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Epigenetic and genetic landscape of uterine leiomyomas:a current view over a common gynecological disease

Antonio Simone Laganà[1] Daniele Vergara[2,3] · Alessandro Favilli[4] · Valentina Lucia La Rosa[5] · Andrea Tinelli[6] · Sandro Gerli[4] · Marco Noventa[7] · Amerigo Vitagliano[7] · Onofrio Triolo[1] · Agnese Maria Chiara Rapisarda[8] · Salvatore Giovanni Vitale[1]

Unit of Gynecology and Obstetrics, Department of Human Pathology in Adulthood and Childhood "G. Barresi", University of Messina, Via C. Valeria 1, 98125 Messina, Italy

2 Department of Biological and Environmental Sciences and Technologies, University of Salento, Lecce, Italy

3 Laboratory of Clinical Proteomic, "Giovanni Paolo II" Hospital, ASL Lecce, Lecce, Italy

4 Department of Obstetrics and Gynecology, University of Perugia, Perugia, Italy

5 Unit of Psychodiagnostics and Clinical Psychology, University of Catania, Catania, Italy

6 Division of Experimental Endoscopic Surgery, Imaging, Technology and Minimally Invasive Therapy, Department of Obstetrics and Gynecology, Vito Fazzi Hospital, Lecce, Italy

7 Department of Woman and Child Health, University of Padua, Padua, Italy

8 Division of Obstetrics and Gynecology, Department of General Surgery and Medical-Surgical Specialties, University of Catania, Catania, Italy

Correspondance to: Antonio Simone Laganà antlagana@unime.it

Abstract

Purpose Despite the numerous studies on the factors involved in the genesis and growth of uterine leiomyo- mas, the pathogenesis of these tumors remains unknown. Intrinsic abnormalities of the myometrium, abnormal myo- metrial receptors for estrogen, and hormonal changes or altered responses to ischemic damage during the menstrual period may be responsible for the initiation of (epi)genetic changes found in these tumors. Considering these elements, we aimed to offer an overview about epigenetic and geneticlandscape of uterine leiomyomas.

Methods Narrative overview, synthesizing the findings ofliterature retrieved from searches of computerized databases.

Results Several studies showed that leiomyomas have a monoclonal origin. Accumulating evidence converges on the risk factors and mechanisms of tumorigenesis: the transloca- tion t (12;14) and deletion of 7q were found in the highest percentages of recurrence; dysregulation of the HMGA2 gene has been mapped within the critical 12q14–q15 locus. Estrogen and progesterone are recognized as promoters of tumor growth, and the potential role of environmental estrogens has been poorly explored. The growth factors with mitogenic activity, such as transforming growth factor-β3, fibroblast growth factor, epidermal growth factor, and insu- lin-like growth factor-I are elevated in fibroids and may have a role as effectors of the tumor promotion.

Conclusion The new clues on genetics and epigenetics, as well as about the growth factors that control normal and pathological myometrial cellular biology may be of great help for the development of new effective and less invasive therapeutic strategies in the near future.

Keywords Uterine fibroid · Myomas · Epigenetics · Genetics · Uterine leiomyoma · Uterine leiomyosarcoma · Growth factors

Introduction

Uterine leiomyomas (ULs), also called myomas or fibroids, are benign smooth-muscle tumors that develop in the myometrium and are characterized by an extremely low malignant potential. They occur in approximately 70% of women of reproductive age, with a 2–3 times higher incidence in Afro-Caribbean women, and are the most common indication for gynecologic surgery [1–3]. ULs may be asymptomatic or responsible of a wide range of symptoms, including abnormal uterine bleeding (AUB), anemia, mass and pressure effects, as well as pelvic pain, infertility and recurrent pregnancy loss. Despite the significant prevalence, the cause of ULs still remains unclear and their biology poorly understood. ULs grow slowly and are surrounded by an extremely dense vascular area, which is made of compressed muscle fibers that supplies the tumor [4, 5]. The concentrations of steroid hormones (especially estrogens and progesterone) and growth factors play a pivotal role in the development and growth of ULs. The early onset of menstrual periods (≤10 years) has been associated with an increased risk of developing myomas, probably in reason of the increased number of divisions that myometrial cells undergo during reproductive age that may predispose to a higher probability of mutation of the genes that control myometrial proliferation [6, 7]. Even women with anovulatory cycles, characterized by increased and prolonged production of estrogen, present frequently fibroids [8, 9]. The incidence of ULs is high in obese women and this is principally due to the massive conversion of adrenal androgens in estrone caused by fatness. In premenopausal women, especially if obese, the decreased metabolism of estradiol reduces its con- version to inactive metabolites, and therefore, the high con- centration of estrogens contributes to an increased growth of fibroids [10–12]. Based on the clinical data, Afro-Caribbean women develop ULs more frequently and at younger age [3, 13]. Moreover, these women have a higher frequency of multiple lesions and increased size of fibroids compared to other ethnic groups [14]. Genetic factors could also affect ULs. For examples, women affected by several specific genetic syndromes such as Alport syndrome, Proteus syndrome, Cowden syndrome and Reed syndrome have an increased predisposition to develop ULs [15, 16]. Currently, clinicians have a wide range of treatment options from medical management to surgical interventions [17]. There are different approved pharmacological thera- pies including gonadotropin-releasing hormone (GnRH) agonist, GnRH antagonist, progestins, aromatase inhibi- tors, and more recently ulipristal acetate and mifepristone, two selective progesterone receptor modulators (SPRMs) [18]. However, hormonal therapies have undesirable side effects, including a rebound growth when the therapy stops. In symptomatic women who wish to preserve fertility, myomectomy is an important clinical option, whereas in perimenopausal women or in women who no longer desire childbearing, more radical surgical approaches can be considered, such as open or laparoscopic hysterectomy [19–24]. Recently, less invasive procedures such as uterine artery embolization and magnetic resonance-guided focused ultrasound (MRgFUS) have been proposed [25]. The development of ULs involves a complex and heterogeneous constellation of factors. Here, we review recent advances in our understanding of the epigenetic and genetic landscape of ULs; and in this context, in addition, we highlight new possible druggable targets or biological processes.

