were analyzed, which were compared with a control group of more than 392,000 individuals. The analysis revealed 22 regions of the genome, the genotypes of which differed in the experimental and control groups. These regions included genes that may well contribute to the development of fibroids, such as the TP53 gene, which affects the stability of the genome, and ESR1, which encodes the estrogen receptor (it is well known that this hormone plays an important role in stimulating the growth of fibroids). Differences in genotypes were revealed for known genes involved in the control of the development of female genital organs.
Rafnar et al 2018	[72]	Variants associating with uterine leiomyoma highlight genetic background shared by various cancers and hormone-related traits
Edwards et al, 2019	[14]	Trans-Ethnic Genome-Wide Association Study of Uterine Fibroids
Gallagher et al, 2019	[73]	Genome-wide association and epidemiological analyses reveal common genetic origins between uterine leiomyomata and endometriosis
Direction 2. Genome-wide transcriptome and DNA-methylome studies		
Wang et al, 2007	[64]	Paired samples of leiomyomas and normal myometrium from 41 patients were examined, which were used to construct banks of micro-RNA and their subsequent sequencing. As a result of bioinformatic analysis, 45 microRNAs with significantly increased or decreased content in leiomyoma nodes compared to the corresponding myometrium were identified ($P < 0.001$). The five undergoing the strongest expression change are the let-7 family: miR-21, miR-23b, miR29b and miR-197.
Navarro et al, 2012	[61]	The objective of the work was to identify abnormally methylated sections of the genome in UL cells *in vivo* using genome-wide analysis methods and to compare the data obtained with the results of the metatranscriptome analysis. Biological material in the form of paired samples of leiomyomas and adjacent normal myometrium was selected from 18 patients of African-American origin. 55 genes with differential methylation of promoter regions were identified, which correlated with differences in the level of expression in uterine leiomyoma compared with normal myometrium. 80% of the identified genes showed an inverse relationship between the status of DNA methylation and the content of the corresponding mRNA in uterine leiomyoma tissues, including 34 genes demonstrated hypermethylation of the promoter region and a corresponding decrease in expression level, and 10 genes demonstrated demethylation and an increase in expression level.
Mehine et al, 2016	[62]	The data of a meta-transcriptomic study of the expression profile of leiomyomas with four types of driver mutations in comparison to the expression profile of the adjacent normal myometrium, are presented. 19 upregulated and a single down regulated markers of leiomiomata (in comparison to the normal myometrium) are reported.
Anjum et al, 2019	[63]	The level of expression of marker transcripts in UL was measured using meta-transcriptomic analysis on the Illumina platform, followed by validation of the most significant results using real-time RT-PCR. Full transcriptome analysis showed an increase in the expression of 128 genes in the nodes of the leiomyoma compared with normal myometrium and a decrease in the expression of 98 genes.
George et al, 2019	[65]	Integrated Epigenome, Exome, and Transcriptome analyses was carried out for revealing molecular subtypes of uterine fibroid nodes. Cases of HER2 overexpression independent chromosomal

