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Experimental Medicine

Detection of Molecular Mechanisms of Angiogenesis during Growth-Development in the Period before the Uterine Leiomyoma Forms in Women in the Reproductive Phase

Mindia Iobashvili[*], Marina Pailodze[*,], Irma Jikia[§], Maia Kharabadze[**], Magda Tortladze[§§], Anzor Gogiberidze[**], Natia Kvesitadze[**]**

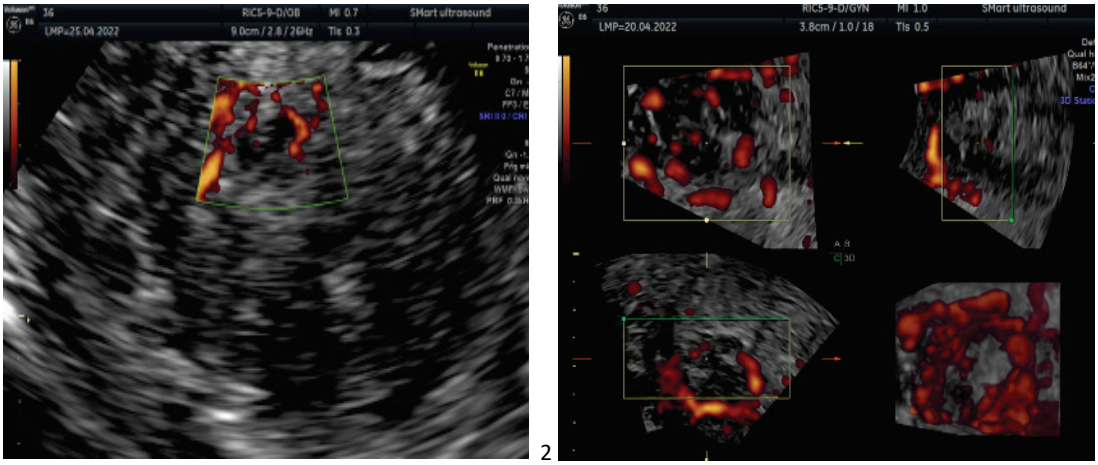
[*]*Department of Gynecology, National Center of Surgery, Tbilisi, Georgia*
[**]*Department of Normal Human Anatomy, TSMU, Tbilisi, Georgia*
[§]*Department of Pathological Anatomy and Forensic Medicine, Ivane Javakhishvili Tbilisi State University, Tbilisi, Georgia*
[§§]*Faculty of Medicine, Caucasus International University, Tbilisi, Georgia*

(Presented by Academy Member Ramaz Khetsuriani)

Angiogenesis and vascularization have been identified as critical elements regulating tumor growth in recent years. Leiomyomas are believed to contain pathological blood vessels, to be less vascularized than the myometrium around them and to be highly susceptible to severe hypoxia. During the reproductive period, prior to the formation of uterine leiomyoma, as a result of the morphological research conducted to reveal the molecular mechanisms of angiogenesis, it was established that uterine leiomyoma is the result of the programmed action of steroids and growth factors under conditions of perverted apoptosis. The opinion about the growth of leiomyocytes from any point of the vascular collector is supported by the presence of progenitor and stem cells in the muscle and adventitia of blood vessels within the vascular collector and the angiogenesis detected with equal frequency in the peripheral and central part of the nodes. Immunohistochemical research determine that, in the 3mm, 4mm and 6mm nodules of intranatal leiomyoma, the full expression of CD34 is revealed in the muscle within the collector. The molecular basis of angiogenesis is the high positivity of progesterone under conditions of minimal focal expression of caspases, which leads to the proliferative activity of leiomyocytes with high SMA concentration and CD34 reduction. *© 2023 Bull. Georg. Natl. Acad. Sci.*

medicine, reproductive medicine, uterine leiomyoma, pathological anatomy, histology, immunohistochemistry

The most frequent tumor in women throughout their reproductive period is a leiomyoma of the uterus [1-4]. The majority of authors indicate that they are single-cell smooth muscle tumors (monoclonal tumors) [5]. In addition, steroids, estrogen and progesterone, are the main regulators of uterine angiogenesis [4,6]. Angiogenesis and vascularization have been identified as critical elements regulating tumor growth in recent years [7]. has been identified [14]. Only in hypoxic environments do stem cells differentiate into mature myometrial cells, and hypoxia has been shown to promote tumor growth and leiomyoma cell transformation [15]. Although leiomyoma is extremely hypoxic, studies by Mayer and other scientists has revealed that its nodules do not contain hypoxia-related genes, which suggests a lack of response to tissue hypoxia [8,16].



