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Uterine fibroids: a new insight into an old problem

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Summary

With the development of molecular methods in cancer biology, the etiopathogenesis of commonly occurring uterine myomas is constantly subject to amendment. It was found that ovarian steroid hormones are involved in the development of these benign tumors, hence the development of new therapies using selective progesterone receptor modulators (uliprystal acetate, UPA), especially in combination with vitamin D3 supplementation. Genetic changes associated with the pathogenesis of myomas, especially mutations in MED12 and related signaling via Wnt / β catenin, may be precursors to the development of aggressive forms of these benign tumors and therefore be influential regarding treatment options. Studies have also revealed the role of microRNA overexpression and angiogenesis in the development of uterine myomas, the inhibition of which may be associated with future therapy of these tumors. In recent years, the role of stem cells (SCs) in myomas and pathways involved in their activation have been described, which may also have clinical implications in the future.

Key words: Uterine fibroids; Steroid hormones; SPERMS; MED12 and HMGA mutations; Angiogenesis; Stem cells.

Introduction

Uterine fibroids are benign tumors and occur in about 80% of women of childbearing age. In 20-50% women, they do not cause any clinical symptoms; in the remainder these tumors cause abnormal menstrual and intermenstrual bleeding, compression symptoms on the adjacent bladder and intestines, and are the cause of reproductive failures: infertility or reduced fertility, miscarriage and complications during childbirth [1-3].

Numerous factors contribute to the pathogenesis and development of fibroids, including: hormones, microRNA, extracellular matrix (ECM) and stem cells (SCs) [1, 4-9].

The paper presents the results of recent research on etiopathogenesis, the development of uterine fibroids, and related therapeutic implications.

Hormones

It has been determined that estrogens and progesterone participate in the development and growth of myomas. Estrogen activity refers to the estrogens secreted by the ovary and by the adrenal glands due to the conversion of local androgens through aromatase, an enzyme belonging to the cytochrome P450 family. Estrogens mainly act through the nuclear α and β receptors (ERα and ERβ), stimulating an increase in the expression of many growth factors. Estradiol, a biologically active estrogen, increases the expression of progesterone receptors via its α receptor, it also induces the transcription of many genes involved in the proliferation and formation of ECM. ECM takes part in the pathogenesis of myomas accumulating growth factors and cytokines. Similarly, progesterone through its receptors regulates the transcription of genes involved in apoptosis, proliferation, and ECM formation. The role of transforming growth factor β (TGFβ) and activin in the formation of connective tissue, a component of myomas, is suggested [3, 5, 10-12].

It has been shown that selective estrogen receptor modulators (SERMS) e.g. raloxifene and selective progesterone receptor modulators (SPERMS) e.g. mifepristone and 2012 ulipristal acetate-(UPA) tested (*Esmya, Fibrista*) are an option for the conservative treatment of uterine fibroids. Comparison of the results of earlier myoma treatment with GnRH analogues, e.g. *Zoladex* or *Decapeptyl depot*, showed UPA was more beneficial as it did not cause menopausal symptoms and was associated with better quality of life. Additionally, aromatase inhibitors (*Letrozole*, *Anastrozol*e) inhibit the development of fibroids. However, all of the presented options reduce the size of myomas and some symptoms associated with them, but they do not eliminate them [2, 3, 10-13].

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Genetic changes

Observational studies showing more frequent occurrence of uterine fibroids in first-degree relatives found confirmation in genetic analyzes [9, 14-17]. The most frequently detected mutations in myomas occur in exon 2 of the MED12 gene (chromosomal location Xq13.11) belonging to the multi-protein complex of the conservatively evolutionary transcription regulator. According to Croce *et al.* [9] and Schwetye *et al.* [14], mutations occur in 50-70% of myomas, but in some studies even 100% of myomas were affected [17].

Studies by Mäkinen *et al.* [15] involving 159 fibroids with different histological staining variants showed differences in the occurrence of mutations in MED12: the most frequent mutations were found in typical myomas, less frequently in cellular tumors (cellulare, highly cellulare), and bizarre myomas (leiomyoma bizzare). Mutations in MED12 occurred in a lower percentage of the surrounding musculature, while they were found in 21.6-30% of uterine myomas [15, 17]. Recent Australian studies have shown that mutations in MED12 leading to the development of myomas take place by signaling the canonical Wnt / β-catenin pathway. Wnt inhibitors and biochemical changes influencing the reduction of β-catenin expression appear to be a promising direction forward in the development of new therapies for fibroids [12]. It has been suggested that fibroids with mutations in MED12 can be treated as precursor changes leading to aggressive uterine tumors [15].

About 50% of uterine fibroids with a mutation in MED12 also have another type of genetic disorder: high mobility group proteins (HMGAs). HMGAs (chromosomal location 6p21) belong to genes which encode low molecular weight proteins with high electrophoretic mobility. According to Galindo *et al.* [16], about 65% of uterine fibroids indicate overexpression of HMGA2, compared to the surrounding muscle tissue. It is believed that this aberration is an early event in the pathogenesis of myomas.