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		rearrangement were found. Hypomethylation of the structural region of the HMGA2 gene was found to be a reason of the overexpression. It was found that MED12 mutations and increased HMGA2 expression can coexist in the same leiomyoma node. Increased expression of IRS4 (insulin receptor substrate undergoing phosphorylation when binding insulin to the receptor) in leiomyoma cells compared to myometrium is found.
Maekawa et al, 2022	[57]	Differences in DNA methylome, transcriptome and histological features in uterine fibroids with and without MED12 mutations were studied. Genes of inflammatory response (CCL2, AOX1, ACKR1), apoptosis (ANXA1, CITED2) and metabolism associated with reactive oxygen species were reported to undergo hypermethylation in all types of leiomyoma cells. It was found that 80-90% of leiomyoma nodes of all types show overexpression of the SATB2 and NRG1 genes compared to the myometrium, and the excess level varies from 1.5 to 20-30 times.
Direction 3. Candidate medicines for UL treatment based on small molecules, vaccines and RNA		
Walker et al, 1983	[74]	Therapeutic potential of the LHRH agonist, ICI 118630, in the treatment of advanced prostatic carcinoma
Hinterhuber et al, 2005	[75]	Expression of RPE65, a putative receptor for plasma retinol-binding protein, in nonmelanocytic skin tumours
Peruzzi et al, 2009	[76]	MMP11 was described as a novel target antigen for cancer immunotherapy
Yin et al, 2010	[77]	Transcription factor KLF11 integrates progesterone receptor signaling and proliferation in uterine leiomyoma cells
Zheng et al, 2014	[78]	Epigenetic regulation of uterine biology by transcription factor KLF11 via posttranslational histone deacetylation of cytochrome p450 metabolic enzymes
Ho et al, 2018	[67]	Resveratrol inhibits human leiomyoma cell proliferation via crosstalk between integrin avß3 and IGF-1R
Grigorkevich et al, 2019	[79]	Matrix metalloproteinases and their inhibitors. Pharmacokinetics and pharmacodynamics
Cao et al, 2019	[80]	H19 lncRNA has been identified as a master regulator of genes that control uterine leiomyomas

There are also a number of papers in the literature focused on the molecular profiling of leiomyoma at the level of transcriptome and DNA-methylome analysis [57,61–63]. Authors [57] and [62] classified the samples used in the work in accordance to the type of driver mutation. The data obtained allow us to draw a number of conclusions regarding the features of gene expression regulation that are important for the development of new leiomyoma therapies.

The work [62] confirms the previously proposed hypothesis about a significant change in the expression of genes involved into Wnt/β-catenin signal pathway: in all types of leiomyoma cells, these genes are suppressed. This is caused by an increase in the expression level of antagonists of Wnt - WIF1 and SFRP1, previously described by [50]. Significantly, this change concerns both types of leiomyomas: those with somatic mutations in MED12 and those with overexpression of HMGA2. This conclusion is fully supported by the work [57]. Therefore, secretory proteins Wif1 and Sfrp1 can be considered as promising targets for removal using therapeutic antibodies that are universal for all leiomyoma types. The pituitary hormone prolactin is another promising target for removal from the organism in order to prevent relapses of the uterine leiomyoma. According to [62], prolactin levels are steadily increased in all leiomyoma types, regardless of the driver mutation. The presence of prolactin in the mother's body is critically important only during lactation, therefore, the use of prolactin inhibitors, including those based on recombinant humanized antibodies at the stage of pregnancy has the prospect of achieving a good effect in terms of preventing the growth of leiomyoma nodes without causing a side damage to the mother's body and fetus. Maekawa et al., 2022 [57] also found demethylation of genes associated with nucleosome assembly, telomerase activity (HIST1H4J, HIST1H4K, HIST1H4F) in all types of the uterine leiomyomata. In contrast, genes

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of inflammatory response (CCL2, AOX1, ACKR1), apoptosis (ANXA1, CITED2) and metabolism associated with reactive oxygen species undergo hypermethylation in all leiomyoma types. This information is important from the point of view of understanding the mechanisms of pathogenesis, however, it does not provide a key to choosing a target for the therapy since most of these genes are active in normal tissues and cannot be switched off without a severe damage for the organism. In a separate direct experiment using RT-qPCR, [57] demonstrated that 80-90% of leiomyoma nodes of all types showed overexpression of the SATB2 and NRG1 genes compared to the myometrium, and the excess level varies from 1.5 to 20-30 times. Cell lines overexpressing SATB2 have a morphologically unusual cell type: they lose the fusiform shape peculiar for smooth muscle tumors and become similar to fibroblasts with elongated pseudopods. This suggests that SATB2 and NRG2 play an important role in the initiation of leiomyoma. We believe that these data allow us to consider the proteins Satb2 and Nrg1 promising targets for the creation of therapies for all leiomyoma types.

