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Regulation of Cell Cycle Regulatory Proteins by MicroRNAs in Uterine Leiomyoma

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Abstract

The objective of this study was to determine whether miR-93, miR-29c, and miR-200c, which we previously reported to be downregulated in leiomyomas, target cell cycle regulatory proteins that influence cell proliferation. Based on TargetScan algorithm 3 cell cycle regulatory proteins namely, E2F transcription factor 1 (E2F1), Cyclin D1 (CCND1) and CDK2 which were predicted to be targets of these miRNAs were further analyzed. In 30 hysterectomy specimens, we found the expression of E2F1 and CCND1 messenger RNA (mRNA) was increased in leiomyoma as compared to matched myometrium, with no significant changes in CDK2 mRNA levels. There was a significant increase in the abundance of all 3 proteins in leiomyoma in comparison with matched myometrium. Using luciferase reporter assay, we demonstrated E2F1 and CCND1 are targets of miR-93 and CDK2 is a target of miR-29c and miR-200c. We confirmed these findings through transfection studies in which transfection of primary leiomyoma cells with miR-93 resulted in a significant decrease in the expression of E2F1 and CCND1 mRNA and protein levels, whereas knockdown of miR-93 had the opposite effect. Similarly, overexpression of miR-29c and miR-200c in leiomyoma cells inhibited the expression of CDK2 protein and mRNA, whereas knockdown of this microRNAs (miRNA) had the opposite effect. Transfection of miR-29c, miR-200c, and miR-93 in primary leiomyoma cells resulted in a time-dependent inhibition of cell proliferation and cell motility. These results collectively indicate that the 3 miRNAs known to be downregulated in fibroid tumors are critical in regulation of cell proliferation because of their effects on 3 key cell cycle regulatory proteins, which are overexpressed in uterine leiomyomas.

Keywords

microRNA, Leiomyoma, fibroids, cell proliferation

Introduction

The pathogenesis of leiomyomas, which are benign tumors affecting 40% to 70% of reproductive women, has been under intense investigation.[1-3] Multiple studies using either microarray or next-generation sequencing have demonstrated dysregulation of a number of protein coding genes involved in cell proliferation and apoptosis that are crucial to fibroid growth and progression.[3,4] MicroRNAs (miRNAs), which are small noncoding RNAs (20-22 nucleotides [nt]), are key posttranscriptional regulators of protein coding genes, and they exert their function through inhibition of translation or degradation of messenger RNA (mRNA).[5] Aberrant expression of miRNAs is associated with a wide range of disorders, including tumorigenesis and tissue fibrosis.[6,7] Previous studies, including our own high-throughput sequencing, identified the expression profile of a large number of miRNAs in leiomyoma and myometrium and provided support for altered expression and regulatory function of a number of them, including let 7, miR-21, miR-29, miR-200, and miR-25-93-106 cluster in leiomyoma and leiomyoma smooth muscle cells (LSMCs).[8-15] More recently, we reported on altered expression of a number of long noncoding RNAs (lncRNAs) that are longer than 200 nt in leiomyomas.[16] Some lncRNAs regulate the expression of miRNAs acting as a sponge.[17]

Our previous work demonstrated a decrease in the expression of miR-29c in leiomyoma which primarily regulates extracellular matrix (ECM)-related genes and epigenetic-related enzymes DNMT3A.[12] Others have reported similar findings with miR-29b, which is also downregulated in fibroids.[15] We also reported a decrease in the expression of miR-200c in leiomyoma[14] and in SKLMS-1, a leiomyosarcoma cell line,[18] compared with normal myometrium. MiR-200c is a key miRNA regulating epithelial to mesenchymal transformation (EMT),[11,14,19] and it has been associated with a whole host of

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cancer types.[14,20-24] Similar to the pattern of miR-29c and miR-200c expression, we reported that miR-93 levels are also downregulated in leiomyoma with an associated increase in its target namely Interleukin-8.[13] The objective of this study was to determine whether the 3 miRNAs (miR-29c, miR-200c, and miR-93) whose expression are downregulated in leiomyoma target cell cycle–associated regulatory proteins, thereby influencing leiomyoma cell proliferation. We selected to investigate 3 cell cycle–associated proteins that are predicted targets of miR-29c, miR-200c, and miR-93 based on TargetScan algorithm. As such, we focused on cyclin-dependent kinase 2 (CDK2), which is a member of serine/threonine protein kinases, which, upon binding to cyclin E, is required for the transition of the cell from G1 phase to the S phase of the cell cycle.[25] Based on TargetScan, CDK2 is predicted to be a target of miR-29c and miR-200c. Cyclin-dependent kinase 2 has been shown to be a target of miR-200c and miR-29 in other cell types.[18,26-28] The next protein of interest was cyclin D1, which in human is encoded by the CCND1 gene.[29] Cyclin D1 is synthesized during the G1 phase of the cycle and forms a complex with CDK4/6, thereby regulating progression of the cell from the G1 phase into the S phase of the cell cycle.[29,30] Cyclin D1 has been shown to be a target of miR-93 in HL-1, a cardiac muscle cell line.[31] E2F1 is a member of the E2F transcription factor family which plays a key role in G1 to S phase transition. This transcription factor binds to several cell cycle regulatory proteins, including the retinoblastoma family and cyclin A/CDK2 complexes.[32] E2F1 is targeted by miR-93 in osteosarcoma cells[33] and hepatocellular carcinoma.[34] Based on TargetScan, both E2F1 and CCND1 are predicted targets of miR-93. In this present study, we determined the expression of these 3 key cell cycle regulatory proteins (CCND1, E2F1, and CDK2) in leiomyomas and compare it to matched myometrium. Our objective was to determine if these cell cycle regulatory proteins are targeted by these 3 miRNAS (miR-93, miR29c, and miR200c) all of which are downregulated in fibroids.