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Figs. 1,2. Ultrasonography 3D reconstruction of the 8 mm and 10 mm nodes in energy Doppler mode. Intranodal angiogenesis.

Leiomyomas are believed to contain pathological blood vessels, to be less vascularized than the myometrium around them [5], and to be highly susceptible to severe hypoxia [8]. The function of angiogenic growth factors is allegedly hindered in leiomyoma tissue. Since they control the growth of the vascular network, the balance between angiogenic promoters and inhibitors is crucial in the formation of tumors [9]. Hypoxia is one of the most potent triggers for angiogenesis in tumors, and avascular leiomyomas are naturally highly hypoxic [8]. Small leiomyomas exhibit less vascularity than myometrium, according evidence from the literature [10,11]. The smallest myoma (1-3 mm) is practically avascular and is surrounded by a capillary network that represents the myometrial vascular network [12,13]. The nodule grows through diffusion. A population of myometrial cells with stem cell-like phenotypic and functional traits

The authors postulate that the myometrium experiences active myocyte proliferation and myometrial hyperplasia (MMH), and that the majority of leiomyomas develop in regions of this MMH. Myometrial hyperplasia (MMH) is thought to be a precursor phase in the growth of leiomyoma [17], whereas hypoxia is the primary feature of MMH [18]. The opinion of scientists about the role of endothelial progenitor cells in the process of vascularization is also significant [19]. Leiomyoma is a significant problem despite the multiple research carried out so far [20]: it goes through rejuvenation; The mechanisms of leiomyoma development remain unknown, as well as the precursor processes; relapses are a characteristic of leiomyoma. It is impossible to finally halt leiomyoma growth and cause it to regress since conservative measures are ineffective, radical hysterectomy remains an effective method of treatment.

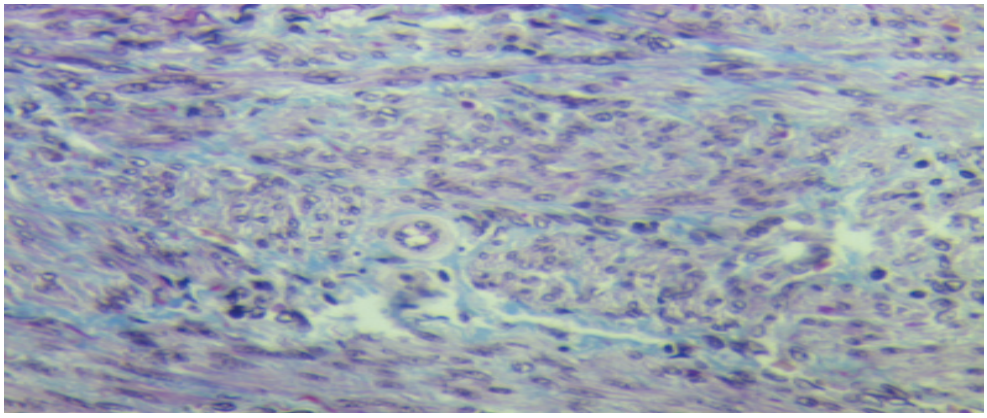

Fig. 3. Masson's trichrome. 3 mm. node. Leika 1000 Led. photo MC170HD, x 0.65.

Exposing the molecular mechanisms of angiogenesis throughout the period before the development of leiomyoma as well as during the growth and development process in females during the reproductive era.

On the basis of the mentioned, the specific tasks were the detection of sequential changes in angiogenesis:

1. In the preceding period of leiomyoma formation
2. Proliferate 3 mm; 4 mm; 6 mm in nodes and bordering muscle.

Materials and Methods

In ultrasonographically proliferative leiomyomas, in the surrounding myometrium around the nodules and inside the nodules, in addition to the growth in their size: vascularization of the muscles around the nodules, which are small-growing and up to 8 mm and 10 mm in size (Figs. 1,2); And whereas the interior vascularization of the nodules has high (RI-0.73) vascularization up to 10 mm in size, it is noticeably low (RI-0.48) at 8 mm. It was crucial to evaluate the issue of angiogenesis and cell development prior to leiomyoma formation. The characteristics of angiogenesis were studied in small nodes (up to 3 mm, 4 mm, and 6 mm). It is widely recognized that unappealing blood vessels changed into the uterine muscle occur prior to the formation of leiomyoma. Leiomyocytes proliferate in the aforementioned region, forming a voluminous structure and fibrosis and collagenization start in the tumor „bulk" that is up to 3 mm in size. As a result, an extracellular matrix (ECM) is created with a volume that steadily increases, giving the tumor its shape and drastically separating it from the surrounding muscle tissue. However, it seems that the majority of the fibroblast growth factors (FGF) are stored in the extracellular matrix components as a reserve (53).

