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Review Article

THE GENETIC CONTRIBUTION, STEM CELLS AND UTERINE LEIOMYOMA: THERAPEUTIC IMPLICATIONS

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ABSTRACT

Uterine leiomyoma is the most common benign tumor in women and is thought to arise from the clonal expansion of a single myometrial smooth muscle cell transformed by a cellular insult. Leiomyomas cause a variety of symptoms, including abnormal uterine bleeding, pelvic pain, bladder or bowel dysfunction, and recurrent pregnancy loss, and are the most common indication for hysterectomy in the USA.

Aim of the work: This review focuses on the most recent discoveries regarding genetic abnormalities and the role of stem-progenitor cells in the pathogenesis of uterine leiomyoma, and discusses the therapeutic implications of the current state of knowledge of these pathways.

Conclusion: The recent shift in focus from hormones to fibroid stem cells and genetic aberrations should lead not only to a deeper understanding of the specific etiology of fibroids, but also to the discovery of new therapeutic targets.

INTRODUCTION

Uterine fibroids occur in up to 80% of reproductive-age women, causing significant morbidity in up to 30% of women (Parker 2007 and Bulun2013).

Leiomyomas can cause a range of symptoms, including abnormal uterine bleeding, pressure-related symptoms, recurrent pregnancy loss and infertility. Interestingly, leiomyoma cause many of these symptoms not only by virtue of the size and mass effect of the tumor itself, but also by modulating gene expression in the endometrium (Cakmak, Taylor 2011).

Uterine leiomyomas represent the most common category of solid pelvic tumors in women (Myers *et al* 2002).

Uterine leiomyomas are tumors that are derived from uterine smooth muscle cells and are most common in gynecologic neoplasms[5].

In the United States, more than 200,000 surgical procedures are performed for the treatment of fibroids, with yearly cost estimates of $5.9-34.4 billion[6].

African-American women develop leiomyomas more frequently and at earlier ages than Caucasian women. Moreover, tumors in African-American women are more aggressive, as they present with larger leiomyomas and more significant symptoms than their Caucasian counterparts [7][8].

The identity of the factor(s) and molecular mechanisms involved in the cellular transformation of myometrial cells into leiomyoma remains unknown. Evidence also exists supporting the involvement of genomic instability influencing genes such as estrogen and progesterone receptors. Several genomic and proteomic studies have also provided evidence for altered molecular environment of leiomyomas compared to the normal myometrium, as a possible biomarker in their proliferation and regression[9].

Global gene expression profiling of uterine leiomyomas (ULMs) revealed that hundreds of genes were dysregulated including those with functional roles in cell proliferation, differentiation and extracellular matrix production. So far, only a few specific genes or cytogenetic aberrations have been identified to be associated with ULMs. While many of the dysregulated genes may function as either effectors or promoters of ULMs growth, they are likely to be secondarily induced and indirectly responsible for tumor growth into morbid and symptomatic ULMs[10].

The recent discovery of a small population of stem-progenitor cells, important in leiomyoma pathophysiology, may provide the opportunity for new therapeutic targets [11].

Aim of the work

This review focuses on the most recent discoveries regarding genetic abnormalities and the role of stem-progenitor cells in the pathogenesis of uterine leiomyoma, and discusses the

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therapeutic implications of the current state of knowledge of these pathways.

METHODS

A comprehensive search of PUBMED was conducted to identify peer-reviewed literature published up to 2016 pertinent to the roles of genetic abnormalities and somatic stem cells in leiomyoma, including literature on therapeutics that target action in leiomyoma. The search included combinations of the following key words: leiomyomastem cells and progenitor cells, Chromosomal translocation, Chromosomal, Gene rearrangement, Reviewed articles were restricted to English language only. Studies in both animals and humans were reviewed for the manuscript.

RESULTS

Leiomyomas as clonal tumors: X-inactivation studies Analyses of multiple leiomyomas from a single uterus have demonstrated that the tumors can harbor different chromosomal changes and suggest that each tumor can develop independently. X-inactivation studies, based on the phenomenon of lyonization, i.e., inactivation of one X-chromosome in normal female cells, have demonstrated that leiomyomas develop as clonal lesions. Initially, glucose-6-phosphate dehydrogenase (G6 PD) isoenzyme analysis was used to demonstrate the independent clonal origin of multiple tumors in a single uterus[12].

Another, more informative, approach based on the CAG repeat polymorphism in the X-linked androgen receptor gene has been used to examine clonality and the results confirmed the monoclonal nature of leiomyoma. A study of a patient with 2 independent leiomyomas, each showing a different pattern of X-chromosome inactivation but with identical del(7)(q21.2q31.2) derivative chromosomes, supports the view that identical cytogenetic changes in multiple leiomyomas from the same patient may represent recurrent chromosomal aberrations in smooth muscles or they may be coincidental. Cytogenetically mosaic tumors were also reported to be clonal[12].
Studies on the inactivation of X-chromosome-linked phosphoglycerokinase (PGK) showed that all studied leiomyomas had a single type of inactive allele and were of unicellular origin but independently generated in the uterus[12].

