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The Life Cycle of the Uterine Fibroid Myocyte

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Abstract

Purpose of Review—Uterine fibroids are common benign tumors of women in the USA and worldwide, yet the biological nature and pathogenesis of these tumors remain largely unknown. This review presents our view of the stages in the life cycle of a subset of uterine fibroid myocytes, introduces hypothetical concepts and morphological data to explain these changes, and relates these changes in individual myocytes to the phases of fibroid tumor development.

Recent Findings—The observations gained from light and electron microscopic, immunohistochemical, and morphometric studies in our laboratory have led to the hypothesis that fibroid changes over time may relate to the excessive production of collagen by phenotypically transformed myocytes. This accumulation of collagen results in decreased microvessel density, followed by myocyte injury and atrophy, with eventual senescence and involution through ischemic cellular degeneration and inanition.

Summary—Uterine leiomyomas, or fibroids, are characterized by two histologic features—proliferation of myocytes and production of an extracellular collagenous matrix. In the larger tumors, the collagenous matrix is often abundant. Within those regions in which the accumulating collagen is excessive, the myocytes are progressively separated from their blood supply, resulting

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Compliance with Ethical Standards

Conflict of Interest Gordon P. Flake, Alicia B. Moore, Deloris Sutton, Norris Flagler, Natasha Clayton, Grace E. Kissling, Benita Wicker Hall, John Horton, David Walmer, Stanley J. Robboy, and Darlene Dixon declare no conflict of interest.

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in myocyte atrophy and eventually cell death. It is within these hypocellular, hyalinized areas that the complete lifecycle of the fibroid myocyte is realized. It begins with the phenotypic transformation of a contractile cell to one characterized by proliferation and collagen synthesis, progresses through an intermediate stage of atrophy related to interstitial ischemia, and eventuates in cell death due to inanition. Lastly, resorption of inanotic cells appears to occur by a non-phagocytic, presumably enzymatic process of degradation and recycling that we refer to as reclamation.

Keywords

Uterine fibroids; Myocyte; Uterine leiomyomas

Introduction

Although patients with uterine leiomyomas, or fibroids, have been followed with serial MRIs over a period of time to determine the progression or regression of the tumors [1], it is only possible to assess each fibroid histologically at one point in time. Thus, the changes that might be occurring over time in the two chief components—the myocytes and the extracellular collagen—are necessarily conjectural. However, based upon our examination of a large number of fibroids histologically, immunohistochemically, ultrastructurally, and morphometrically, we have developed hypotheses regarding the natural history of these tumors. Our observations over many years rest largely on two sources of material—the NIEHS Uterine Fibroid Study [2], and the collection of fibroids in the operating room for ultrastructural examination [3•].

The NIEHS Uterine Fibroid Study was initiated to gain insight into the pathogenesis of these tumors and to correlate their molecular characteristics with their histology. The light microscopic appearance of the tumors was cataloged on the basis of multiple proliferative, degenerative, and inflammatory features, and these observations were then entered into a large, searchable database. During the course of this review of 2151 microscopic sections from 460 fibroid tumors, we noted that the smaller fibroids were usually composed predominantly of myocytes with little collagen, while the larger tumors often contained abundant extracellular matrix. Mitotic activity also seemed to be more frequent in the smaller tumors, but the number of smaller tumors (< 2 cm) in the study was limited. These observations and impressions led us to hypothesize that the early development of fibroids might be predominantly proliferative, and that the collagenous matrix was variable from one tumor to another but sometimes was remarkably abundant with reduction in the myocyte cellularity. On this basis, we arbitrarily divided fibroid growth into four phases, with phase 1 tumors having the least collagen and phase 4 tumors the most [3•] (Fig. 1 and Table 1). Our hypothesis was that the collagenous matrix would accumulate as the tumor enlarged. A visual estimate of the percentage collagen based on the hematoxylin and eosin (H&E) slides correlated very well with the subsequently determined image analysis of Masson trichrome stained slides on a subset of tumors [4•].

As the light microscopic analysis in these studies proceeded, we extended our observations with other ancillary techniques. These included image analysis of Masson's trichrome

stained fibroids to more precisely quantify collagen content, immunohistochemistry for proliferating cell nuclear antigen (PCNA) for analysis of myocyte proliferative rates, and electron microscopic studies for the elucidation of fine details. With regard to the ultrastructural studies, we focused our attention primarily upon the fibrotic, hypocellular areas of the tumors, with the objective of gaining insight into the fate of the fibroid myocyte.