The ECM is not only a tissue that provides density, separates the nodule from the surrounding muscle tissue and contains fibroblast growth factors, but it is also likely one of the factors that inhibits the free, uncontrolled spread of tumors in the muscle of the uterine body and lowers the risks of malignancy. It is not usually supported by evidence that the smallest leiomyoma (1-3 mm) is almost avascular is surrounded by a vascular network of myometrium represented by capillaries and grows via diffusion (Fig. 3).

On sagittal dissection, was found a 2 mm-long white nodule with extracellular matrix (ECM) which, on sagittal dissection, protruded freely from its smooth crystal-surfaced „nest".

This finding shows that other tumors can also be larger than the surrounding muscle than leiomyomas with their capsule. The smallest leiomyomas (1-3 mm) are nearly avascular, surrounded by a capillary-based vascular network of the myometrium and exhibit nodal growth by diffusion, claim the researchers. A little growing leiomyoma is

encapsulated and isolated from a muscle that is not differentiated by acute angiogenesis, contrary facts was demonstrated in material. The previously mentioned fact suggests that multifactorial mechanisms are present during the formation and development of the leiomyoma as well as the significance of the modified blood vessels inside the vascular collector in the process of growth of the leiomyocytes in the node.

It is debatable whether or not altered blood vessels can cause leiomyoma. Based on the characterristics of the leiomyocytes, the majority of researchers think that a single cell multiplied into a tumor.

Given the abundance of stem and progenitor cells in the muscularis and adventitia of blood vessels in the leiomyoma nodule and the fact that leiomyoma cells exhibit progenitor like characteristics, it is likely that the proliferation of leiomyoma cells starts in the reshaped vascular collector area from all of its points.

The activation of endothelium in the wounded zone's intima, which, like leiomyocytes, also derives from stem and progenitor cells, lends further credence to this opinion. Based on the founded indicators, leiomyoma can grow anywhere along the modified vascular collector.

CD34, ER, PR, and SMA immunohistochemical characteristics of expression.

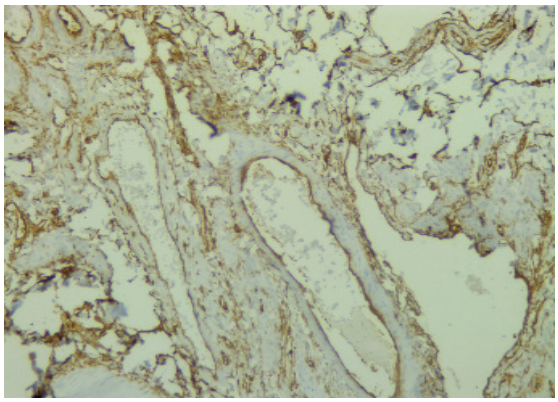

Fig. 4. Node 3 mm. Vascular collector. Without muscular myoma, expression of CD34 up to 80%. Leika 1000 Led. photo MC170HD, x 0.65.

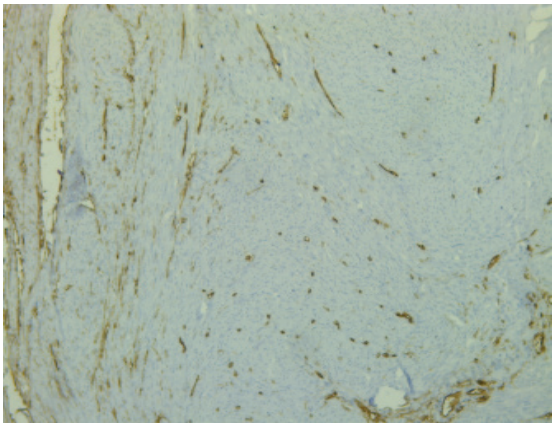

Fig. 5. Node 3 mm, expression of CD34 up to 20%. Leika 1000 Led. photo MC170HD, x 0.65.