Pathophysiology of uterine leiomyomas

ULs are benign mesenchymal tumors of variable size from a few millimeters to several centimeters, rounded shape. They are made up of smooth muscle fibers with concentric spiral pattern and fibrous connective tissue, which tends to form a pseudo capsule anchored to the myometrium by fibro- muscular bridges. ULs can usually be located throughout the uterus. Because of location, they can be classified in [26]: intramural, in case of developing in the myometrial wall, they can cause distortion of the uterine shape and cavity; subserosal, in case of developing under the serosa of the uterus, and may be sessile or pedunculated; submucosal, in case of developing beneath the endometrium, they can protrude into the endometrial cavity, and may be sessile or pedunculated; intraligamentous, in case of developing through the pages of the broad ligaments.

Fibroids possible alterations are hyaline, fatty and cystic. Deposit of calcium salts determines calcification. There is a consensus in the literature that ULs are more common in black women than in white women [27]. The incidence of ULs is estimated to be about 2–3 times higher among them [28]. The endogenous estrogen metabolism is primarily oxidative and involves hydroxylation of the steroid or carbon-2 (2-OHE1) or carbon-16 (16-OHE1). The 2-OHE1 metabolites are primarily responsible for the biological activity device, while 16-OHE1 is agonist for the estrogen receptor. The CYP1A1 gene appears to play a key role in the estra- diol hydroxylation on carbon 2, and black women with the wild-type CYP1A1 gene showed in fact an increased ratio of estradiol derivatives hydroxylated in position 2, compared to derivatives hydroxylated in position 16. This position couldexplain the higher incidence of ULs in black women [29].

Estrogens have traditionally been proposed as the main promoters of the growth of ULs. The hypothesis of the role of estrogen has been supported by clinical studies to evaluate drug therapies with GnRH agonists, resulting in effective

hypoestrogenism and subsequent regression of fibroids [30]. The levels of progesterone, similar to those of estrogen, are also cyclically elevated during reproductive years and significantly decrease after menopause [31]. On this basis, numerous pharmacological therapies to reduce progesterone action at cellular level have been investigated and clinically approved. Treatment with progesterone antagonist mifepristone (RU486) induces regression of ULs by the reduction of progesterone receptors (PR) and inhibiting extracellular matrix formation [32, 33]. Ulipristal acetate significantly reduces ULs volume and fibroid-associated bleeding; its mechanism of action involves the modulation of progesterone signaling pathway, thus promoting remodeling of the extracellular matrix and the reduction of collagen synthesis[34, 35].

Regarding the high plasticity and regenerative capacity of the uterus during female reproductive lifetime and pregnancy, experimental studies demonstrated the prominent role of adult stem cells in endometrial and myometrial compartments for uterine tissue maintenance and function. Several pathological conditions, such as ULs, endometriosis and endometrial cancer, are driven by alterations in this pool of cells that display stem properties [36]. It has been pro- posed that ULs originate in hypoxic conditions from a single mutated myometrial smooth muscle stem cell. Another critical role in the tumorigenesis of uterine leiomyoma stem cells is played by the paracrine activation of the wingless- type (WNT)/β-catenin pathway mediated by estrogen and progesterone [37]. The presence of steroid hormones stimulates the secretion of WNT ligands and the nuclear translocation of β-catenin in leiomyoma stem cells, leading to the expression of genes with a critical role in ULs growth and development. Targeting these stem-progenitor cells and their paracrine interactions with more differentiated cell populations within leiomyoma may lead to a significant decrease of tumor growth and recurrence [38].

In a recent work, the combination of a high-throughput
proteomic approach with an in silico analysis enabled a deep profiling of ULs [39]. This proteomic study identified molecular differences between normal and tumor samples, related to energy metabolism, cell–cell communication, extracellular matrix remodeling, and estrogen and progesterone receptors status. This highlights the molecular complexity of ULs and cellular processes involved in the etiology and pathogenesis of the disease.

Histological aspects of uterine leiomyomas

The histological characterization of ULs represents a key aspect for the differential diagnosis between leiomyoma and leiomyosarcomas. UL cells can be distinguished from more aggressive tumor type's cells by microscopic examination and by tumor growth rate. From the histological standpoint ULs can be differentiate in: atypical or "bizarre" leiomyoma; hemorrhagic cellular leiomyoma; epithelioid leiomyoma; myxoid leiomyoma.