Genesis of Fibroids

The events that cause a leiomyoma to develop remain an enigma despite the fact that uterine fibroids are the most common gynecologic tumors, occurring with an estimated cumulative incidence of > 80% of African-American women and nearly 70% of Caucasians in one study [5], and that many risk factors for their occurrence are known [6]. Some believe the tumors are a response to injury [7]. And we and others have speculated that ischemic changes related to the hypercontractility of dysmenorrhea, which is estimated to occur in up to 70% of women by the fifth year after menarche [8], might initiate an injury response [3•, 7]. Since it has been shown that the smooth muscle cells of blood vessels respond to injury of the vascular intima by proliferating and synthesizing extracellular matrix [9, 10], we have suggested that a similar reaction might occur in the uterus in response to ischemic injury during dysmenorrhea. When it is considered that in the response to arterial injury, vascular smooth muscle cells convert from contractile cells to proliferating, collagen-synthesizing cells, this experimental phenomenon seems to be at least morphologically analogous to the proliferation and collagen production that occurs in uterine fibroid myocytes.

Phenotypic Transformation: Proliferation and Synthesis (Phases 1 and 2)

Several differences are apparent when comparing the histologic architecture of fibroids to the myometrium, and the cytology of fibroid myocytes to myometrial myocytes. First, the fascicular pattern of the myocytes in the myometrium, and their parallel, linear orientation, stands in contrast to the haphazardly fascicular pattern and disorganized arrangement of the myocytes in fibroids (Fig. 2a, b). The loss of this well-defined fascicular pattern and parallel orientation of muscle fibers represents a significant deviation from the norm since the muscle fascicle is important to the normal coordinated contraction of smooth muscle. We have also noted that the lateral bars between myocytes in the myometrium, which we suspect are gap junctions allowing for the influx of ions from cell to cell during contraction, are no longer seen between the myocytes in fibroids [3•] (Fig. 2c, d). These observations are consistent with a loss of myocyte contractility during the phenotypic shift.

Previous work has indicated an increase in the proliferation index of fibroids as compared to the myometrium [11]. This finding was supported in the NIEHS Uterine Fibroid Study in which the mitotic index of fibroids exceeded that in the myometrium in a larger number of samples [3•]. In addition, the number of mitoses correlated with the fibroid size, with increased mean mitotic counts in the smaller tumors and fewer mitoses in the larger tumors. The latter was supported on a subset of these tumors by the finding of a higher mean PCNA proliferation index in the smaller tumors of phases 1 and 2 compared to the lower index in larger tumors of phases 3 and 4.

Further, the myocytes in fibroids often appear to have less eosinophilic cytoplasm than those in the myometrium, and to have nuclei which are thinner and more pointed at the poles (Figs. 2c, d and 3e, f). Transmission electron microscopy corroborated these findings, revealing a reduction in thin myofilaments and more abundant endoplasmic reticulum in fibroid cells, with the latter suggestive of an increase in synthesizing capacity (Fig. 2e, f).

In summary, we have morphologic evidence in fibroids of the conversion of myocytes to cells with diminished contractile organelles, a higher proliferation rate, and increased synthesizing capability. This phenotypic transformation represents the initial stage in the lifecycle of the fibroid myocyte.

Once initiated, the transformation results in growth of the fibroid nodule because of the increased proliferation rate of the transformed myocytes, probably coupled with the deposition of extracellular matrix [3•, 4•]. The growth rate and eventual tumor size are variable. In patients with more than one fibroid, the majority will have tumors in more than one phase [3•], which may reflect origin at differing times, different growth rates, or differences in collagen production.

