GEORGIAN BIOMEDICAL NEWS

ISSN (Online): 2720-8796 ISSN (Print): 2720-7994 VOLUME 1. ISSUE 1. JANUARY-MARCH 2023

Peculiarities of Angiogenesis of the Uterine Body Leiomyomas in Women of Reproductive Age

Mindia Iobashvili[1], Marina Pailodze[2], Irma Jikia[3], Anzor Gogiberidze[2], Nikoloz Vachadze[2], Magda Tortladze[4], Evgeni Asatiani[2]

DOI: 10.52340/GBMN.2023.01.01.07

ABSTRACT

BACKGROUND

Despite recent achievements regarding the pathogenesis of leiomyoma, the exact mechanisms and role of angiogenesis are not well understood. According to the existing evidence, the excess of steroids, their influence on the apoptosis mechanisms, and the expression of local growth factors may stimulate the formation of leiomyomas in women of reproductive age.

OBJECTIVES

The present study aimed to ascertain the pathophysiological mechanisms underlying angiogenesis disorders and shed light on the function of steroids in this process.

METHODS

The central and peripheral parts of the 42 postsurgical uterine leiomyoma nodes were examined with the aim of assessment of angiogenetic changes in the proliferating, recurrent, and latent leiomyomas, using (i) hematoxylin and eosin (H and E), and Masson's trichrome stain of formalin-fixed paraffin-embedded sections, and (ii) immunohistochemistry evaluation of estrogen receptor (ER), progesterone receptor (PR), and CD34 (transmembrane phosphoglycoprotein protein encoded by the CD34) markers.

RESULTS

The small proliferative leiomyomas (1-2 cm) are characterized by prominent angiogenesis with a dominance of small and medium-caliber aberrant vessels. The tendency to decrease intranodal blood vessels with the growth of tumor nodes (3-4 cm in size) indicates to depletion of resources of the autonomous vascular collector (tumor bed), after which the leiomyomata may enter the recurrence phase with the formation of a new locus of angiogenesis. The uniform distribution of the blood vessels throughout the proliferative leiomyoma node indicates that tumor growth occurs throughout the whole vascular collector. The depletion of the capacity of angiogenesis has an inhibitory effect on the growth of latent nodes and increases the risk of the secondary lesion because of blood supply alterations.

CONCLUSIONS

Angiogenesis plays an important role in the progression of leiomyoma. The tumor growth occurs throughout the whole vascular bed (in the central and peripheral parts of the leiomyomata) and not from a single smooth muscle cell.

KEYWORDS

CD34 marker; estrogen receptor (ER); latent leiomyoma; progesterone receptor (PR); proliferating leiomyoma; recurrent leiomyoma; uterine body leiomyoma.

BACKGROUND

According to the existing evidence, angiogenesis is an essential element in the regulation of tumor growth.[1] It is well known that leiomyomas are characterized by markedly remodeled, diminished number of vessels compared to the surrounding healthy myometrium,[2] which causes severe hypoxia of the tumorous tissue.[3] There is evidence of a decrease in the function of angiogenic growth factors in the case of leiomyoma as well.

There are various viewpoints regarding the growth of leiomyomas in the uterine body.[4-8] The myometrium experiences active myocyte proliferation and myometrial hyperplasia (MMH). The majority of leiomyomas develop in hypoxic sites of MMH, which is recognized as a precursor of leiomyoma.[9,10] The small leiomyomas are less vascular compared to myometrium,[11,12] and the smallest (1-3 mm) ones are almost avascular with nodular growth due to diffusion from the surrounding myometrial vascular network.[13] According to existing considerations the excess of steroids, alterations of the apoptosis mechanisms, and expression of local growth factors may increase the frequency of leiomyomas in women of reproductive age.[14-17]

Despite recent achievements in the knowledge of the pathology exact mechanisms of leiomyoma is still obscure, the precise role of leiomyoma precursor cells is not well understood, and the radical hysterectomy is the only successful treatment option because of the ineffectiveness of conservative measures.

In the present study, we aimed to ascertain the pathophysiological mechanisms underlying angiogenesis disorders and shed light on the function of steroids in this process.

