MicroRNAs in fibroid biology: the lens for pathogenesis and functional significance?

Uterine fibroids or leiomyoma are the most common benign neoplasms of reproductive-aged women and have a lifetime incidence of approximately 60% to 70% in the general population. They confer an estimated total annual societal cost (medical/surgical care and loss of work days) of approximately $34.4 billion. Due to the significant morbidity associated with fibroids, this disease has become a major public health issue over the past decade and remains the leading indication for hysterectomy in the United States. Although uterine fibroids are among the most commonly observed and treated gynecologic disorders, there is no curative medical treatment, and the likelihood of development and risk for recurrence cannot be predicted.

The most commonly accepted etiology is the transformation of myometrial smooth muscle (comprising the majority of uterine muscle mass) fibroblasts and dysfunctional extracellular matrix (ECM) formation via aberrant transforming growth factor β (TGF-β) signaling. However, the specific functional role of genetic regulators in cellular transformation and hypertrophy is incompletely understood. With the recent focus of investigation on epigenetic causes of human disease, small (22–25 base pairs) noncoding RNAs, microRNAs (miRNA), have garnered mounting interest as therapeutic targets, and have been implicated as possible mediators of the pathobiology of uterine fibroids.

Marsh et al. (1) present findings from a well-designed laboratory study that investigates the functional significance of the miRNA 29 family (29a, 29b, and 29c) members in primary fibroid and myometrial cells. Specifically, these investigators assessed the effect of miRNA 29 overexpression and underexpression, respectively, via oligonucleotide transfection of miRNA mimics and inhibitors on major fibrillar collagen expression (types I, II, and III). After overexpression of miRNA 29b and miRNA 29c, a significant reduction of all major collagen types was observed compared to miRNA 29a in fibroid cells. Conversely, down-regulation of miRNA 29 family members resulted in increased collagen type 3 species. A distinct strength of this study is that the investigators validated their prior findings of miRNA 29 down-regulation in fibroid specimens (compared with myometrium) (2). Furthermore, their study used primary cultures of patient-matched myometrium and fibroids derived from surgical samples of a tissue repository.

It is noteworthy that this is the first study to report a functional role of a miRNA species in fibroid ECM production. This finding is of particular significance because it implies that the signature fibroid phenotype, aberrant ECM formation, may be due to epigenetic modifications of gene translation. The findings of this study raise several fundamental questions: [1] Does aberrant miRNA expression contribute to the transformation from myometrium (normal state) to a uterine fibroid (disease state)? [2] If aberrant miRNA expression plays a role in fibroid development, can miRNAs be targeted to prevent fibroid development and/or recurrence?

Approximately 2,000 distinct miRNA species have been identified and are estimated to regulate 30% to 50% of mRNA translation in mammals via either translational inhibition or mRNA degradation. However, investigation of the role of miRNAs in human disease has been challenging. A single miRNA species may have several hundred related or unrelated simultaneous mRNA targets and may impact multiple signaling pathways. It is difficult to confirm direct targeting by specific miRNA species even when the expression of a predicted target(s) changes after overexpression or underexpression of that particular miRNA. This point was thoughtfully raised by Dr. Marsh and colleagues, when they relayed that their findings do indicate a role for miRNA 29 in major collagen fibrillar expression, but future studies will be required to confirm the pathways by which the miRNA 29 family may mediate collagen gene and protein expression.

Also, it cannot be assumed that the biologic function of a specific miRNA is similar in different diseases and/or organs systems. This is especially true when interpreting the findings of studies that use predicted miRNA targets that have not been confirmed in the tissue/organ of interest. For example, miRNAs that target mediators of cell proliferation, ECM formation, or angiogenesis in cancer models may not have the same targets and/or biologic impact in fibroids. As a result, the findings of in vitro functional studies need to be cautiously interpreted as they may not be translatable to in vivo disease.

Novel approaches to develop miRNAs as noninvasive diagnostic and predictive biomarkers in cancer biology have been promising for improving clinical diagnosis and surveillance. The successful use of a miRNA-122 inhibitor to treat active hepatitis C infection in the first-ever human multicenter, randomized, placebo-controlled phase 3 trial has propelled the therapeutic application of miRNAs to the forefront of clinical medicine (3). However, in contrast to other areas of human disease, investigation of miRNAs as therapeutic targets in fibroids is in the incipient stage. There does not seem to be a unique serum or urine miRNA signature to distinguish patients with and without uterine fibroids. As a result miRNAs have not been established as biomarkers in this gynecologic disorder.