Macroscopically, the atypical ULs are characterized by yellow to tan areas, hemorrhage, focal softening, cavitation, or myxoid change; microscopically, they present bizarre pleomorphic cells, prominent nuclear pseudo inclusions and atypical nuclei, which can be focal or diffuse throughout the tumor. Atypical ULs have a benign clinical course and they are distinguished from leiomyosarcomas by the absence of cell necrosis and low mitotic counts (<10MFs/10HPFs) [40]. Features of hemorrhagic ULs are hemorrhage, edema, myxoid change, focal hyper cellularity, nuclear pleomorphism and high mitotic activity (8MFs/10HPFs). Nevertheless, these suspicious morphologic changes may be observed also in pregnant women and in those taking progestin or oral contraceptives [41]. Epithelioid leiomyoma of uterus is a rare variant of typical leiomyoma that present high cellularity and foci of hemorrhage and necrosis. Microscopically this tumor consists of polygonal clear cells with central or eccentric nuclei [42]. Immunohistochemically, these tumors show positivity for cytokeratins [43]. Myxoid ULs are usually circumscribed and composed by a gray jelly-like material; microscopically the cells present an elongated or stellate shape. The differential diagnosis with myxoid leiomyosarcoma may be difficult, but usually typical features such as the absence of tumor cell necrosis and severe cytological atypia in association with a mitotic index of <2MFs/10HPFs are consistent with a benign pathology [40]. Even if rare, intravenous leiomyoma are characterized by invasive growthtypical of malignancy that can create problems of differential diagnosis.

Uterine leiomyosarcoma is a very rare and highly malignant neoplasm, characterized by the presence of severe nuclear atypia, necrosis of tumor type and high mitotic index. The survival rate of patient with leiomyosarcoma is estimated between 15 and 25% at 5 years, and poses significant clinical implications [44]. It is still not clear if leiomyosarcoma arises from ULs or as an independent entity. Moreover, it is not easy to determine if a uterine tumor is benign or malignant prior to treatment, and the misdiagnosis of uterine leiomyosarcoma is of great concern, especially in reason of intrapelvic dissemination after the use of laparoscopic uterine morcellation [45].

Genetic and epigenetic features of uterine fibroids

There is a general agreement in the literature that myomas are of monoclonal origin. The clonality of ULs was studied using X-linked glucose-6-phosphate dehydrogenase (G6PD) isoenzymes, for the discrimination between active and inactive alleles of the X-linked genes [46–49]. Even if the monoclonality of ULs seems sufficiently studied, there are reports of some

biclonal or oligoclonal cancers [50]. It has also been suggested that monoclonality may be the result of a selective original polyclonal proliferation. Cytogenetic studies have highlighted the presence of clonal chromosome rearrangements involving mostly deletions, duplications and translocations involving chromosomes 6, 7, 12 and 14 in approximately 40–50% of ULs [51]. Additional abnormalities, such as monosomy 22 and rearrangements involving chromosome X, 1, 3 and 13 occur less frequently and often in conjunction with other chromosomal abnormalities [51, 52]. The fact that in 40% of ULs cytogenetic analysis is apparently nor- mal can be explained by sub-microscopic changes of the karyotype of the normal subgroup. It is widely supported the hypothesis that the clonal expansion of tumor cells pre- cedes the development of cytogenetic aberrations. However, it seems certain that the correlation between the location and cytogenetic abnormality is not dependent by the diameterof the fibroid.

Chromosomal abnormalities

ULs are characterized by non-random and tumor-specific chromosome abnormalities. This suggests a link between these chromosomal alterations and tumor physiology. A comparison between several studies led to the identification of main cytogenetic anomalies in ULs, including chromosomal abnormalities t (12;14) (q14–15; q23–24), translocations t (1;2) (p36; p24) and mutations in the chromosome7 (q22–31).

The most common translocation found in cytogenetic studies is located on chromosomes 12 and 14, in particular t (12;14) (q14–q15; q23–q24) presents in about 20% of the karyotype of abnormal ULs [53, 54]. Interestingly, HMGIC was mapped within the critical region of chromosome 12q14–q15: this gene encodes the high mobility protein HMG. The presence of this translocation causes up-regulation of HMGA2 expression. The HMG proteins are the most abundant non-histone chromatin-associated proteins with a specific role in the regulation of gene transcription during development and cancer; worthy of note, the expression of this protein was detected in ULs but not in correspondence with normal myometrium [55].

In normal female tissues, HMGA2 shows a high expression at amniocyte, ovary, and rectum level (Fig. 1a). In human tumor samples, the gene is expressed at higher levels in esophageal, head and neck, and ovarian cancers compared to other tumor types (Fig. 1b). The functional role of HMGA2 may also derive from the selective inter- action with specific cellular regulators, and a protein–pro- tein interactions (PPIs) network may help to determine this role (Fig. 1c). As shown, the protein is a central hub in a molecular network of proteins controlling cell proliferation, drug resistance and response to chemotherapy. It is clear that alterations of HMGA2 can influence a wide range of cellular functions.