Above 40 genes are totally listed in [57,61,62], the products of which are represented in the leiomyoma nodes of Med12-dependent and HMGA2-dependent types at the level significantly exceeding those in the normal myometrium. They should be considered as promising targets for therapy. However, for experimental verification of the effectiveness of candidate therapeutic agents, it is advisable to narrow down a fairly wide range of these targets, giving preference to two categories of proteins:

1. Secreted proteins that do not remain the surface of the cells producing them;

2. Proteins present exclusively in leiomyoma cells and are absent in any cells of normal tissues.

Such an approach to the selection of targets is dictated by the need of minimizing the side toxicity of candidate drugs, which can be achieved only if normal organs and tissues remain unaffected. In the first case (the secreted molecules), proteins themselves not producer cells are targeted, and the therapy leads only to temporary reducing their concentration in the extracellular space, while producer cells remain alive. Therefore, requirements for the absence of the targets in normal cells may be not absolute. As for proteins present strictly in leiomyoma cells, they are found mainly among embryonic proteins that are absent in adult cells, but often act as cancer markers. After sorting potential targets according to the proposed principle, the following list of genes (proteins) can be presented:

1. From the work [61]: PCP4 - Purkinje cell protein 4, expressed in embryogenesis; CHRDL2 - growth factor, a chordin-like antagonist of BMP; RPE65 - retinol transporter from the retinal epithelium; MMP11 - matrix metalloproteinase 11 - stromelysin 3, expressed in embryogenesis, causes metastasis of breast tumors; MFAP2 - microfibrillar-associated protein 2, affects the motility of cancer cells in gastric cancer, regulates the expression of a5ß1 integrin via ERK1/2 pathway.

2. From the work [62]:

a. for HMGA2-dependent node type: HMGA2, GRPR – gastrin precursor; PLAG1 - proto-oncogene, the main participant of HMGA2-dependent signaling; PAPPA2 - pappalysin, a protease that destroys IGFBP5, a marker of osteoblasts; MB21D2 - nucleotidyltransferase, an anti-virus protection gene induced by interferon;

b. for MED12-dependent node type: ADAM12 - membrane-bound metalloproteinase responsible for shedding – cleavage of extracellular receptor domains and proteoglycans; MMP11 - matrix metalloproteinase 11 - stromelysin 3, expressed in embryogenesis, causes metastasis of breast tumors; MMP16 - matrix metalloproteinase 16, has a transmembrane domain, expressed in embryogenesis; RAD51B - protein of the DNA reparation system, similar to RecA, having ATPase activity, not expressed in normal tissues; PCP4 is a protein of 4 Purkinje cells, expressed in embryogenesis; RUNDC1 is a DNA-binding protein with a RUN-type domain at the C-terminus; THSD4 (Adamtsl6-β) matrix metalloproteinase with a thrombospondin domain associated with the cell surface; participates in the formation of ECM microfibrils, ensuring elasticity and releasing of deposited growth factors. Adamtsl6-β expression increases during the formation of the periodontal ligament, which consists of a fibrillin-1 microfibril.

An interesting finding of [61] is the detection of an increased level of IL17B mRNA in leiomyoma. IL17B is a cytokine derived from T-lymphocyte and involved in the initiation and maintenance of

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inflammation, fibrosis and keratinization. An advantage of IL17B as a target for leiomyoma therapy is availability of recombinant humanized antibodies (*e.g.*), approved for the treatment of psoriasis and psoriatic arthritis [81]. These drugs demonstrate moderate side effects, which facilitate their clinical trials.

The protein RPE65 (retinol transporter from the retinal epithelium) was identified in the same work as a marker of differential expression in leiomyoma may also be considered as a target available for therapy with pre-existing drugs. It can be assumed that the superexpression of RPE65 in leiomyoma cells makes them sensitive to synthetic retinol analogues used in pharmaceuticals for the treatment of acne: adapalene, tretinoin, isotretinoin and tazarotene [75]. These products are indicated for topical use for the treatment of acne of mild and moderate severity, pilar keratosis, as well as other skin diseases.