Cytogenetic studies

Standard karyotyping has been used to detect chromosomal aberrations such as deletions, duplications and translocations which require the culture of leiomyoma cells to obtain metaphase preparations.

An alternative method that has been employed in a few studies is comparative genome hybridization, which permits the recognition of cytogenetic changes such as deletions and amplifications without the need for cell cultures, although it does not allow detection of balanced rearrangements. Neither standard karyotyping nor comparative genomic hybridization permits the detection of small submicroscopic chromosomal abnormalities such as point mutations or epigenetic changes such as methylations [13].

Approximately 40% of UL have non-random and tumor-specific chromosome abnormalities. This has allowed classification of some UL into well-defined subgroups which include deletion of portions of 7q, trisomy 12 or rearrangements of 12q15, 6p21 or 10q22. Additional abnormalities, which appear consistently but not as frequently, include rearrangements of chromosomes X, 1, 3 and 13. The variety of chromosomal rearrangements, including but not limited to translocation, deletion and trisomy, predict different molecular genetic mechanisms for UL formation and growth (24). A tendency for karyotypically abnormal leiomyomas to be more cellular and to have a higher mitotic index than do chromosomally normal tumors, has been reported[13].

Although no relationship between the patient age or parity and the type of chromosomal abnormality has been identified, a few studies have found a positive correlation between the presence of a cytogenetic abnormality and the anatomic location of uterine leiomyomas, i.e., intramural (35%) and subserous (29%) leiomyomas are more likely to have abnormal karyotypes than the submucous (12%) type. Another study, showed a relationship between karyotype and leiomyoma size, with the largest tumors carrying t(12;14) abnormalities. In contrast, tumors with del(7) were found to be smaller and those with mosaic karyotypes were intermediate in size[14].

Genetic Abnormalities

Recent research suggests that most fibroids fall into one of four categories of mutations: mediator complex subunit 12(MED12) mutations, fumarate hydratase(FH) inactivation, COL4A6-COL4A5 deletions, or High-mobility groupA2(HMGA2) over expression[15][16].

In one study of HMGA2 and MED12 mutations in fibroids, the two mutations appear to be mutually exclusive, raising the possibility that different genetic abnormalities in fibroids actually represent separate pathophysiology[17].

In support of this hypothesis, HMGA2 aberrations are highly correlated with big fibroid tumors, whereas tumors with MED12 mutations tend to be smaller[18][19].

Because of their possible role in stem cell action, we will focus on HMGA2 and MED12 mutations in this review.

HMGA2

Mutations involving HMGA2 are found in approximately 7.5% of fibroid tumors and HMGA2 overexpression is due to rearrangements involving chromosome 12q14-15[19].

In mouse neural stem cells, HMGA2 expression inhibits senescence by downregulating p16INK4a, a suppressor of stem cell self-renewal[20].

Similarly, HMGA2 has been shown to downregulate p14Arf, also a negative regulator of self-renewal, in fibroid cells[21].
Finally, uterine fibroids exhibit underexpression of Let-7, which is known to suppress HMGA2[22].

MED12

In the largest study of MED12 mutations in fibroids, specific MED12 mutations were found in 70% of fibroids, although smaller studies have reported a prevalence anywhere from 48% to 92%[23].

It has been shown that stem cells from fibroid tissue, but not from myometrial tissue, carry MED12 mutations, supporting our hypothesis that a genetic hit may explain the transformation of a myometrial stem cell to a fibroid stem cell [24].

MED12 regulates Wnt signaling by binding to β-catenin, making it possible that absence of or defects in MED12 in fibroid stem cells could lead to unregulated Wnt/β-catenin pathway-stimulated tumor growth[25].

Moreover, MED12 deficiency, possibly in somatic stem cells, releases negative regulation of TGFβ signaling, resulting in increased proliferation in cancer cells[26][27].

Taken together, this evidence suggests that MED12 deficiency could lead to activation of the Wnt/β-catenin and TGFβ pathway, thereby supporting stem cell renewal, proliferation, and fibrosis in uterine fibroids[28].

Stem-progenitor cells

Somatic stem cells (also called adult stem cells or tissue-specific stem cells) are a small group of cells present throughout the body that undergo asymmetric division, allowing self-renewal and the production of daughter cells that can go on and differentiate into tissue-specific cell types. These cells are necessary for tissue regeneration and repair, which is critical for maintaining organ function [29].

Similarly, tumor-initiating cells (also called cancer stem cells or tumor progenitor cells) are a small group of cells within a tumor also capable of asymmetric division, and thereby have the ability for self-renewal and tumor maintenance and growth [30].

Stem cells derived from leiomyoma tissue, but not myometrium, carry mediator complex subunit 12 (MED12) mutations, leading some to hypothesize that at least one genetic hit may transform a myometrial stem cell into a leiomyoma tumor progenitor cell, which then interacts with the surrounding myometrial tissue to give rise to a leiomyoma tumor[31].

Interestingly, mutations in the MED12 gene have been reported in ~70% of uterine leiomyomas[32][33].