METHODS

We have studied the postsurgical uterine leiomyoma biomaterials of 42 patients with an average age of 31±10 years. The central and peripheral parts of the tumor nodes (including modified small, medium, and large caliber blood vessels) were examined with the aim of assessment of angiogenetic changes in the proliferating, recurrent, and latent leiomyomas by the following methods:

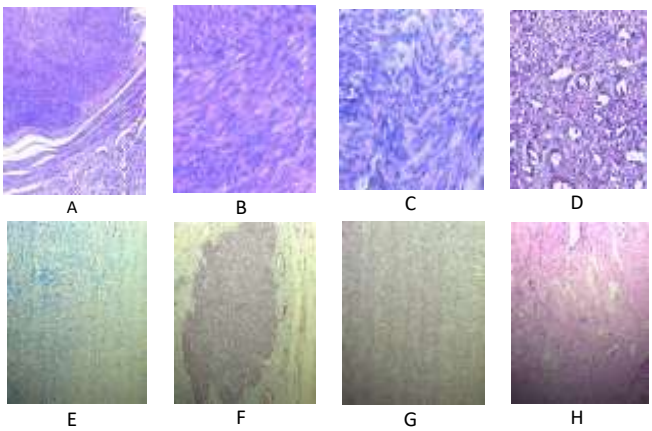
- Hematoxylin and eosin (H and E), and Masson's trichrome stain of formalin-fixed paraffin-embedded sections for the evaluation of histological architecture of biomaterials;
- Immunohistochemistry evaluation of angiogenesis and estrogen receptor (ER), progesterone receptor (PR), and CD34 (transmembrane phosphoglycoprotein protein encoded by the CD34) markers.

SPSS version 22 software was used for statistical analysis. The p-values were provided by Mann-Whitney tests. A value of $p<0.05$ was considered to be statistically significant.

RESULTS

The active proliferation and hypercellularity (Fig1A, Fig1B, and Fig1C) with the intense angiogenesis (Fig1D) were detected in the small nodes of leiomyoma. The initial phase of nodular growth was characterized by the tendency of fibrosis and collagenization (Fig1E). The new proliferation (Fig1F), formation of the abundant anastomoses, and growing capillary net (Fig1G) were typical for the period of recurrence. Finally, the diffuse collagenosis and areas of the secondary lesions were denoted in the latent period (Fig1H).

FIGURE 1. Hematoxylin and eosin, and Masson's trichrome stained formalin-fixed paraffing-embadded sections of uterine body leiomyoma nodes



A, B, C, and D: 0.3-2 cm size nodes with proliferation, hypercellularity, and intense angiogenesis; E: up to 3 cm size growing node with fibrosis and collagenization; F and G: up to 6 cm size node with new proliferation, anastomoses, and capillary net; H: 7-15 cm size nodes with the diffuse collagenosis and areas of secondary lesions. *Leika 1000 Led. MC170HD, x 0.65*

On the periphery and in the central part of the proliferative nodules in size of 1-2 cm, a large number of blood vessels were detected. With the growth of nodes (up to 3-4 cm), the percentage of avascular areas increased dramatically.

A large number of vessels were detected in the central part and periphery of the recurrent tumorous nodes (in size of 4-8 cm) and surrounding muscles as well. However, in the case of latent leiomyomas, there was an evident decrease in the number of vessels compared to proliferative and recurrent nodules.

The quantitative assessment of unusual/aberrant blood vessels in the central and peripheral parts of leiomyomata showed the following:

- Abundant vascularization of nodes and surrounding muscle tissues, with the presence of aberrant blood vessels, the number of which progressively decreased with the growth of nodes, to absolute disappearance in latent forms of leiomyoma;
- The equal number of aberrant vessels in the periphery and the central parts of the proliferative and recurrent nodes. It should be noted that in case of recurrent leiomyomas, the density of aberrant vascularization was higher in the center of the node than in the periphery;
- Low number of aberrant blood vessels in the latent leiomyoma nodes.

The quantitative assessment of small, medium, and large blood vessels in the central and peripheral parts of leiomyomata showed the following:

- The high number of small blood vessels in small proliferation nodes (up to 1 cm);
- The high number of medium-caliber blood vessels in proliferation nodes up to 2 cm in size;
- The leaping decrease in the number of small blood vessels with the growth of the node;
- The high number of large blood vessels in the surrounding muscular tissue compared to small size leiomyoma nodes;
- The high number of large blood vessels in both the periphery of the recurrent nodes and surrounding muscle tissue;
- The low number of large blood vessels in the latent nodes, compared to other types of nodes and surrounding muscle tissue.

An immunohistochemical study revealed a high content of SD34 marker in a small node (up to 1 cm) of proliferative leiomyoma (Fig.2A), with a tendency to decrease with node growth up to 2 cm (Fig.2B) and a dramatic reduction in nodes up to 3 cm in size (Fig.2C). As for recurrent nodes, the concentration of the SD34 marker is high (Fig.2D), and in latent nodes (Fig.2E) decreased, on the contrary.