6. Approaches to development of new candidate therapeutics for the prevention and treatment of relapses of leiomyoma, including patients preparing for pregnancy or in the process of gestation

Development of new therapeutic agents should be started by testing those pharmaceutical substances that are already available for use and have passed preclinical clinical trials to the maximum extent (Table 3). Among those listed in Chapter 5, this category includes:

(1) The immunobiological drug Secukinumab, Ixekizumab, Brodalumab and Netakimab, a recombinant humanized antibody against IL17 - for all leiomyoma types;

(2) Synthetic analogues of retinoids: adapalene, tretinoin, isotretinoin and tazarotene – potential blockers of the RPE65 receptor - for all leiomyoma types;

(3) Olaparib and niraparib - synthetic inhibitors of poly-ADP-ribosopolymerase (PARP1, also known as NAD+-ADP-ribosyltransferase 1 and PARP2) – for leiomyoma with biallelic inactivation of the fumarate hydratase gene as a driver mutation;

(4) Resveratrol - for all leiomyoma types.

The development of inhibitors based on amino acid hydroxamates, carboxylates, thiols, phosphonates and sulfonamides, flavonoids or doxycyclin derivatives against matrix metalloproteinases ADAM12, MMP11, MMP16 and THSD4 (Adamtsl6-β) should be considered a quick and easily accessible way to suppress their activity *in vivo*. The review [75] contains a survey of the structure and functions of metalloproteinases. This work provides no data on the importance of these four proteinases for maintaining the normal functioning of human tissues and organs. In the work [79] clinical trials of the following synthetic matrix protease inhibitors are reported: Marimastat (Pfizer, phase III, for the treatment of breast and lung cancer), Batimastat (British Biotech, phase II, for the treatment of malignant tumors), S-3304 (Shionogi, Phase II, for the treatment of solid lung cancer tumors), COL-3 (NSC-683551) (National Cancer Institute, phase I, refractory metastatic cancer), CGS-27023A (Novartis, phase I, for the treatment of arthritis and malignancies).

Table 3. Candidate targets for development of the uterine leiomyoma therapeutic agents.

Nosological type of the tumor	Chemical substances	Humanized monoclonal antibodies	Vaccines	RNA delivery
MED12-dependent	ADAM12, MMP11, MMP16, KCNAB3, CACNA1C, RAD51B	ADAM12, MMP11, MMP16, RUNDC1	ADAM12, MMP11, MMP16, RUNDC1, RAD51B	miR-200c, miR-93
HMGA2-dependent	PAPPA2, MB21D2,	GRPR, PLAG1, PAPPA2, MB21D2	HMGA2, PLAG1, PAPPA2, MB21D2	miR-21, miR-23b, miR-29b, miR-197, mir-106b

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FH-dependent	PARP (Olaparib*, Niraparib*), TNFRSF21, NQO1, SLC7A11, FAM46C, ABCC3	-	-	-
All types together	Resveratrol**, RPE65: adapalene**, tretinoin**, isotretinoin** and tazarotene**	IL17*, WIF1, SFRP1, SATB2, NRG1, PCP4, CHRDL2, MFAP2	PCP4, CHRDL2, MFAP2	Anti-H19, miR-182

* - substance is approved for treatment of some conditions besides leiomyoma; ** - substances is used as a para-pharmaceutics.

7. Discussion

In period since 1987, development of medicines for therapy of the uterine leiomyoma and basic researches of molecular mechanisms of this disease initiation were carried out in parallel without proper mutual coordination. For instance, Goserelin acetate was patented in 1976 and approved by FDA in 1983 [74]. Mifepristone clinical trials for therapy of the fibroid were completed in 2009 [40]. Cetrorelix acetat for the first time described in 1997 [38] was allowed by FDA for therapy of the uterine leiomyoma in 2010. Ulipristal acetate for the first time described in 2011 [41] remains under clinical trials since 2009 until now [45]. Clinical trials of Asoprisnil were discontinued in 2005 at phase III due to registered endometrial changes in patients [82]. Last generation proteinkinase inhibitors pf pAKT/PIC3/mTOR signal pathway Capivasertib and Ipatasertib have not been clinically tested for therapy of the uterine leiomyoma apparently due to an obviously high side toxicity. This brief survey clearly demonstrates that recent achievements in meta-transcriptomics, meta-methylomics and GWAS studies of the uterine leiomyoma almost were not used for development of the novel therapeutics. Moreover, the firmly established metabolic differences between leiomyoma types caused by different driver mutations (misfunction of MED12, overproduction of HMGA2 and biallelic inactivation of FH) were not used for development of specific medicines directed against specific targets of this different nosological forms of the uterine leiomyomata except relatively rare FH-dependent cases.