Compared to ULs, leiomyosarcoma showed aberrations identical or closely related to the deviations of recurring structural myomas; these observations indicate a similar evolution pattern with early leiomyomatosis that may progress in both benign and malignant pathologies [56]. The cytogenetic similarities found between leiomyoma, leiomyosarcoma and rhabdomyosarcoma are few and may be random; cytogenetic profiles are similar in both tumor types and are produced by random rearrangements of 12q13–15, t (12;14) in leiomyoma and t (3;12) in lipoma, while the myxoid lipo- sarcoma has the t (12;16) (q13, p11) as a specific rearrangement [54]. A considerable percentage of ULs are cytoge- netically characterized by clonal chromosome abnormalities, including t (12;14) (q14–15; q23–24) and other 12q14–15 rearrangements that occur without obvious changes in 14q. The mapping of the breakpoint in 12q15 has been identified and at present, it seems that the breakpoint on 12q may be cytogenetically identical to the benign tumors but more proximal to the myxoid liposarcomas [57]. A cytogenetic analysis performed on two short-term cultures of leiomyosarcomas revealed that these tumors are characterized by complex karyotype changes, including modifications in the region of chromosomes 12 and 14 that are observed in benign ULs. This raises a hypothesis about the role of genes in 12q and 14q in the progression from benign to malign tumors [58]. Data from a cytogenetic analysis of 224 ULs performed by Nilbert and collaborators [59] showed 138 patients with an insufficient number of mitosis in 35 tumors, normal karyotype in 145, and clonal chromosomal aberrations in 44; these results suggest that while some multiple ULs originate independently, others can derive from the same neoplastic clone. Another study [60] showed no evi- dence of clonal evolution in the form of subclones in eight tumors, and moreover, monosomy 22 found in three second- ary tumors may reflect a preferential way for the evolution of the karyotype of ULs. About 40% of the tumors in this last study are associated with clonal chromosomal abnormali- ties: in addition, five different subgroups characterized by trisomy 12, t (12;14) (q14–15;q23–24) and re-modulation of chromosome 7 have been identified [61]. Recurrent cytogenetic abnormalities are common in ULs (7), especially of (q11.2–22q31–32) and t (12;14) (q14–15; q23–24); leiomyosarcomas present different alterations of the karyotype and often involve chromosomes 1, 7, 13 and 14 [62]. Moreover, many myxoid liposarcomas (MLS) are characterized cytogenetically by aberrations t (12;16) (q13;p11) [63].

Genomics and genetics of uterine leiomyomas

Technological advances in high-throughput genomic analysis greatly helped the recent understanding of the pathogenic pathways involved in ULs formation, especially the role of biallelic inactivation of fumarate hydratase (FH), media- tor complex subunit 12 (MED12), HMGA1 and HMGA2 mutations.

FH is a Krebs cycle enzyme that acts as a tumor sup- pressor. Hereditary leiomyomatosis and renal cell carcinoma (HLRCC) is an autosomal-dominant hereditary syndrome caused by germline mutation in the FH gene, localized to chromosome 1q42.2.