Our understanding of the functional role of miRNAs in leiomyoma has been guided by a limited number of studies with gene targets and end points adapted from cancer models. Two previous studies that used overexpression and underexpression have evaluated the functional significance of several miRNAs in fibroids. The findings of Chuang et al. (4) implicated the miR93/106b cluster as a regulator of interleukin 8 (*IL8*), connective tissue growth factor (CTGF), plasminogen activator inhibitor (PAI-1), and pentraxin-related protein (PTX3) through F3. Chuang et al. (5) also demonstrated that miRNA 200c is a regulator of epithelial to mesenchymal transition and tumorigenesis (*ZEB1/2* [zinc finger E-box-binding homeobox], *FBLN5* [fibulin 5]), cellular transition (E-cadherin), angiogenesis (*VEGF-A* [vascular endothelial growth factor A]), and matrix remodeling (*TIMP2* [tissue inhibitor

of metalloproteinase 2] and *FBLN5*). Although these studies provide insight into the possible role of miRNAs in the genesis of fibroids, they are not definitive. Future studies in murine and nonhuman animal models will be essential to defining the role of miRNAs in pathogenesis of fibroid development.

Congratulations to Dr. Marsh and colleagues for an expertly conducted seminal study evaluating the functional significance of the miRNA 29 family in the signature phenotypic characteristic of uterine fibroids. On review of this high-impact study and prior studies, it is evident that several areas of future investigation need to be prioritized. Firstly, it will be necessary to determine whether the miRNA 29 family and other miRNAs regulate the complex endocrine, paracrine, and autocrine genes that control the responsiveness of fibroids to sex steroids. Since TGF-β signaling has been a well-described regulator of ECM dysregulation, it will be necessary to investigate whether aberrant miRNA 29 expression regulates or is regulated by the TGF-β signaling pathway. As proof of concept, it will be crucial to assess whether miRNAs may be developed further as therapeutic targets to recapitulate the fibroid disease state in normal tissue and to induce regression of the fibroid disease state back to the normal state in both three-dimensional in vitro culture systems and animal models.

If miRNAs prove to be viable therapeutic targets, future studies will also need to determine whether the most feasible application will be for primary prevention before clinically evident disease or as secondary prevention to reduce the risk of recurrence after myomectomy. Although much investigation still needs to be done to evaluate the role of miRNAs in fibroid development, these small noncoding RNAs may provide the molecular lens to delineate epigenetic regulation of clinical disease.

Aaron K. Styer, M.D.
Vincent Center for Reproductive Biology, Vincent Department of Obstetrics and Gynecology, Massachusetts General Hospital, and Department of Obstetrics, Gynecology, and Reproductive Biology, Harvard Medical School, Boston, Massachusetts

http://dx.doi.org/10.1016/j.fertnstert.2016.05.017

You can discuss this article with its authors and with other ASRM members at
https://www.fertstertdialog.com/users/16110-fertility-and-sterility/posts/10904-micrornas-in-fibroid-biology-the-lens-for-pathogenesis-and-functional-significance

REFERENCES

1. Marsh EE, Parker JB, Wu J, Chakravarti D, Bulun SE. Decreased expression of microRNA-29 family in leiomyoma contributes to increased major fibrillar collagen production. Fertil Steril 2016;106:781–9.
2. Marsh EE, Lin Z, Yin P, Milad M, Chakravarti D, Bulun SE. Differential expression of microRNA species in human uterine leiomyoma versus normal myometrium. Fertil Steril 2008;89:1771–6.
3. Janssen HL, Reesink HW, Lawitz EJ, Zeuzem S, Rodriguez-Torres M, Patel K, et al. Treatment of HCV infection by targeting microRNA. N Engl J Med 2013;368:1685–94.
4. Chuang TD, Luo X, Panda H, Chegini N. miR-93/106b and their host gene, MCM7, are differentially expressed in leiomyomas and functionally target F3 and IL-8. Mol Endocrinol 2012;26:1028–42.
5. Chuang TD, Panda H, Luo X, Chegini N. miR-200c is aberrantly expressed in leiomyomas in an ethnic-dependent manner and targets ZEBs, VEGFA, TIMP2, and FBLN5. Endocr Relat Cancer 2012;19:541–56.