Decreased expression of microRNA-29 family in leiomyoma contributes to increased major fibrillar collagen production

Erica E. Marsh, M.D., M.S.C.I.,[a,b] Marissa L. Steinberg, M.D.,[a] J. Brandon Parker, Ph.D.,[a] Ju Wu, M.D.,[a] Debabrata Chakravarti, Ph.D.,[a] and Serdar E. Bulun, M.D.[a,b]

[a] Division of Reproductive Biology Research and [b] Division of Reproductive Endocrinology and Infertility, Department of Obstetrics and Gynecology, Feinberg School of Medicine, Northwestern University, Chicago, Illinois

Objective: To determine the expression and function of the microRNA-29 family (miRNA-29a, miRNA-29b, miRNA-29c) in human leiomyoma and myometrium.
Design: Basic science experimental design.
Setting: Academic medical center.
Patient(s): Women undergoing surgery for symptomatic uterine fibroids.
Intervention(s): Overexpression and knockdown of miRNA-29a, miRNA-29b, and miRNA-29c in primary leiomyoma and myometrial cells.
Main Outcome Measure(s): [1] Expression of the miRNA-29 family members in vivo in leiomyoma versus myometrium; [2] Major fibrillar collagen (I, II, III) expression in leiomyoma and myometrial cells with manipulation of miRNA-29 species.
Result(s): Members of the miRNA-29 family (29a, 29b, 29c) are all down-regulated in leiomyoma versus myometrium in vivo. The expression of the miRNA-29 family can be successfully modulated in primary leiomyoma and myometrial cells. Overexpression of the miRNA-29 family in leiomyoma cells results in down-regulation of the major fibrillar collagens. Down-regulation of the miRNA-29 species in myometrium results in an increase in collagen type III deposition.
Conclusion(s): The miRNA-29 family is consistently down-regulated in leiomyoma compared to matched myometrial tissue. This down-regulation contributes to the increased collagen seen in leiomyomas versus myometrium. When miRNA-29 members are overexpressed in leiomyoma cells, protein levels of all of the major fibrillar collagens decrease. The miRNA-29 members are potential therapeutic targets in this highly prevalent condition. (Fertil Steril® 2016;106:766–72. ©2016 by American Society for Reproductive Medicine.)
Key Words: Leiomyoma, fibroids, microRNA, collagen, extracellular matrix

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Leiomyomas, or fibroids as they are more commonly known, are benign uterine smooth muscle tumors that represent the most common tumor in reproductive-aged women. By the age of 50 years, these tumors have a prevalence of 60%–65% in white women and >80% in African-American women in the United States. Although they are clinically symptomatic in only 20%–40% of women who have them, most women who have symptoms from fibroids have multiple symptoms, which include heavy uterine bleeding, pelvic pain, infertility, and recurrent pregnancy loss (1). Due to the prevalence and the sequelae of these tumors, they continue to be the leading cause of hysterectomy in the United States and annually represent ≤$34 billion dollars in cost nationally (2). Despite the public health impact of these tumors, we are still early in our understanding of their pathogenesis.

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Reprint requests: Erica E. Marsh, M.D., M.S.C.I., Feinberg School of Medicine, Northwestern University, 676 North St. Clair, Suite 1845, Chicago, Illinois 60611 (E-mail: erica-marsh@northwestern.edu).

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The primary distinguishing factor between these tumors and their adjacent normal myometrial tissue is the abundance of extracellular matrix. Multiple gene and tissue microarrays have demonstrated that the matrix consists largely of the major fibrillar collagens (types I, II, and III) (3). Although advances have been made in understanding the pathophysiology of the growth of these tumors, there is still not a good understanding of the molecular basis of the extracellular matrix dysregulation seen in leiomyomas (4).

MicroRNAs (miRNAs) have been found to be novel regulators of fibrosis in many disease processes including liver fibrosis, lung fibrosis, and cardiac fibrosis. MicroRNAs are 20–25 nucleotide long noncoding RNAs that are involved in regulation of gene expression by translational repression (5). This repression is the result of either destruction of messenger RNA or destabilization and prevention of translation of messenger RNA. More than 3,000 miRNAs have been identified (6) and bioinformatic estimates place the number of miRNA target sites in the human genome at >45,000 (7). Furthermore, it is now speculated that >60% of human protein coding genes are regulated by miRNAs (7). We and other investigators have demonstrated that, in addition to differential gene expression between leiomyoma and myometrium, there is also a differential expression of miRNAs (8–10), suggesting that miRNAs play a role in gene regulation in these tumors. Although several studies have demonstrated that hormonal and growth factor regulation of miRNAs in leiomyomas alters cell proliferation (11), few have demonstrated a functional role for them in terms of extracellular matrix overproduction (12).