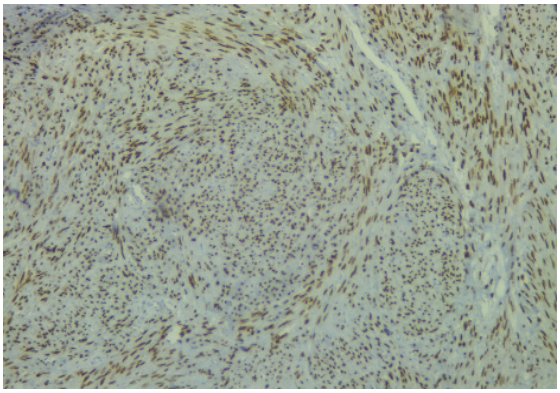

Fig. 6. Node 3 mm. PR expression. Bordering muscle Leika 1000 Led. photo MC170HD, x 0.65.

Comparing the muscular tissue surrounding the nodes of 3 mm, 4 mm and 6 mm to the muscular tissue of the uterus without leiomyoma (Fig.4), a significant number (up to 100%) of transformed, unsightly blood vessels with high expression of CD34 were found. Additionally, CD34 is expressed up to 20% in the nodule (Fig. 5.). ER- hardly detectable in the adjacent muscle, but completely absent from the node. Progesterone is substantially more prevalent in the node, than in the surrounding muscle (Fig. 6) with the 6 mm node showing the greatest positivity at up to 90%. Caspase is expressed locally in leiomyoma nodules. SMA- In nodes, positivity is prominently represented (Fig. 7.).

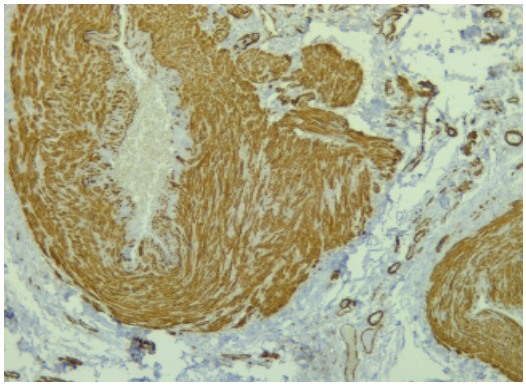

Fig. 7. Node 6 mm. SMA expression. Leika 1000 Led. photo MC170HD, x 0.65.

For research there was used**:** Ultrasonography of small nodules of uterine body leiomyoma with color Doppler ultrasonography mapping, detection of Sonoanatomic peculiarities, assessment of peripheral and internal blood circulation in nodules and determination of resistance index; Slides are stained with hematoxylin and eosin to detect morphological changes; Masson's trichrome lumen staining to assess the extracellular matrix (fibrosis and collagenization); Assessment of immunohistochemistry alterations utilizing the markers CD34, ER, PR, SMA and caspase.

Discussion

It is well recognized that angiogenesis and vascularization constitute significant elements that regulate the development of tumors [7]. The development of leiomyoma from remodeled blood vessels is controversial. Based on the characteristics of leiomyocytes, the majority of researchers think, that the proliferation of a single cell is what generates the tumor. Considering this, it was critical to evaluate the issue of the production of leiomyoma progenitor cells and to pinpoint the dynamical and molecular mechanisms behind the growth of leiomyomas.

According to our theory, the deformed blood vessels in the uterine body's muscularis that exist before to the commencement of leiomyoma are created as a vascular collector, an autonomous unit that is distinct from the muscularis. It is assume that the proliferation of leiomyocytes commences in the area of the vascular collector from all its points due to the muscle and adventitia of blood vessels in the leiomyoma nodule are rich in stem and progenitor cells and leiomyoma cells exhibit progenitor like characteristics [21]. In the collector, there were few localized caspases and high of SD34 and PR expression, according to immunohistochemical examination. Which suggests that in these circumstances, progesterone can stimulate the mitotic activity and proliferation of leiomyocytes in the secretory phase of the cycle by forming a leiomyoma nodule.