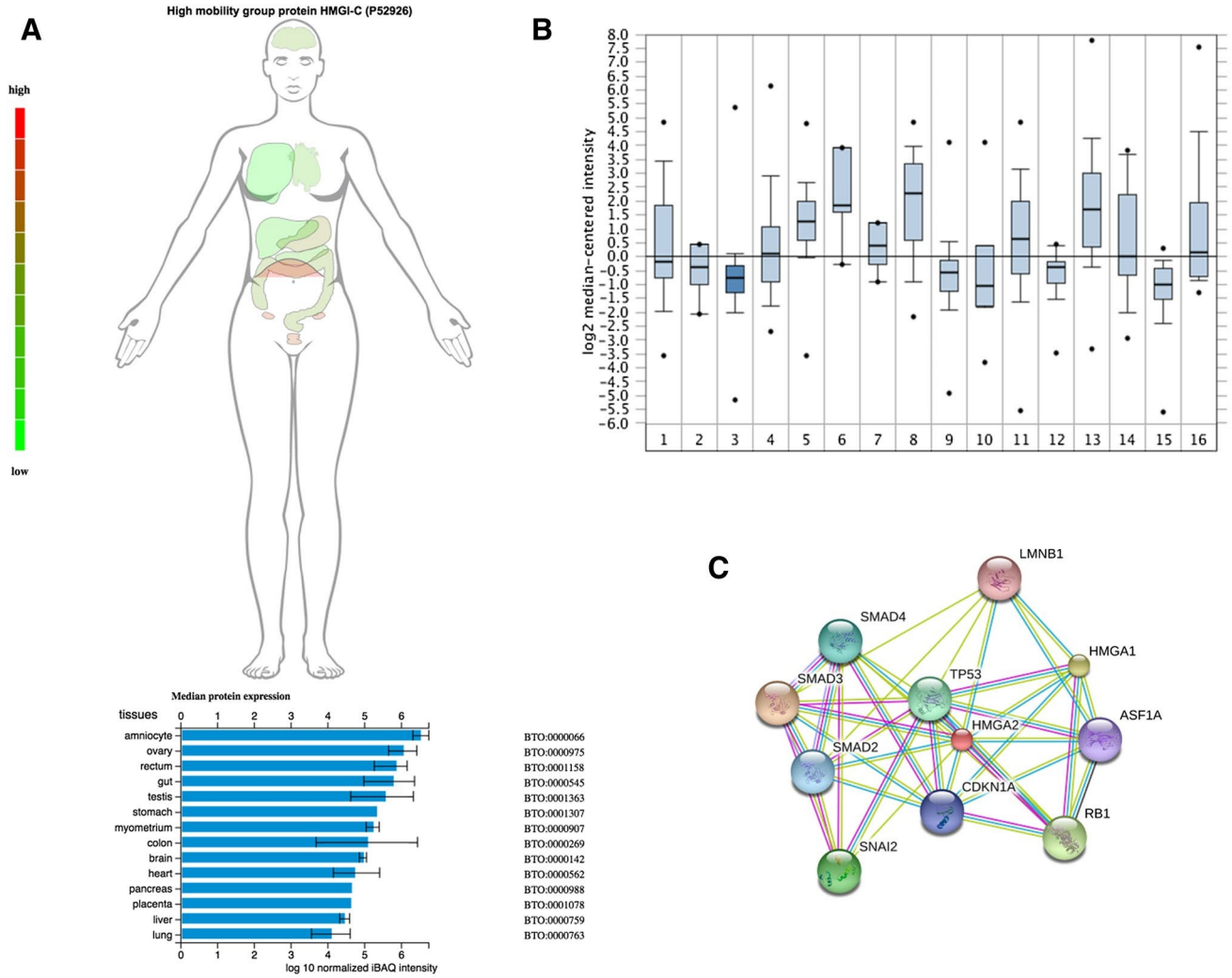

Fig. 1 HMGA2 expression in normal and cancer tissues. **a** Data regarding the HMGA2 protein expression in normal tissues were obtained from the Proteomics BD website (http://www.proteomicsdb. org). **b** Gene expression analysis of HMGA2 in a panel of cancer tis- sues: *1* bladder cancer, *2* brain and CNS cancer, *3* breast cancer, *4* cervical cancer, *5* colorectal cancer, *6* esophageal cancer, *7* gastric cancer, *8* head and neck cancer, *9* kidney cancer, *10* liver cancer, *11* lung cancer, *12* lymphoma, *13* ovarian cancer, *14* pancreatic cancer, *15* prostate cancer and *16* sarcoma. **c** The protein–protein interac- tion network of HMGA2 was determined using the online software STRING (http://string-db.org/). The network nodes are proteins. The edges represent the predicted functional associations. An edge may be drawn with up to seven differently *colored lines*—these *lines* repre- sent the existence of the seven types of evidence used in predictingthe associations

In these patients, a somatic alteration of the wild-type copy of the FH allele (the so-called "second hit") results in loss of FH activity, and thereby, in a fumarate accumulation in target tissues (skin, uterus and kidney) [64, 65]. Women with HLRCC develop ULs at an earlier age, they are usually multiple (up to 20) and of bigger dimension (from 1.5 to 10 cm). These women are symptomatic and are at high risk for hysterectomy even in childbearing age [66]. Oncogenic mutations in codon 44 of exon 2 of the chro- mosome Xq13, encoding for gene MED12, occur in 50–70% of ULs, representing the most common genetic anomalies in these benign tumors [67]. MED12 is a subunit of the mod- ule CDK8, multi-protein complex mediator composed byMED12, MED13, cyclin-dependent kinase 8 (CDK8) and cyclin C [68]. MED12 is an evolutionary-conserved regula- tor of transcription that has a regulatory role by forming a molecular bridge between DNA elements and RNA poly- merase II initiation complex [69]. Accumulating evidence suggests that MED12 is involved in the activation of Wnt/β- catenin and p53 pathway leading to an impaired regulation of cell growth and tumorigenesis [70, 71].

Several studies showed no MED12 mutations in ULs with HMGA2 overexpression, suggesting MED12 and HMGA2 mutations are two independent genetic events in the UL tumorigenesis [71, 72]. In general, benign leiomyoma is not considered precursor of highly aggressive leiomyosarcoma, however, a small rate (2–20%) of leiomyosarcoma and smooth muscle tumors of uncertain malignant potential (11%) present MED12 mutations [73–76]; these findings suggest that leiomyoma and leiomyosarcomas may have a different and independent tumorigenic pathway, and a possible epigenetic mechanism may be responsible for the malignant transformation of a benign precursor [77–79].

Deletions, amplifications and rearrangements of HMGA genes are detected in a wide variety of benign tumor of mes- enchymal origin [80]. HMGA2 is a target gene of tumor carrying 12q15 and 6q21 rearrangements [81–84]. It is a transcriptional regulator that encodes a small protein that belongs to the family of non-histone chromatin-binding proteins. This protein contains structural domains binding to DNA, called AT-hooks, and can act as factors for transcriptional regulation, resulting in an activation of the $p14^{Arf}$-p53 network [85]. HMGA2 overexpression has been identified in 7.5–10% of leiomyoma, and after MED12, is the second most common genetic alteration in these tumors. ULs with HMGA2 overexpression displayed highly significant up-reg- ulation of the proto-oncogene pleomorphic adenoma gene 1 (PLAG1), suggesting that HMGA2 promotes tumorigenesisthrough PLAG1 activation [86].