Materials and Methods

Tissue Collection and Leiomyoma and Myometrial Smooth Muscle Cell Isolation

With prior approval from institutional review board (#036247), portions of uterine leiomyoma and matched myometrium were collected from patients (n = 30) who were scheduled to undergo hysterectomy at Harbor-UCLA Medical Center. The patients' age ranged from 27 to 53 years (median = 43 ± 7.1), and they were not taking any hormonal medications for at least 3 months prior to surgery. All leiomyomas used in this study were 2 to 5 cm in diameter. Tissues were snap frozen and stored in liquid nitrogen for further analysis or used for isolation of LSMCs as previously described.[12,13,35] Briefly, LSMCs were cultured in Dulbecco Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum until reaching confluence with a change of media every 2 to 3 days. Cells at passages p1 to p3 were used for all experiments. Cell culture experiments were performed at least 3 times using LSMCs obtained from different patients. All supplies for isolation and cell culture were purchased from Sigma-Aldrich (St Louis, Missouri) and Fisher Scientific (Atlanta, Georgia).

Gain or Loss of Function of miR-93, miR-200c, and miR-29c

Leiomyoma smooth muscle cells were seeded at a cell density of 3.5×10^4/well in 6-well plates and at subconfluence transfected with 50 nM of pre-miR-93, pre-miR-200c, and pre-miR-29c, anti-miR-93, anti-miR-200c, anti-miR-29c, pre-miR negative control (NC), or anti-miR negative control (aNC) (Applied Biosystems, Foster city, California) for 24 to 96 hours using PureFection transfection reagent (System Biosciences, Inc, Mountain View, California), according to the manufacturer's protocol.

RNA Isolation and Quantitative Real time-Polymerase Chain Reaction (RT-PCR) Analysis

Total RNA was extracted from LSMCs using TRIzol (Thermo Fisher Scientific, Waltham, Massachusetts), and their quantity and quality were determined (ND-1000 Spectrophotometer; NanoDrop Technologies, Wilmington, Delaware) as previously described.[16,36,37] Subsequently, 1 μg was reverse transcribed using random primers according to the manufacturer's guidelines (Applied Biosystems, Carlsbad, California). Primers for E2F1, CCND1, and CDK2 detection were designed by Primer Express Software (Applied Biosystems, Foster city, CA). Quantitative RT-PCR was performed using SYBR gene expression master mix (Applied Biosystems). Reactions were incubated for 10 minute at 95°C followed by 40 cycles for 15 seconds at 95°C and 1 minute at 60°C. The mRNA levels were determined using the Invitrogen (Carlsbad, CA) StepOne System with FBXW2 used for normalization.[38] All reactions were run in triplicate, and relative expression was analyzed with the comparative cycle threshold method ($2^{-\Delta\Delta CT}$) according to the manufacturer (Applied Biosystems, Foster city, CA). Values were expressed as fold-change compared to the control group. The primer sequences used were as follows: E2F1 (sense, 5′-GGACTCTTCGGAGAACTTTCAGATC-3′; antisense, 5′-GGGCACAGGAAAACATCGA-3′); CCND1 (sense, 5′-GCCCTCTGTGCCA CAGATGT-3′; antisense, 5′-CCCC GCTGCCACCAT-3′); CDK2 (sense, 5′-TTCCCCTCATCAA-GAGCTATCTGT-3′; antisense, 5′-ACCCGATGAGAATGG-CAGAA-3′); and FBXW2 (sense, 5′-CCTCGTCTCTAAAC AGTGGAATAA-3′; antisense, 5′-GCGTCCTGAACAGAAT-CATCTA-3′).

Immunoblotting

Total protein isolated from leiomyoma and paired myometrium and LSMCs transfected with pre-miR-93, pre-miR-200c, pre-miR-29c, anti-miR-93, anti-miR-200c, anti-miR-29c, pre-miR

negative (NC), and aNC were subjected to immunoblotting as previously described.[37,39] Specific antibodies generated against E2F1 (sc-193; Santa Cruz Biotechnology, Dallas, Texas), CCND1 (60186-1-Ig; Proteintech Group, Inc, Chicago, Illinois), and CDK2 (sc-6248; Santa Cruz Biotechnology) with concentration of 1:1000, 1:000, and 1:250, respectively, were used to detect specific protein expression. The membranes were also stripped and probed with Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) antibody (Santa Cruz Biotechnology) to serve as loading control. The band densities were normalized by GAPDH using ImageJ program (http://imagej.nih.gov/ij/), and they are expressed as a ratio relative to the control group designated as 1.