The expression of the estrogen receptors (ERs) was insignificant in the small (1-2 cm in size) proliferative nodes (Fig.3A and Fig3B), with a tendency to increase in the nodes up to 3 cm in size (Fig.3C), and the significant increase in the

The Georgian Biomedical News
ISSN (Online): 2720-8796 ISSN (Print): 2720-7994

recurrent nodes (Fig.3D). No estrogen receptors (ERs) were found in the latent nodes.

FIGURE 2. Expression of transmembrane phosphoglycoprotein protein encoded by the CD34 in the different types of leiomyoma nodes

A B C D E

A and B: The high content of SD34 marker in a small node (up to 1 cm) of proliferative leiomyoma; B: The decreased expression of SD34 in the node up to 2 cm in size; C: The dramatic reduction of SD34 in the node up to 3 cm in size; D: The high concentration of SD34 in the recurrent nodes; E: The decreased concentration of SD34 in the latent nodes. *Leika 1000 Led. MC170HD, x 0.65*

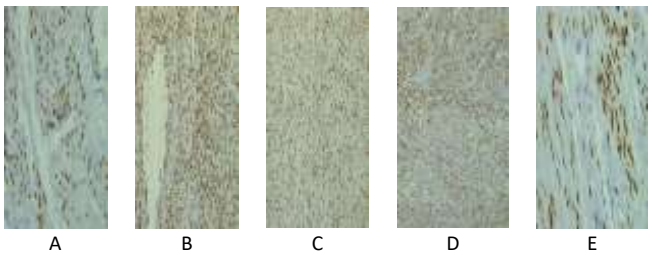
FIGURE 3. Expression of estrogen receptors (ER) in the different types of leiomyoma nodes

A B C D

A: Insignificant expression of ERs in the small (1-2 cm in size) proliferative nodes; C: Increased expression of ERs in the nodes up to 3 cm in size; D: Significant expression of ERs in the recurrent nodes. *Leika 1000 Led. MC170HD, x 0.65*

The expression of the progesterone receptors (PRs) was high in all types of leiomyomata with a tendency to increase with node growth (Fig.4).

FIGURE 4. Expression of progesterone receptors (PRs) in the different types of leiomyoma nodes



A B C D E

A: The expression of PRs in the proliferative leiomyoma node up to 1 cm in size; B: The expression of PRs in the proliferative leiomyoma node up to 2 cm in size; C: The expression of PRs in the proliferative leiomyoma node up to 3 cm in size; D: The expression of PRs in the recurrent leiomyoma node; E: The expression of PRs in the recurrent leiomyoma node. *Leika 1000 Led. MC170HD, x 0.65*

DISCUSSION

The proliferative leiomyomas up to 1 cm in size were characterized by a well-developed (45%), evenly distributed network of blood vessels, 24.3% of which was aberrant and 16.2% of small caliber vessels.

47.4% of proliferative nodes up to 2 cm in size were supplied with medium caliber blood vessels, 23.7% of which were localized in the center of the node and 21.1% - on the periphery. The share of aberrant vessels was 13.2%.

The index of vascularization of the proliferative leiomyomas 3-4 cm in size was only 15%. The tumorous tissue was mainly supplied with small and large vessels. The proportion of medium-caliber vessels was 13.2% and the aberrant vessels 2.6%.

An immunohistochemical study showed a tendency to decrease the SD34 expression and increase the number of estrogen receptors (ERs) with the growth of proliferative leiomyomas. The expression of the progesterone receptors (PRs) was high in all types of leiomyomata with a tendency to increase with node growth.

Proliferative leiomyomas in size of 1-2 cm are characterized by prominent angiogenesis with a dominance of small and medium-caliber aberrant vessels, which indicates a crucial role of angiogenesis in the progression of leiomyoma.

The tendency to decrease intranodal blood vessels with the growth of tumor nodes (3-4 cm in size) indicates to depletion of resources of the autonomous vascular collector (tumor bed), after which the leiomyomata may enter the recurrence phase with the formation of a new locus of angiogenesis.

The uniform distribution of the blood vessels throughout the proliferative leiomyoma node indicates that tumor growth occurs throughout the whole vascular collector. Our finding contradicts the theory of leiomyoma development "from a single smooth muscle cell."[1,7,8,11,14]

The depletion of the capacity of angiogenesis has an inhibitory effect on the growth of latent nodes and increases the risk of the secondary lesion because of blood supply alterations.

CONCLUSIONS

Angiogenesis plays an important role in the progression of leiomyoma.

The tumor growth occurs throughout the whole vascular bed (in the central and peripheral parts of the leiomyomata) and not from a single smooth muscle cell.