The pathogenesis of ULs is associated with changes in different class of genes as supported by several experimen- tal data. In particular, gene expression profiling provided atmolecular level a detailed comparison of normal and ULs tissues.

Using high-density microarrays, changes in mRNA expression level of 68 genes were observed between ULs and normal myometrium samples. These genes were assigned to six different functional categories, including signal trans- duction genes, growth factors, transcriptional factors, ECM genes, and prostaglandin-related genes. In this gene dataset, alcohol dehydrogenase 1 (ADH1) and ionotropic glutamate receptor 2 (GluR2) mRNA showed the largest changes, with a 17-fold decrease and fivefold increase, respectively [87, 88]. The up-regulation of GluR2 was also confirmed at protein level [87]. Authors hypothesize that this up-regulation may increase the flux of Ca^{2+} into tumors with effects on cell signaling and tumor vascularization. To further extent, localization of GluR2 seems to confirm this hypothesize, since this protein is localized in the endothelial cells of the blood vessels. This highlights a possible role of this receptor in supporting angiogenesis in tumor tissues [88].

Other studies confirmed that ULs express a specific gene signature compared to normal myometrium. In particular, the gene expression of a set 46 gene involved in different cellular processes clustered ULs and normal tissues into two groups. Among the genes analyzed, four genes (doublecortin, calpain-6, IL-17B, and proteolipid protein-1) were identified as specific for ULs samples, because not expressed or expressed at very low levels in a group of 18 human tissues including normal and cancer samples [89].

Another study by Ahn et al. [90] used both cDNA micro- array and electrophoretic techniques to investigate the inter- actions of multiple genes and proteins involved in the patho- physiology of ULs. The screening, carried out on 17,000 genes, identified 21 genes up-regulated and 50 inhibited. On a further extent, Catherino et al. [91] performed a simultaneous comparison of the expression of thousands of genes using microarrays, to highlight the specific pathways that may play a role in the development of ULs. Such findings suggested that even if the hormonal control of leiomyoma growth is predominant, there are other critical pathways involved in the development of leiomyoma cell phenotype. In particular, the expression of genes of the extracellular matrix of ULs is unbalanced and this could be a new source of therapeutic targets for this disease. Other authors have identified several deregulated genes associated with apoptosis; of particular interest was TRAIL and ASK1, as well as proliferation of numerous genes differentially expressed, including TGFB-1, PDGFC, and two phosphatases [92]. Considering these elements, it was hypothesized that deregulation of apoptosis and proliferation is crucial for the development of fibroids. Similarly, Arslan et al. [93] used Affymetrix GeneChip U133A, which analyzed the expression profiles of 22,283 genes in paired samples of leiomyoma and adjacent normal myometrium, identifying 80 genes with different expression in the two tissue types: the analysis of gene expression revealed consistent changes in genes that regulate the synthesis of retinoids, the metabolism of IGF signaling mediated by TGF-β and the formation of the extra-

cellular matrix.

The main results of these studies are summarized in Table 1. The most frequently recurring changes were: IGF2 up-regulation (reported in 7 studies); CRABP2 up-regula- tion (reported 5 studies); GRIA2 up-regulation (reported in 5 studies); MEST up-regulation (reported in 5 studies); ADH1 down-regulation (reported in 7 studies); ATF3 down- regulation (reported in 6 studies); Cyr61 down-regulation (reported in 5 studies); TPD down-regulation (reported in5 studies).

Epigenetic mechanisms

Epigenetic mechanisms play a crucial role in the pathogenesis of ULs. Epigenetics refers to change in phenotype mediated by altered gene expression. In humans, three main epigenetics mechanisms play a crucial role in modulating the gene expression in fibroids formation: DNA methylation, histone modifications and microRNAs (miRNAs) [94]. Aberration of DNA methylation occurs in many diseases and is involved in tumorigenesis. Studies on genome-wide DNA methylation analysis reveal higher hyper methylation levels

Table 1 Up- and down-regulated genes in uterine leiomyomas

Gene symbol	Function	Tsbris et al. [84]	Wang et al. [85]	Skubitz et al. [86]	Ahn et al. [87]	Catherino et al. [88]	Hoffman et al. [89]	Arslan et al. [90]	Anania et al. [103]
Up-regulated genes									
IGF2	Growth factor	16.80	2.60	2.90	2.20	13.50	3.00	3.50	
CRABP2	RA-binding protein	5.10	2.60	3.60		11.60	3.10		
GRIA2	Angiogenesis	38.80	4.90	3.60			7.50		730
MEST	Growth factor	11.60	2.40	3.10			3.90	6.10	
Down-regulat d genes									
ADH1	RA synthesis	−40.00	−16.60	−5.80	−18.60	−9.20		−9.40	−10.00
ATF3	Transcription factor	−6.00		−4.00		−2.80	−8.80	−5.20	−16.70
CYR61	IGFBP/angiogenesis	−5.30			−4.90		−5.80	−5.10	−6.30
DPT	TGF-modulator	−18.70	−4.00		−11.00	−4.30		−7.40	

of several tumor suppressor genes in ULs compared to the adjacent normal tissue. DNA methylation is regulated by at least three DNA methyltransferases (DNMT1, DNMT3A, and DNMT3B). DNA methyltransferase 1 (DNMT1) maintains DNA methylation during DNA replication; instead DNMT3A and DNMT3B act establishing methylation pat- terns [94]. Interestingly, ULs are associated with alterations of DNA methylation at multiple genomic loci with increased DNMT1 and DNMT3a mRNA expression in tumor samplescompared to myometrium [95].