Analysis of the data by [62] allows suggestion that leiomyomas with a driver mutation in the MED12 gene should be considered as a separate relevant object for development of therapeutics. This follows from the greatest prevalence of this type of leiomyoma, as well as from the large number of target proteins in it, which are completely absent in normal tissues of the adult human body, but necessary for the survival and growth of the tumor. This category should include, first of all, free and membrane-bound metalloproteinases: ADAM12, MMP11, MMP16 and THSD4 (Adamtsl6-β). The development of specific inhibitors to them is a routine procedure, delivery to the target is facilitated by its extracellular location, and the absence of targets in normal tissues will ensure the absence of undesirable side effects. It also seems promising to create inhibitors of the potential-dependent channels KCNAB3 (potassium-specific) and CACNA1C (calcium-specific), as well as hyaluronidases that stimulate cell migration (a product of the KIAA1199 gene).

MMP11 seems to be the most promising target for development of target therapy of leiomioma, since it is not expressed in any of the normal tissues of the body, however sustainable therapeutic effect of leiomyomata is expected only if MMP11 inhibitor is combined with blockers of some other targets. Trials of these inhibitors should be carried out mainly on patients with MED12-dependent leiomyomas. It can be assumed that these compounds will have low side toxicity and will be suitable for patients at all stages of pregnancy. Developing an inhibitor of PAPPA2 (pappalizin), a protease that destroys IGFBP5, IGF1 mRNA binding protein, a marker of osteoblasts is also technically accessible. In this case, the trials should be carried out on a group of patients with HMGA2-dependent leiomyoma.

Creating inhibitors of membrane receptors: potential-dependent channels KCNAB3 and CACNA1C is also technologically easy task. Trials of these inhibitors should be carried out on patients with MED12-dependent leiomyoma.

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A modern and effective way to remove unwanted proteins from the body (blocking their activity) is the development of immunobiological drugs based on humanized recombinant antibodies. It is advisable to start their development with the antagonist proteins of the Wnt - Wif1 and Sfrp1 pathway, since they are secretory and universal in terms of the type of driver mutation that caused the development of the node. The development of immunobiological drugs for the removal of MMP11 and MMP16 metalloproteases from the body also has good prospects. Their trials should be carried out on groups of patients with MED12-dependent leiomyomata. Besides these drugs can be effectively used for the treatment of a number of malignant solid tumors, in particular to prevent metastasis of colon carcinoma. The prospects of this approach are confirmed by the data of [76], which provides the results of testing of a DNA vaccine based on MMP11 to protect mice from colon carcinoma. Immunization not only protected the animals from tumor progression, but also did not show undesirable side effects.

In addition, an economically affordable to protect patients against leiomyoma onset is the creation of preventive and protective vaccines based on proteins with high specificity for leiomyoma cells. A large set of markers can be attributed to this category: Satb2, Nrg1, PCP4, CHRDL2, MFAP2 - to combat all leiomyoma types; HMGA2, GRPR, PLAG1, PAPPA2 and MB21D2 - to combat HMGA2-dependent leiomyoma; ADAM12, MMP11, MMP16, RAD51B, THSD4 (Adamtsl6-β) and RUNDC1 - to combat of the MED12-dependent leiomyoma type. Vaccines can be either protein or genetic (based on DNA or RNA). Vaccines of the first type require longer development and testing, but have a significantly lower manufacturing cost per dose, which makes them more accessible to patients. The great advantage of vaccination is safety for patients compared to chemical agents, which allows them to be used during pregnancy. The advantage of vaccines is also the possibility of immunization of patients planning pregnancy with a high individual risk of leiomyoma onset, whereas the effect of immunization will be manifested during pregnancy, when the use of any medications is limited. An indirect argument confirming the effectiveness of vaccination as a means to combat the occurrence of leiomyomata is the fact that the risk of developing leiomyoma decreases significantly with each full-term pregnancy, whereas aborted pregnancies, on the contrary, increase the risk of the disease [3]. The reason for this, along with powerful hormonal effects occurring during pregnancy in the mother's body, may be the factor of immunization of the maternal organism with embryonic proteins, among which there are many antigens specifically expressed on the surface of leiomyoma cells, in particular: Satb2, Nrg1, PCP4, CHRDL2, MFAP2, HMGA2, PLAG1, PAPPA2, MB21D2, ADAM12, MMP11 and MMP16. The immunogenicity of the products of these proteins is probably not high by itself and due to the presence of the placental barrier, but due to repeated boosting during the multiple pregnancies, the production of antibodies specifically suppressing the growth of leiomyoma nodes can gradually reach a significant level.