Interstitial Ischemia: Myocyte Atrophy and Injury (Late Phase 3)

Fibroids probably continue to enlarge, particularly in phases 2 and 3, as a result of both the increased proliferation of the myocytes as well as the synthesis and deposition of extracellular matrix. The two components, myocytes and matrix, are present in variable quantities from one tumor to another, and also within the same tumor. In those fibroids that we place into the phase 3 category, in which the collagen comprises 10–50% of the mass of the tumor, the collagenous component begins to accumulate and to exceed that of the normal myometrium [4•] and is clearly excessive in the latter part of phase 3. At this point the accumulated extracellular matrix causes many myocytes to separate from each other, and also appears to increase the distance between some myocytes and the nearest capillaries (Fig. 3a–c), apparently without sufficient compensatory angiogenesis [4•]. We believe that this eventually leads to a state of interstitial ischemia since nutrients and oxygen must now diffuse a longer distance from the capillaries to the myocytes, as well as passing through a more dense and compact collagenous stroma. Since the smooth muscle of the blood vessels in fibroids often exhibits changes similar to those occurring in the fibroid myocytes themselves, such as hyperplasia and fibrosis, vascular ischemia is probably also a contributing factor [3•].

The consequence of this interstitial and vascular ischemia is myocyte atrophy [3•]. When later phase fibroids are examined histologically, the most obvious microscopic feature is the abundant collagenous stroma separating and surrounding the myocytes (Fig. 3f). Less obvious, but apparent when compared to the myometrium, is the reduced size of some of the fibroid myocytes, which appear to have less cytoplasm and shorter nuclei than those of the myometrium (Fig. 3e, f) [3•]. Since the myocyte atrophy is most apparent in areas of fibrosis and hyalinization, we hypothesized that the dense extracellular matrix surrounding the myocytes might impede the diffusion of nutrients and oxygen from the capillaries. Further, we questioned whether the microvessel density (MVD) of fibroids might be reduced in these areas of hyalinization, thereby increasing the distance between some myocytes and the

nearest capillaries. With the use of Factor VIII-related antigen (von Willebrand factor) immunohistochemical stains on a subset of fibroids, we found a progressive fall in the MVD from an average of 92 in phase 1 to a low of 33 in phase 4 tumors [4•], a roughly threefold decline in MVD. Utilizing the MVD, and the collagen content determined by image analysis for each of the four phases, we estimated the mean distances of myocytes from vessels with Monte Carlo simulations. These simulations showed a progressive increase in the mean distance from a low of 34.9 μm in phase 1 to a high of 58.0 μm in phase 4, thus supporting the concept of interstitial ischemia and its probable contribution to the myocyte atrophy of late stage fibroids.

In addition to myocyte atrophy in late phase 3 and phase 4, some tumors also exhibit clusters or large foci of myocytes with cytoplasmic vacuolization [3•]. This may represent a form of degenerative change or injury. Ultrastructural examination reveals evidence of both autophagocytosis (Fig. 3d), which may be initiated by nutritional deprivation, and injury. Swollen endoplasmic reticulum and mitochondria, and fragmented mitochondrial cristae, may be seen, and lysosomes and autophagic vacuoles appear to be increased [3•]. The autophagic vacuoles contain membranous and granular debris, and sometimes lie adjacent to degenerating mitochondria or myofilaments, suggesting that these structures are targeted for engulfment. We favor the concept that autophagy, at least in this circumstance, is cytoprotective to cells deprived of nutrients and represents a mechanism by which starving cells can degrade myofilaments and other nonessential organelles for the utilization of amino acids and other molecules.

Thus, during late phase 3 and phase 4, we hypothesize that the excessive elaboration and accumulation of collagen in some regions of fibroids results in a state of interstitial ischemia, leading to myocyte atrophy and injury. This marks a fundamental change, or intermediate stage, in the lifecycle of this population of fibroid myocytes.

Inanosis: Myocyte Death (Phase 4)

All cells must lie within reasonable proximity of capillaries in order to receive adequate diffusion of nutrients and oxygen from capillaries. According to Guyton and Hall [12], cells ordinarily lie within 20–30 μm of capillaries. Although we are limited by the two-dimensional nature of histologic sections, the Monte Carlo simulation value of 58.0 μm for the average myocyte to vessel distance in phase 4 tumors [4•] was substantially greater than this literature figure of 20–30 μm for normal tissue. Since most hyalinized areas in fibroids are either hypocellular or acellular, and vascularity in these hyalinized areas is limited or absent, it seems reasonable to conclude that the myocyte to vessel distance necessary for survival has been exceeded in these hyalinized, hypocellular areas by the excessive deposition of collagenous matrix.