Other epigenetic modifications include acetylation and methylation of the histone tails. Histone methylation can determine either activation or repression of gene transcription; instead, histone acetylation determines gene activation [94]. miRNAs are small non-coding RNA molecules that play a pivotal role in transcriptional and non-transcriptional regulation process binding within mRNA molecules [96]: although their role as epigenetic regulators has been already extensively demonstrated in different gynecological disease, the information about ULs to date remains still elusive and does not allow to draw firm conclusion.

Growth factors and hormonal regulation

Growth factors are polypeptides or small proteins that act as signaling molecules over short distance in an autocrine and/ or paracrine manner, interacting with specific receptors on the cell surface. Several growth factors, such as transform- ing growth factor-β (TGF-β), basic fibroblast growth factor (bFGF), vascular endothelial growth factor (VEGF), plate- let-derived growth factor (PDGF) and insulin-like growth factor (IGF), play a central role in the mechanism involved in myometrial pathophysiology and development of ULs, mostly by their capability of modulating cellular growth, proliferation and differentiation [97].

The TGF-β family consists of three major isoforms of particular interest as regards the fibroids because of their ability not only to promote mitogenesis, but also to up-regulate the synthesis of many extracellular matrix components, with consequent increase of the fibrous component [98]. Interestingly, a significant increase of mRNA levels of TGF- β3 in the samples of leiomyoma in the luteal phase respect to the follicular phase suggests a central role of progesterone in the regulation of expression of TGF-β3 [99]. In contrast, no change was observed in the expression of TGFβ mRNA and protein in myometrial tissue during the menstrual cycle [100]. In view of the probable role of this growth factor in the pathophysiology of fibroid, is of particular interest to know that the gene encoding the TGF-β3 is close to the point of Drive cut 14q23–24, one of the sites identified in studiesof cytogenetics of fibroids [101].

The bFGF induces the proliferation of smooth muscle cells, including leiomyoma and myometrial cells, and also promotes angiogenesis [102, 103]. Expression of bFGF-mRNA is increased in ULs compared with normal myometrium, and the extracellular matrix of ULs shows immune-reactivity to the fibroblast growth factors-1 (FGF) receptor-1 [104]. Since TGF-β3 and bFGF are significantly overexpressed in ULs respect to normal myometrium, both factors can contribute to enhance growth of ULs [105]. The mitotic activity of ULs is highest during the luteal phase of the cycle: this finding suggests that the production of EGF (epidermal growth factor) may be a mechanism by which progesterone stimulates mitotic activity of fibroids. Not surprisingly, the mRNA level of the EGF receptor has been found increased in leiomyomatous cells [106].

The PDGF is a potent mitogen for smooth muscle cells and stimulates the binding via the heparin to bFGF and VEGF growth factors. Due to the ability of these factors to bind heparin, they may be retained in the extracellular matrix, which is generally abundant within fibroids, and can therefore serve as a reservoir for these growth factors [107]. When the myometrial cells are treated with PDGF and EGF, there is a synergic decrease of DNA synthesis, while the treatment of leiomyoma cells with both factors results in an increase in DNA synthesis [108]. Although VEGF appears to be a highly specific mitogen for vascular endothelial cells, high levels of VEGF-mRNA and the expression of VEGF protein have also been identified in smooth muscle cells of ULs, but also in normal myometrium [102]. The levels of VEGF-mRNA in ULs, in fact, are not significantly differ- ent from the level found in the myometrium; furthermore, the levels of VEGF-mRNA do not differ between the prolif- erative and secretory phases of the cycle and show similar levels after treatment with a GnRH analogue [109]. There is evidence that VEGF acts in synergy with FGF [110] and can also release the angiogenic factor bFGF from his “deposit” presented by residues of heparin in the extracellular matrix [111]. The increase of the peptide insulin-like growth factor (IGF) has been detected in some ULs compared with normal myometrium [102]. Several authors agree that IGF-I may play a role in mitogenic growth of uterine fibroids due to an increase in the levels of its receptor and overexpression of the growth factor itself. The finding that the IGF binding protein-3 is increased in ULs compared to myometrium disease may be of great significance, as this would increase the bioavailability of “free” IGF, which would act as a stimulant for the fibromatous proliferation [112]. Progesterone can hypo-regulate the expression of IGF-I through progesterone receptor A (PRA) and B (PRB), thus the action of progesterone on leiomyomatous growth may depend (at least in part) on this mechanism [73, 74]. Estrogen and progesterone influence ULs development by regulating growth factors and their signaling pathways [113]. The activation of steroid hormone receptors may have a myriad of effects which include

up-regulation of growth factors and receptor tyrosine kinase (RTK) that through the downstream effector proteins, such as mitogen-activated protein kinase (MAPK) p44/42 (ERK½), are capable of mediating transcription, translation, and cell proliferation [114].