Noteworthy, the effectiveness of the use of preventive vaccines to prevent the development of leiomyomas will depend on the development of diagnostic tools that allow determining the individual risk of developing a certain type of leiomyoma in a patient. This task is currently still waiting for its resolution.

Finally, a promising area of leiomyoma therapy is the delivery of RNA and DNA constructs. For example, in the case of HMGA2-dependent leiomyomas, the delivery of miR-21, miR-23b, miR-29b and miR-197 micro-RNAs described in [64], which suppress the expression of HMGA2. 45 micro-RNAs with significantly increased or decreased content in leiomyoma nodes compared to the corresponding myometrium were identified.

A good effect can be expected from the delivery of miRNA to induce the specific degradation of the long non-coding RNA H19 described in [80]. This RNA is a universal marker of MED12- and HMGA2-dependent leiomyomata, participating in the regulation of the expression of the chromatin demethylation factor TET3.

CEACAM1 (carcinoembryonic antigen - cell adhesion molecule 1), described by [42] and KLF11 genes [74,78] should be suggested as candidate DNA moieties the delivery of which to the leiomyoma cells *in vivo* is highly likely to contribute to their degradation due to the induction of endogenous apoptosis mechanisms as it happens in normal myometrium after a childbirth.

The production of RNA and DNA structures is a routine technological task, well-developed during the fight against the pandemic of the SARS-CoV-2 virus. However, for targeted delivery of such molecules to the uterine leiomyoma cells, it is advisable to use functionalized vectors with specific affinity to the surface markers of these. In the case of HMGA2-dependent leiomyomata, GPR20 and IL11RA can be specific receptors for solving this problem, since according to [62], their hyperproduction on cells of this type is observed. In the case of MED12-dependent leiomyomas, this function could be carried out by the membrane proteins POPDC2, PLP1 (proteolipid protein, lipofilin, expressed in the central nervous system, responsible for the interaction of axon membranes with myelin), THSD4 (Adamtsl6-β - transmembrane protein 4 with thrombospondine domain).

We are looking forward that our study will promote implementation of the data obtained by the modern high through-put molecular and bioinformatic methods to development of efficient specific methods for mitigating negative impact of leiomyomata on the life quality of the population.

8. Conclusions

Analysis of the results of metatranscriptomic and metamethylomic analysis suggests that the proteins Satb2, Pcp4, Wif1, Mmp11, Mmp16 and Adam12 are the most unique molecular markers expressed in all types of leiomyomatous nodes (with a driver mutation in the MED12 gene, null-mutation in the FH gene and with overexpression of the HMGA2 gene), but silent in the normal myometrium. These proteins should be investigated as candidate vaccine substances for the prevention of relapses of leiomyomatosis in patients with hereditary predisposition to this disease, particularly to those who plan pregnancy. Additional candidate markers for development of preventive and curative vaccines against leiomyomatosis can be identified as a result of immunological screening of blood sera from women who gave many births. Resveratrol and synthetic retinol analogues: adapalene, tretinoin, isotretinoin and tazarotene should be investigated as a promising therapeutic agent that allows slowing the growth of leiomyomatous nodes, including in patients with pregnancy.

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