Although not readily apparent in microscopic sections, almost invariably a few small, pale shrunken myocytes are found in these hyalinized areas, if examined closely with the $\times 40$ objective (Fig. 4a) [3•]. The nuclei of these cells are very small, rounded to ovoid, pale, and never fragmented. Nucleoli are not seen. The cytoplasm often retains an ovoid to fusiform shape and is very pale to lightly eosinophilic. In some of these cells, the nuclei retain slight basophilia, while others are so pale as to be barely visible. The smallest of these nuclei are

but 1 to 2 μm in diameter, using an ocular micrometer. Those cells exhibiting the most extreme degree of nuclear and cytoplasmic atrophy, with nuclei so pale as to be barely visible, are assumed to be nonviable (Fig. 4a–c). Although it is always difficult to exclude tangential sectioning of larger cells, when the nuclear pallor is marked and the nuclear size is truly reduced to 2 μm or less, then it is likely that the cell is nonviable. This is based upon the fact that if all of the DNA of a human nucleus was condensed, it would occupy the volume of a cube measuring 1.9 μm on a side [13]. Converting this volume to that of a sphere to more nearly simulate the shape of a cell nucleus, the sphere would have a diameter of 2.356 μm , indicating that any nucleus of smaller size would have lost a portion of its requisite DNA.

We refer to these pale, shrunken cells as myocyte tombstones and to the process leading to their demise as inanosis. The term inanosis refers to the slow, gradual deprivation of nutrients and oxygen resulting in cellular inanition, with extreme atrophy and eventual death of the cell. Although apoptosis and necrosis both occur in fibroids, the inanotic smooth muscle cells do not have the morphologic features of apoptotic cells or apoptotic bodies. Necrotic cells may be karyolytic with pale nuclei, but the cells do not usually shrink and the necrotic process usually involves clusters of cells, is more rapid, and is usually associated with an inflammatory reaction [3•].

Inanotic cells are usually noted only in areas of hyalinization, in late phase 3 and in phase 4 tumors. Our use of the term involution in phase 4 tumors thus refers to the process of inanosis occurring in these focal areas of hyalinization, rather than diffusely throughout the tumor. In fact, a higher percentage of phase 4 tumors are ≤ 2 cm in size than in the other phases, although the PCNA proliferation index is lower than in the other phases [3•, 4•].

Reclamation: Myocyte Dissolution and Resorption (Phase 4)

Inanotic cells sometimes exhibit irregular, angulated nuclear shapes suggestive of a loss of membrane integrity [3•]. Occasionally, inanotic cells with hollow or empty nuclei are noted. More commonly seen is the presence of clear spaces in the stroma around inanotic cells (Fig. 4c, d) [3•]. Although these spaces could be artifacts of processing, we suspect that they are resorption pits. One reason for favoring this view is that the hyalinized areas of some phase 4 tumors exhibit occasional foci with numerous clear circular, empty spaces imparting a spongiform appearance (Fig. 4c, d) [3•]. We believe that these empty spaces represent foci of complete resorption of inanotic cells.

Of note is that there is no inflammatory reaction, nor any macrophages, around either the inanotic cells or around these empty spaces that we believe to be resorption pits. In other words, it seems that the inanotic cells are being resorbed without the involvement of phagocytic cells, which suggests that the resorption process is distinct and differs from that associated with reaction to necrotic or apoptotic cells. We have speculated that the dissolution of these cells may be the result of both lysosomal enzymes from the cells themselves and proteinases in the surrounding stroma, and we refer to this process of non-phagocytic, presumably enzymatic, resorption as reclamation since the molecular contents are being reclaimed and recycled for use in other cells.

Based on these concepts developed from our light microscopic studies, we then focused our attention on the hyalinized areas of our ultrastructural fibroid specimens. Ultrastructural analysis of hyalinized fibrotic areas that appeared relatively acellular by light microscopy revealed a graveyard of wispy atrophic cells, degenerating cells, and remnants of dead cells entrapped within a dense collagenous matrix (Fig. 4e) [3•]. Fine filaments were prominently reduced, and some cells contained swollen endoplasmic reticulum and degenerating mitochondria. Lysosomes often appeared increased, and double-membraned autophagic vacuoles with membranous debris, ribosomes, and/or degenerating myofilaments were occasionally encountered [3•]. These features supported the concepts of atrophic downsizing and injury related to interstitial ischemia.