Because of their dependence on hormones, ULs can also be affected by environmental chemicals, whose biological effects are mediated through increased/decreased levels of estrogen and/or progesterone receptors. As far these elements are concerned, the development of ULs may be significantly influenced by the "cross-talking" between the estrogen receptor and signaling pathways RTK [114]. Last but not least, UL cells presenting overexpression of aromatase-P450 are able to synthesize estrogen sufficient to accelerate their own cell growth. In this regards, the over- expression of P450 aromatase affects the growth of leio- myoma and surrounding myometrium through an autocrine mechanism [115].

Association between leiomyoma and leiomyosarcoma

The debate on the association between leiomyoma and leiomyosarcoma is still open. Uterine smooth muscle tumors are, in general, benign, however, several studies tested the hypothesis that uterine leiomyosarcoma may arise from existing areas or from small subgroup of UL cells. On this basis, Quade et al. [116] performed a functional analysis on 146 genes using microarrays. Analyzing uterine leiomyosarcomas, the authors found a slight overrepresentation of genes on 1p and 2q and a down-regulation of other specific genes. In addition, four extrauterine leiomyosarcomas had a very similar gene profile to uterine leiomyosarcomas.

Uterine leiomyosarcomas are characterized by a large number of chromosomal aberrations [40], and thus genomic instability, confirming the hypothesis that the molecular pathways in leiomyoma and leiomyosarcoma are distinct. Nevertheless, it was showed that a small subgroup of UL cells with loss of the short arm of chromosome 1 may undergo to malignant transformation into uterine leiomyo- sarcoma [117].

On a further extent, Mittal et al. [118] tested 18 cases of uterine leiomyosarcoma studying the expression of p53, estrogen receptor, PRA, PRB and Ki-67. In six cases, the areas of the UL and leiomyosarcoma were similar, and were tested using the oligonucleotide array-CGH high density to determine possible genetic aberrations in the two areas. Almost all of the genetic aberrations in areas of the leiomyoma were also found in the relevant sectors of uterine leiomyosarcoma. In addition, areas of uterine leiomyosarcoma showed more genetic aberrations. These profiles of immunohistochemical and genetic aberrations suggested that uterine leiomyosarcoma may arise from areas of pre-existing leiomyoma and symplastic cell type [118].

Partially confirming these findings, Dastranj Tabrizi et al. [119] studied 32 uterine smooth muscle tumors (10 cases of leiomyoma with bizarre nuclei, 4 cases of smooth muscle tumor of uncertain malignant potential and 12 cases of leio- myosarcomas). Six cases of leiomyosarcomas (50%) showed strong and diffuse nuclear staining with p53 antibody and only one case of bizarre leiomyoma showed a focal positive reaction with p53. Regarding the other findings, the percent- age of positive cell for ki67 was 14.92% in leiomyosarcomas and only 0.85% in bizarre ULs. Based on their data analysis, those authors concluded that the loss of inhibitory function of wild-type p53 gene was a pivotal event in the genesis of leiomyosarcoma [119].

About this point, mitotically active ULs are character- ized by low cytologic atypia with a number of 5–20 mitotic figures per ten fields, without coagulation necrosis of tumor cells and tend to have a benign clinical course. Although the majority of mitotically active ULs have a benign clinical course, these lesions may recur and have the potential for malignant transformation. Therefore, patients with mitoti- cally active ULs require careful follow-up [120].

Finally, the possible role of hypoxia as a key factor in the pathophysiology of leiomyosarcoma was investigated: according to a recent study [121], hypoxia-inducible fac- tor (HIF)-1alfa, HIF-2alpha, glucose transporter-1, carbonic anhydrase IX, were not expressed in the tissues of leiomyoma. In contrast, the expression of these markers has been abundantly detected in leiomyosarcomas that showed a phenotype with high turnover with significant increase of proliferative and apoptotic factors. The uterine leiomyoma may therefore represent a state of proliferation restricted by the presence of oxygen; on the contrary, the hypoxia can contribute to perpetuate the aggressive phenotype [121].

Conclusion

In the last decade, the investigations about molecular mechanism occurring within ULs have considerably improved knowledge of the topic, even if many aspects are still very far from be fully elucidated. Genetic abnormalities are pre- sent in a large percentage of ULs, especially translocation t (12;14) and the deletion of 7q. Estrogen and progester- one have been identified as growth promoters. The lack of estrogen metabolizing enzyme 17β-hydroxysteroid dehydrogenase causes an accumulation of estradiol in the fibroids resulting in promoter activity. The TGF-β3 and bFGF are associated with the

production of extracellular matrix. During the luteal phase, with maximum mitotic activity, EGF has been identified as a growth factor, whereas IGF-I showed mitogenic activity and overexpression of both peptide and its receptor. The (epi)genetic aberrations found in leiomyoma were also found in uterine leiomyosarcoma, suggesting that the uterine leiomyosarcoma may arise from areas of pre-existing leiomyoma. The new clues on genetics and epigenetics, as well as about the growth factors that control normal and pathological myometrial cellular biology may be of great help for the development of new effective and less invasive therapeutic strategies in the near future.

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Compliance with ethical standards

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