Mutations affecting the expression of the high mobility AT-hook 2 (HMGA2) gene have also been reported, and appear to be mutually exclusive with MED12 mutations, suggesting the possibility of different pathophysiologies behind leiomyomas harboring different mutations[34].

Evidence suggests that leiomyomas possess much smaller populations of stem cells compared with the myometrium [35]. Making it unclear whether these genetic alterations cause the transformation of a myometrial stem cell or simply support already existing leiomyoma stem-progenitor cells. Importantly, it has alternatively been hypothesized that uterine hypoxia, aberrant methylation or abnormal estrogen signaling could play a critical role in the transformation of a myometrial stem cell into a leiomyoma[36][37].

Further research into the cellular insult leading to this transformation event in myometrial stem cells could reveal important therapeutic targets. The leiomyoma stem cell population is likely essential for Steroid-dependent leiomyoma growth[11].

When cell suspensions containing leiomyoma stem-progenitor cells and mixed myometrial cells are injected under the kidney capsules of immunodeficient mice treated with estrogen and progesterone, they grow into significantly larger tumors than those containing differentiated leiomyoma cells and mixed myometrial cells. The tumors derived from leiomyoma stem-progenitor cells also have a much higher proliferation index than the tumors that do not contain these cells[24].

Interestingly, leiomyoma stem-progenitor cells appear to be deficient in ERs and PRs, but have tumorigenic capabilities when stimulated by estrogen and progesterone. Moreover, leiomyoma stem-progenitor cells seem to require the presence of either mature leiomyoma or myometrial cells for proliferation and growth. We hypothesize that these cells rely on strikingly higher levels of steroid hormone receptors in surrounding differentiated myometrial and leiomyoma cells to mediate estrogen and progesterone action via paracrine signaling[24].

Recently, reported a critical role for the wingless-type (WNT)/β-catenin pathway in the communication between leiomyoma stem-progenitor cells and the surrounding differentiated cells. Treatment of mature myometrial cells with estrogen and progesterone resulted in secretion of WNT ligands, which induced nuclear translocation of β-catenin in neighboring leiomyoma stem-progenitor cells and ultimately activated the expression of genes critical for growth and proliferation. Moreover, selective inhibition of WNT binding or β-catenin in leiomyoma stem-progenitor cells, but not in fully differentiated leiomyoma cells, significantly decreased tumor growth[31].

Additionally, MED12 has previously been shown to regulate β-catenin/WNT signaling, further supporting its role in leiomyoma pathogenesis [25].

On the other hand, treatment with progesterone has previously been shown to decrease WNT expression in the ovine uterus[38].

Much remains to be explored in fibroid stem cells. Originally, fibroid stem cells were isolated using the Hoechst dye exclusion technique for side populations (SP)[30][39]. However, the SP technique is expensive, exhibits significant sensitivity to minor staining variations, and is detrimental to cell survival, making further study of fibroid cells difficult[40]. As a solution to these pitfalls, we recently reported a novel way of isolating fibroid stem cells using cell surface markers CD34 and CD49b[41].

Cell sorting using antibodies to these cell surface proteins revealed 3 distinct cell populations: CD34+/CD49b+, CD34+/CD49b-, and CD34-/CD49b- cells. CD34+/CD49b+ cells were highly enriched with stem cells whereas the other two groups did not contain any stem cells. Moreover, genes specific to stem cells, such as KLF4, NANOG, OCT4 were over expressed in the CD34+/CD49b+ cells further suggesting that these cells are indeed stem cells[41].

Interestingly, CD34+/CD49b- cells had intermediate levels of these stem cell factors compared to CD34-/CD49b- cells. Additionally, ER-alpha and PR were significantly under expressed in CD34+/CD49b+ cells, consistent with prior studies on SP, and CD34+/CD49b- cells again showed

intermediate expression levels between CD34+/CD49b+ and CD34-/CD49b- cells[41].

Taken together, these results led us to hypothesize that CD34+/CD49b+ cells are largely fibroid somatic stem cells, capable of asymmetric division allowing both self-renewal and the production of intermediary daughter cells, or CD34+/CD49b-cells, which ultimately develop into fully differentiated fibroid cells, or CD34-/CD49b- cells. An unbiased genome-wide investigation to better characterize the three populations on a molecular level is currently underway and will hopefully lead to new therapeutic targets.[Molly B. Moravek and Serdar E. Bulun., 2015].

CONCLUSIONS

Historically, the vast majority of fibroid research has focused on the role of steroid hormones in fibroid pathogenesis. The result of this work has been the development of medical treatment options targeting steroid hormones, such as GnRH agonists, aromatase inhibitors and anti-progestins. To date, we have not found a medical treatment for uterine fibroids that results in permanent tumor shrinkage or eradication, or that can be used long-term with minimal side effects. Finding an effective, long-term treatment for fibroids could have great public health implications, given their high prevalence and associated medical costs. The recent shift in focus from hormones to fibroid stem cells and genetic aberrations should lead not only to a deeper understanding of the specific etiology of fibroids, but also to the discovery of new therapeutic targets. Targeting the products of genetic mutations or fibroid stem cells has the potential to achieve both better control of current tumors and the prevention of the development of new fibroids.

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