In addition, occasional cells were found that exhibited entire segments of cytoplasm with degeneration of contents and sometimes with lack of a well-defined plasma membrane (Fig. 4f). Particles of cellular debris were sometimes abundant within the stroma between the degenerating cells (Fig. 4e, f). Fine filaments and cytoplasmic organelles such as mitochondria and ribosomes were sometimes found lying free within the surrounding collagen fibers. Naked nuclei with discontinuous nuclear envelopes were occasionally seen. These features were indicative of both cell degeneration and disintegration, with release of cellular debris into the stroma.

Equally notable was the lack of inflammatory infiltrate or phagocytosis in the midst of such extensive cell death and disintegration. Thus, the ultrastructural analysis reinforced our impression from the light microscopy that resorption was occurring in the absence of phagocytic cell recruitment, presumably as the result of intracellular and stromal enzymatic degradation. The electron-lucent stroma noted around the degenerating cells is believed to correspond to the clear spaces around the inane cells in light microscopic sections and to represent enzymatic resorption associated with the reclamation process.

In summary, the two processes that we refer to as inanosis and reclamation, or cell death and cell resorption, represent the final stages in the lifecycle of the fibroid myocyte.

Although we believe that involutional changes occur focally in many later-phase fibroids, we do not propose that these changes occur in all fibroids, nor do we propose that these changes result in shrinkage of the tumors or necessarily have clinical relevance. And since the largest number of tumors in our series were categorized as phase 3 (10–50% collagen) [3•], it may be that many fibroids would never involute (phase 4) [3•]. However, phase 4 tumors, in particular, do have the lowest PCNA proliferation rate, the lowest microvessel density, and evidence of both cell death (inanosis) and cell resorption (reclamation). And, thus, it is interesting that in Peddada's MRI-based uterine growth study [1], regression (> 20% shrinkage) occurred in 7% of the fibroids, a figure that is similar to our percentage (6%) of phase 4 tumors [3•] in which we have observed involutional changes morphologically.

Summary

These are our views and suppositions, based upon our observations and our attempts to explain those observations. Our primary goal in these studies has been to gain a better

understanding of the developmental biology and pathogenesis of fibroid tumors, during the course of which we have developed a few hypothetical concepts which we have here presented. We have attempted to illustrate these hypothetical concepts of phenotypic transformation, interstitial ischemia, inamosis, and reclamation in a graphic representation in Fig. 5 [3•].

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Fig. 1.

Fibroid phases 1–4. Left: Masson's trichrome stained 1× images (muscle red and collagen blue). Note the progressive increase in bluestaining collagen from phase 1 to phase 4. Right: corresponding 10× images (H&E). Phase 1: tumor consists entirely of myocytes with no apparent collagen. Phase 2: scattered collagen fibers (arrow) appear. Phase 3: moderate collagen formation (10 to 50%). Phase 4: predominance of hyalinized collagen with interspersed myocytes. Note also the abundance of microvessels (small ovoid spaces) in phase 1, and the paucity of vessels in phases 3 and 4. Image taken from [3•]

Fig. 2.

Phenotypic transformation. **a, b** Masson's trichrome stains of **a** myometrium with uniform fascicular pattern, parallel linear arrangement of the myocytes, and collagen (arrow) limited primarily to interfascicular zones and **b** fibroid with haphazardly arranged muscle fascicles and abundant interfascicular collagen (asterisk), as well as intrafascicular collagen between the myocytes (arrow). **c, d** H&E stains of **c** myometrium showing lateral bars between adjacent myocytes, believed to be gap junctions that permit ion passage during contraction and **d** fibroid with absent lateral bars. **e** Myometrial myocyte with abundant actin fine filaments (asterisk), interspersed dense bodies (short arrow), clusters of mitochondria (long arrow), and nucleus with a rounded end. **f** Transformed fibroid myocyte with extensive, dilated endoplasmic reticulum (short arrow), swollen mitochondria (long arrow), greatly reduced myofilaments limited to the periphery of the cell (asterisk), and nucleus with a pointed end. Original objective magnification of **a** and **b** = $\times 10$. Original magnification of **c** and **d** = $\times 330$. Original EM magnification of **e** and **f** = $\times 11,500$. Images taken from [3•]; images **b** and **c** modified from [3•]

Fig. 3.