AUTHOR AFFILIATION

[1] David Agmashenebeli University of Georgia;
[2] Tbilisi State Medical University (TSMU);
[3] Tbilisi State University (TSU);
[4] Caucasus International University (CIU).

The Georgian Biomedical News
ISSN (Online): 2720-8796 ISSN (Print): 2720-7994

REFERENCES

1. Luo F, Liu X, Yan N, Li S, Cao G, Cheng Q, et al. Hypoxia-inducible transcription factor-1alpha promotes hypoxia-induced A549 apoptosis via a mechanism that involves the glycolysis pathway. BMC Cancer 2006;6:26.
2. Wullschleger S, Loewith R, Hall MN. TOR signaling in growth and metabolism. Cellб 2006; 124:471 – 484.
3. Ikeda K, Arao Y, Otsuka H, Kikuchi A, Kayama F. Estrogen and phytoestrogen regulate the mRNA expression of adrenomedullin and adrenomedullin receptor components in the rat uterus. Mol Cell Endocrinol 2004; 223:27-34.
4. Uluer ET, Inan S, Ozbilgin K, Karaca F, Dicle N, Sancı M. The role of hypoxia related angiogenesis in uterine smooth muscle tumors. Biotech Histochem 2015;90:102-10.
5. Mayer A, Hockel M, Wree A, Leo C, Horn LC, Vaupe lP. Lack of hypoxic response in uterine leiomyomas despite severe tissue hypoxia. CancerRes2008;68:4719–4726.
6. Sadick, Jakob Richers, Benjamin Tuschy, Lothar R. Schad, Stefan O. Schoenberg, Frank G. Zöllner, Feasibility of quantitative MR-perfusion imaging to monitor treatment response after uterine artery embolization (UAE) in symptomatic uterus fibroids, Magnetic Resonance Imaging, 10.1016/j.mri.2019.02. 008, (2019).
7. Hassan MH, Eduardo Eyzaguirre E, Arafa HMM, Hamada FMA, Salama SA, AlHendy A. Memy I: a novel murine model for uterine leiomyoma using adenovirusenhanced human fibroid explants in severe combined immune deficiency mice. Am J Obstet Gynecol 2008;199: 156.e1–6.e8.
8. Drosch M, Bullerdiek J, Zollner TM, Prinz F, Koch M, Schmidt N. A novel mouse model that closely mimics human uterine leiomyomas. Fertil Steril 2013;99:92735.e6.
9. Folkman J. Angiogenesis. Annu Rev Med 2006;57:1 – 18.
10. Zhang L, Xiong W, Li N, Liu H, He H, Du Y. Estrogen stabilizes hypoxia-inducible factor 1α through G protein-coupled estrogen receptor 1 in eutopic endometrium of endometriosis. Fertil Steril 2017;107:439-47.
11. Silverberg SG, Tabbara SO. The uterine corpus. In: Silverberg SG, Delellis RA, Frable WJ, editors. Principles and Practice of Surgical Pathology and Cytopathology. 3rd ed. Vol. 3. New York: Churchill Livingstone; 1997. pp. 2459–516.
12. Kusumbe AP, Ramasamy SK, Itkin T, Mäe MA, Langen UH, et al. Agedependent modulation of vascular niches for haematopoietic stem cells. Nature 2016;532:380-4.
13. Watanabe H, Takahashi E, Kobayashi M, Goto M, Krust A, Chambon P, et al. The estrogen-responsive adrenomedullin and receptor-modifying protein 3 gene identified by DNA microarray analysis are directly regulated by estrogen receptor. J Mol Endocrinol 2006;36:81-9.
14. Catherino WH, Malik M. Uterine leiomyomas express a molecular pattern that lowers retinoic acid exposure. Fertil Steril 2007;87:1388-98.
15. Ishikawa H, Xu L, Sone K, Kobayashi T, Wang G, Shozu M. Hypoxia Induces Hypoxia-Inducible Factor 1α and Potential HIF-Responsive Gene Expression in Uterine Leiomyoma. Reprod Sci 2019;26:428-35.
16. Suo G, Sadarangani A, Lamarca B, Cowan B, Wang JY. Murine xenograft model for human uterine fibroids: an in vivo imaging approach. Reprod Sci 2009;16:827
17. Fritsch M, Schmidt N, Gröticke I, Frisk AL, Keator CS, Koch M, et al. Application a patient derived xenograft model for predictive study of uterine fibroid disease. PLoS One 2015;10:e0142429.

ISSN (Online): 2720-8796 ISSN (Print): 2720-7994