MicroRNAs

MicroRNAs are a family of small [19-22] low-coding nucleopeptide RNA particles involved in the regulation of the expression of both oncogenes and suppressor genes. Cardozo *et al.* [18] in studies on fibroid cell lines and in myoma tissue showed overexpression of miR-21a-5p, which caused an increased expression of TGF-β and increased proliferation and migration of myoma cells. At the same time, the overexpression of this microRNA induced changes in extracellular matrix gene expression, including fibronectin, collagen, metalloproteinases (MMP2, MMP9, and MMP11) and serpins associated with EC remodeling. This finding suggests a functional role for miR-21a-5p in the development of fibroids [8, 18].

Angiogenesis

Research results suggest that angiogenic growth factors play an important role in the pathomechanism of growth, survival and abnormal myomas' vascularization: vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF), platelet-derived growth factor (PDGF), and TGF-β. There is a greater expression of these factors in myoma tissue compared to the surrounding myometrium [19].

According to Ciebiera *et al.* [8], aside from the significant influence of steroid hormones ,TGF-β plays a key role in the pathophysiology of uterine fibroids. Research conducted by Uluer *et al.* [20] on the tissues of various histological types of myomas and sarcomas on the influence of angiogenic factors, including VEGF, confirm its stimulating effect on the development of uterine fibroids. However, it was not found that angiogenesis is associated with its main hypoxia inducible stimulator: hypoxia inducible factor α (HIF-1α). Rather, the contribution of other molecular factors, including cyclooxygenase enzyme isoform (COX-2), *Ras* oncogene, and the nuclear transcription factor NF-kB are under consideration. This does not, according to the authors, exclude the therapeutic significance of anti-angiogenic factors. According to a study involving the fibroids of 80 women (of reproductive and perimenopausal age), the expression of the VEGFA isoform and its receptor was confirmed [20]. According to the above authors, this overexpression may be related to malignant transformation in the myoma.

Recently published research results indicate that angiogenesis in myomas is related to the expression of endothelin ET-1, a mediator involved in angiogenesis, cell proliferation, and migration. Inhibition of ET-1 and its ET-A receptor leads to a reduction in cell proliferation in myomas. Such inhibition may be a future therapeutic option for fibroids [21].

Vitamin D

Vitamin D is a steroid compound involved in the maintenance of homeostasis of many tissues. It regulates cell proliferation and differentiation, inhibits angiogenesis, and stimulates apoptosis. In addition to activating tyrosine kinases with the activation of different signaling pathways, vitamin D acts via the nuclear receptor (VDR) regulating the expression of many genes. It is believed that vitamin D deficiency may be an important risk factor in the development of uterine fibroids. Vitamin D and its analogues seem to be an effective and affordable treatment for uterine myomas. This vitamin together with other drugs may be a modern treatment option [22]. The *in vitro* research by Ali *et al.* [23] supports this thesis. The combination of UPA with vitamin D3 significantly reduced cell proliferation compared to treatment with UPA alone. The authors noted a decrease in the expression of Ki-67 and PCNA prolifera-

tion markers and Cyclin D1 in more than 50% of cells, as well as an increase in cell apoptosis. Concentrations of fibronectin, TGF-β and proinflammatory cytokines, interleukin 6,8,1α and 1β, decreased. This combined action of UPA and vitamin D3 seems to be a promising myoma treatment option.

Stem cells

Somatic stem cells are a small subpopulation of cells in the body with unique features: asymmetric division, self-renewal, and generation of daughter cells capable of differentiating into different tissues. They are necessary for the regeneration and repair processes of tissues, which assist the preservation of organ function [6, 24].

In malignant tumors, the presence of cancer stem cells (CSCs) which are associated with poor prognosis, has been described [25]. Stem cells were also isolated from the fibroid tissue by flow cytometry: three populations of stem cells: CD34 + / CD49b +, CD34 + / CD49b-, and CD34- / CD49b- [5]. According to Carneiro [26], stem myoma cells may play a role in the formation and development of myomas. Similarly, other authors believe that progesterone-dependent myoma is possible due to stem cell activity. Myoma SCs are deficient in ERα, PR and depend on steroid receptors present in mature myoma or myometrial muscle cells, as well as the signaling of the paracrine Wnt / β-catenin pathway [7]. The paracrine signaling of fibroid growth is also mediated by insulin-like growth factor IGF2, a polypeptide hormone with a structure homologous to insulin and insulin receptor A (IR-A) [5]. Theories related to the presence of SCs in fibroids and the inhibition of signaling pathways that they use [3.6] are under consideration.

Shalaby *et al.* [27] developed a method of local treatment for uterine fibroids using magnetic nanoparticles combined with adenovirus. Decreased proliferation and induction of apoptosis in myoma cells was observed. It can be assumed that methods of conservative treatment of myomas based on molecular diagnostics as well as pharmacological and genetic treatment will be developed.

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