Interstitial ischemia and myocyte atrophy. **a–c** Microvessel density decreases. Vessels are far more numerous in the phase 1 fibroid (**a**), compared to the few, more widely spaced vessels in the phase 3 fibroid (**b**), and the absence of capillaries in the hyalinized, hypocellular area on the left of the phase 4 fibroid (**c**). **d** Autophagocytosis. An autophagosome (long arrow) contains apparent ribosomes (short arrows), membranous debris of possible endoplasmic reticulum origin (arrowhead), and finely granular material probably from degenerated myofilaments. The nucleus is in the upper right (asterisk). **e, f** Myocyte Atrophy (**e** myometrium, **f** fibroid). The myocytes in the fibroid (**f**) are separated by abundant extracellular matrix (asterisk) and have shorter nuclei and less cytoplasm (arrows) than the myocytes (arrow) in the myometrium (**e**). Factor VIII-related antigen (von Willebrand factor) immunostaining of **a–c**. Original objective magnification of **a–c** $\times 10$. Images taken from [4•]. Original EM magnification of **d** $\times 43,000$. Original objective magnification of **e, f** $\times 40$. Images **d–f** taken from [3•]

Fig. 4.

Inanosis and reclamation. **a** The shrunken inanotic fibroid myocytes are characterized by small, pale nuclei (arrows), are surrounded by other atrophic myocytes, and are among the cells most distant from the two capillaries (arrowheads). **b** Oil immersion image of inanotic cells with pale nuclei in a fibrotic, atrophic field of a phase 3 tumor. The cell denoted by the arrowhead is located 76 μm from the capillary (arrow). **c** Clear circular spaces (arrows) are sometimes noted within hyalinized areas of phase 4 tumors and are believed to be the end stage of reclamation in which complete cellular resorption has occurred. Inanotic dead cells (arrowheads) are also noted. **d** Numerous clear spaces (arrows) are occasionally noted in areas of hyalinization, resulting in a spongiform appearance. These clear spaces are believed to be sites of complete cellular resorption. **e, f** Ultrastructure of hyalinized, fibrotic fibroids revealing much more than can be seen with the light microscope. **e** Numerous tiny shrunken cells (arrowheads) are widely spaced in the fibrotic stroma. A cluster of myocytes which are less atrophic (arrow) and have retained some cytoplasm provide a frame of reference for comparison. **f** Inanosis and reclamation. This cell exhibits features of both inanosis and reclamation. The cytoplasmic contents are degenerated, granular, and vacuolated (short arrow), and the cell membrane is disrupted in some areas, allowing the extrusion of cytoplasmic debris (long arrow). Extruded degenerate material (arrowhead) can also be seen in the surrounding stroma. Note that the stroma surrounding the cell is loose and electron-lucent, which is believed to correspond to the clear space often seen around inanotic cells with the light microscope, and thought to represent a resorption pit associated with

reclamation. Original magnification: **a** = $\times 132$, **b** = $\times 330$, **c** = $\times 132$. Original objective magnification of **d** = $\times 40$. Original EM magnification of **e** = $\times 1700$, **f** = $\times 9,900$. Images **a–e** taken from [3•]; image **f** modified from [3•]

Fig. 5.

Life cycle of the fibroid myocyte. The four hypothetical stages in the life of fibroid myocytes which are entrapped within areas of excessive collagenous matrix. Phenotypic transformation (fibroid phases 1 and 2)—initial stage in which contractile myocyte transforms to a proliferating, collagen synthesizing cell. Interstitial ischemia with myocyte atrophy (fibroid phase 3)—intermediate stage in which excessive production of collagen by the transformed myocyte results in decreased microvessel density with deprivation of nutrients and atrophy of fibroid myocytes. Inanosis (fibroid phase 4)—final stage in which cell death occurs due to extreme deprivation of nutrients and oxygen (inanition), followed by reclamation (fibroid phase 4)—disintegration, dissolution, and resorption of dead cells by non-phagocytic, presumably lysosomal and stromal enzymatic mechanisms, as the body reclaims and recycles molecular contents. Illustration by David Sabio. Image modified from [3•]

Table 1

Fibroid phases

Phase	Estimated collagen content	Functional status
Phase 1	No, or insignificant, collagen matrix	Proliferation of myocytes
Phase 2	< 10% collagen	Proliferation of myocytes and synthesis of collagen
Phase 3	10–50% collagen	Proliferation, synthesis of collagen, and early senescence in late phase 3
Phase 4	> 50% collagen	Involution

Table taken from [3•]