In previously published microarray analysis, we identified 81 differentially expressed miRNAs between leiomyomas and myometrial tissue. The miRNA-29b and miRNA-29c were identified as being among the most significantly down-regulated in leiomyoma versus myometrium (8). The down-regulation of these particular miRNAs is special as the miRNA-29 family of miRNAs have been implicated in fibrosis and in other disease processes including fibrosis after myocardial infarction (13), pulmonary fibrosis (14), and systemic sclerosis (15). Based on recent studies in other fibrotic diseases (16–20), we believe that miRNAs may play a functional role in the aberrant extracellular matrix components found in leiomyomas. Although a previous study has investigated miRNA-29b, to our knowledge, the entire miRNA-29 family has not been considered (21).

The goal of this project is to validate the differential expression of the entire miRNA-29 family (miRNA-29a, miRNA-29b, and miRNA-29c) in leiomyoma versus myometrium and to determine whether these miRNAs have a functional role in leiomyoma extracellular matrix pathogenesis. Based on miRNA microarray studies done by this laboratory and others, as well as studies done in other fibrotic diseases, we hypothesize that all the members of the miRNA-29 family will be down-regulated in leiomyoma versus myometrium. We further hypothesize that this down-regulation contributes to the increased collagen production in these tumors and that overproduction of the miRNA-29 species will lead to decreased collagen production in leiomyoma cells.

MATERIALS AND METHODS

Study Subjects

Uterine leiomyoma and matched myometrial tissue were collected from subjects (n = 20) undergoing hysterectomy for symptomatic uterine leiomyoma, and leiomyoma alone was collected from an additional 10 subjects. The subjects were all premenopausal women 27–49 years old, who were on no hormonal medications within 3 months of surgery and who were nonsmokers. All of the subjects gave written informed consent for participation in the study. The study protocol was approved by the Institutional Review Board of Northwestern University and all surgeries were performed at Northwestern Memorial Hospital.

Tissue Specimens

The resected tissue was collected in the operating room and taken directly to the pathology department where samples were provided to the research team within 1 hour of being removed from the subject. Leiomyoma ranged from 4–12 cm in greatest dimension and samples were routinely obtained at 1–2 cm from the outer capsule of the leiomyoma to avoid variation of findings due to location within the tumor. All of the fibroids were either subserosal or intramural. No submucosal fibroids were used in this study. Myometrium was collected from within 2 cm of the excised leiomyoma. The tissues were rinsed in cold phosphate-buffered saline (PBS) three times and were either flash frozen and stored at -80°C, cut into 2–3 mm^3 pieces, and placed in vials containing RNALater (Ambion) for nucleic acid preservation, or were immediately digested for primary cell isolation.

Nucleic Acid Isolation

Ribonucleic acid was isolated from either tissue or cells using the protocol previously described (8). Briefly, flash frozen tissue specimens were homogenized using a mortar and pestle and liquid nitrogen. The crushed tissue was allowed to incubate in Tri-Reagent (Sigma) for 5 minutes then mixed with one-fifth volume of chloroform. This mixture was then kept on ice for 15 minutes and then centrifuged at 14,000 rpm for 20 minutes. The resultant clear aqueous layer was transferred and mixed with an equal volume of isopropanol. The mixture was then incubated at 4°C for 10 minutes and then centrifuged at 14,000 rpm for 20 minutes. The liquid was removed from the vial, and the formed pellet was resuspended and 1 mL of 75% ethanol was added and centrifuged at 8,000 rpm for 10 minutes. The liquid was again removed and the pellet was allowed to air dry at room temperature for 5 minutes at which time it was resuspended in diethylpyrocarbonate-treated sterile water. The RNA purity and concentration were determined by spectrophotometry using the NanoDrop ND-1000 (NanoDrop Technologies).

Cell Culture

Surgical tissue specimens were rinsed in PBS and placed in a mixture of Hanks' balanced salt solution (HBSS) containing DNase at a concentration of 150 mg/mL and collagenase at