Conclusions

Uterine body leiomyoma results from the programmed action of steroids and growth factors under conditions of aberrant apoptosis, by forming a collector from remodeled, distorted (clearly separated from muscle) blood vessels in the pre-leiomyoma period, which is an ideal environment for the growth and development of leiomyoma and gives a reason to assume that the growth of leiomyocytes begins in the entire width of the vascular collector, from any of its points. This opinion is supported by the same frequency of angiogenesis in the peripheral and central regions of the nodes, the presence of stem and progenitor cells in the vascular collector, in the muscle and adventitia of blood arteries and the existence of stem and progenitor cells in these tissues. Sharp hypoxia, which distinguishes small nodules and performs a significant role in tumor development, as well as the observation that myometrial cells can only undergo the transformation into leiomyoma cells under hypoxic conditions, support this theory. On the other hand, some researchers contend that the absence of associated genes in the nodules causes the tissue to be less susceptible to hypoxia. The above-mentioned conflicting views on the role of hypoxia in the development of leiomyoma and the characteristics of angiogenesis that are discovered, lead to consider the variety of mechanisms underlying leiomyoma development. As a result, it

is difficult to identify and treat leiomyoma's developmental challenges. Immunohistochemical research determine that, in the 3mm, 4mm and 6mm nodules of intranatal leiomyoma, the full expression of CD34 is revealed in the muscle within the collector. The molecular basis of angiogenesis is the high positivity of progesterone under conditions of minimal focal expression of caspases, which leads to the proliferative activity of leiomyocytes with high SMA concentration and CD34 reduction.

ექსპერიმენტული მედიცინა

რეპროდუქციული ასაკის ქალებში საშვილოსნოს ლეიომიომის ჩამოყალიბების პერიოდსა და ზრდა-განვითარების პროცესში ანგიოგენეზის მოლეკულური მექანიზმების გამოვლენა

მ. იობაშვილი[*], მ. ფაილოძე[*,**], ი. ჯიქია[§], მ. ხარაბაძე[**], მ. თორთლაძე[§§], ა. გოგიბერიძე[**], ნ. კვესიტაძე[**]

[*]ქირურგიის ეროვნული ცენტრი, გინეკოლოგიის დეპარტამენტი, თბილისი, საქართველო
[**]თბილისის სახელმწიფო სამედიცინო უნივერსიტეტი, ადამიანის ნორმალური ანატომიის დეპარტამენტი, თბილისი, საქართველო
[§]ივანე ჯავახიშვილის სახ. თბილისის სახელმწიფო უივერსიტეტი, პათოლოგიისა და სასამართლო მედიცინის კათედრა, თბილისი, საქართველო
[§§]კავკასიის საერთაშორისო უნივერსიტეტი, მედიცინის ფაკულტეტი, თბილისი, საქართველო

(წარმოდგენილია აკადემიის წევრის რ. ხეცურიანის მიერ)

ანგიოგენეზი განიხილება, როგორც სიმსივნის ზრდის გადამწყვეტი ფაქტორი. ვარაუდობენ, რომ ლეიომიომებს აქვს პათოლოგიური სისხლძარღვები, გამოირჩევიან სისხლძარღვოვანი სიმწირით, ვიდრე მიმდებარე მიომეტრიუმი. რეპროდუქციულ პერიოდში, საშვილოსნოს ლეიომიომის ჩამოყალიბების წინამორბედ პერიოდში, ანგიოგენეზის მოლეკულური მექნიზმების გამოვლენის მიზნით ჩატარებული მორფოლოგიური კვლევის შედეგად დადგინდა, რომ საშვილოსნოს ლეიომიომა წარმოადგენს გაუკუღმართებული აპოპტოზის პირობებში სტეროიდებისა და ზრდის ფაქტორების დაპროგრამებული მოქმედების შედეგს. სისხლძარღვოვანი კოლექტორის ნებისმიერი წერტილიდან ლეიომიოციტების ზრდის შესახებ მოსაზრებას ამყარებს სისხლძარღვოვანი კოლექტორის ფარგლებში სისხლძარღვების კუნთოვანსა და ადვენტიციაში პროგენიტორული და ღეროვანი უჯრედების არსებობა და კვანძების პერიფერიულ და ცენტრალურ ნაწილში თანაბარი სიხშირით გამოვლენილი ანგიოგენეზი. იმუ-

ნოჰისტოქიმიური კვლევის საფუძველზე დადგინდა, რომ ლეიომიომის მცირე ზომის (3 მმ, 4 მმ, 6 მმ) კვანძების ირგვლივ ვლინდება CD34-ის სრული ექსპრესია კოლექტორის ფარგლებში არსებულ კუნთოვანში; ანგიოგენეზის მოლეკულურ საფუძველს წარმოადგენს კასპაზების მინიმალური ფოკალური ექსპრესიის პირობებში პროგესტერონის მაღალი პოზიტივიზმი, რაც იწვევს ლეიომიოციტების პროლიფერაციულ აქტივობას SMA- მაღალი კონცენტრაციითა და CD34-შემცირებით.

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