Luciferase Reporter Assays

Leiomyoma smooth muscle cells were seeded in 6-well plates until reaching subconfluence and transiently cotransfected with 50 nM pre-miR-93, pre-miR-200c, pre-miR-29c oligonucleotides, or NC and a luciferase reporter plasmid (1 μg/well) containing 3′-untranslated region [UTR] sequences for E2F1, CCND1, or CDK2 (GeneCopoeia, Inc, Rockville, Maryland) using PureFection transfection reagent. Firefly and Renilla luciferase activities were measured after 48 hours of transfection using the dual-luciferase reporter assay system (Promega, Madison, Wisconsin). Firefly luciferase activity was normalized to Renilla luciferase activity, and the level of induction was reported as mean ± standard error of the mean (SEM) of 3 experiments performed in duplicate and compared with a ratio in cells transfected with NC independently set at 1.

Cell Proliferation Assay

Leiomyoma smooth muscle cells were seeded at 1000 cells/well in 96-well plates and cultured for 48 hours. The cells were then transfected with 50 nM pre-miR-93, pre-miR-200c, and pre-miR-29c oligonucleotides or NC as described above. The rate of cell proliferation was determined using the MTT assay and cells were photographed. Briefly, 3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide (MTT), (Sigma, St Louis, MO) was added to the culture medium at a final concentration of 1 mg/mL and was incubated for 2 hours at 37°C. The medium was aspirated, and the formazan product was solubilized with dimethyl sulfoxide and the absorbance at 570 nm was determined and subtracted from the absorbance at 630 nm (background) for each well. The assay was performed in 6 replicates per condition, and it was repeated 3 times.

Cell Motility Assay

The cell motility activity was determined using Radius 24-well assay kit (Cell Biolabs, San Diego, California) consisting of a circular biocompatible gel in each well according to the manufacturer's instructions. Briefly, LSMCs were seeded in the assay plates and were cultured for 48 hours and then were transfected with pre-miR-93, pre-miR-29c, pre-miR-200c, and NC as described above. After 72 hours of incubation, the biocompatible gels were removed, and the cells were incubated for additional 21 hours, and the images of migratory cells were captured using an Olympus IX70 microscope equipped with digital camera (Olympus Inc, Melville, New York).

Statistical Analysis

Wherever appropriate, the results were reported as mean ± SEM and analyzed by PRISM software (GraphPad, San Diego, California). Data set normality was determined by the Kolmogorov-Smirnov test. Comparisons involving 2 groups were analyzed using Student *t* tests. For comparisons involving multiple groups, analysis of variance followed by a Tukey honest significance difference (HSD) test for post hoc pairwise analysis was conducted. Statistical significance was established at $P < .05$.

Results

Initially, we determined the abundance of CCND1, E2F1, and CDK2 mRNA and protein in leiomyoma and matched myometrium in 30 hysterectomy specimens in subjects on no hormonal medications. As shown in Figure 1A, the expression of E2F1 and CCND1 mRNA was increased in leiomyoma as compared to matched myometrium; however, no significant changes were observed in CDK2 mRNA levels. Representative immunoblots and mean protein levels for CCND1, E2F1, and CDK2 are shown in Figure 1B. As demonstrated in Figure 1B, there was a significant increase in the abundance of all 3 proteins in leiomyoma as compared to matched myometrium. The differential expression of E2F1, CCND1, and CDK2 is independent of race and menstrual cycle phase (data not shown).

We then determined whether the 3 cell cycle regulatory proteins are targets of miR-93, miR-29c, and miR-200c in leiomyomas using the luciferase assay (Figure 2). Figure 2A demonstrates the sequence alignment of seed regions of miRNAs with their target genes at the 3′-UTR region. In Figure 2B, we demonstrate that both E2F1 and CCND1 are targets of miR-93 in leiomyoma cells because overexpression of miR-93 in these cells reduced the luciferase activity of E2F1 and CCND1. Overexpression of miR-29c and miR-200c reduced the luciferase activity of CDK2, indicating miR-29c and miR-200c target CDK2 in leiomyoma cells (Figure 2C).

Upon establishing as to which cell cycle regulatory protein is regulated by which miRNA, we proceeded to over- and underexpress the miRNA of interest in leiomyoma cells through transfection of LSMCs with pre-miR or antisense oligo, respectively; then, we determined their effects on the expression of target mRNAs (Figure 3A and B) or protein (Figure 3C). As shown in Figure 3A and supporting the results from the luciferase assay (Figure 2), overexpression of miR-93 resulted in decreased expression of E2F1 and CCND1 mRNA

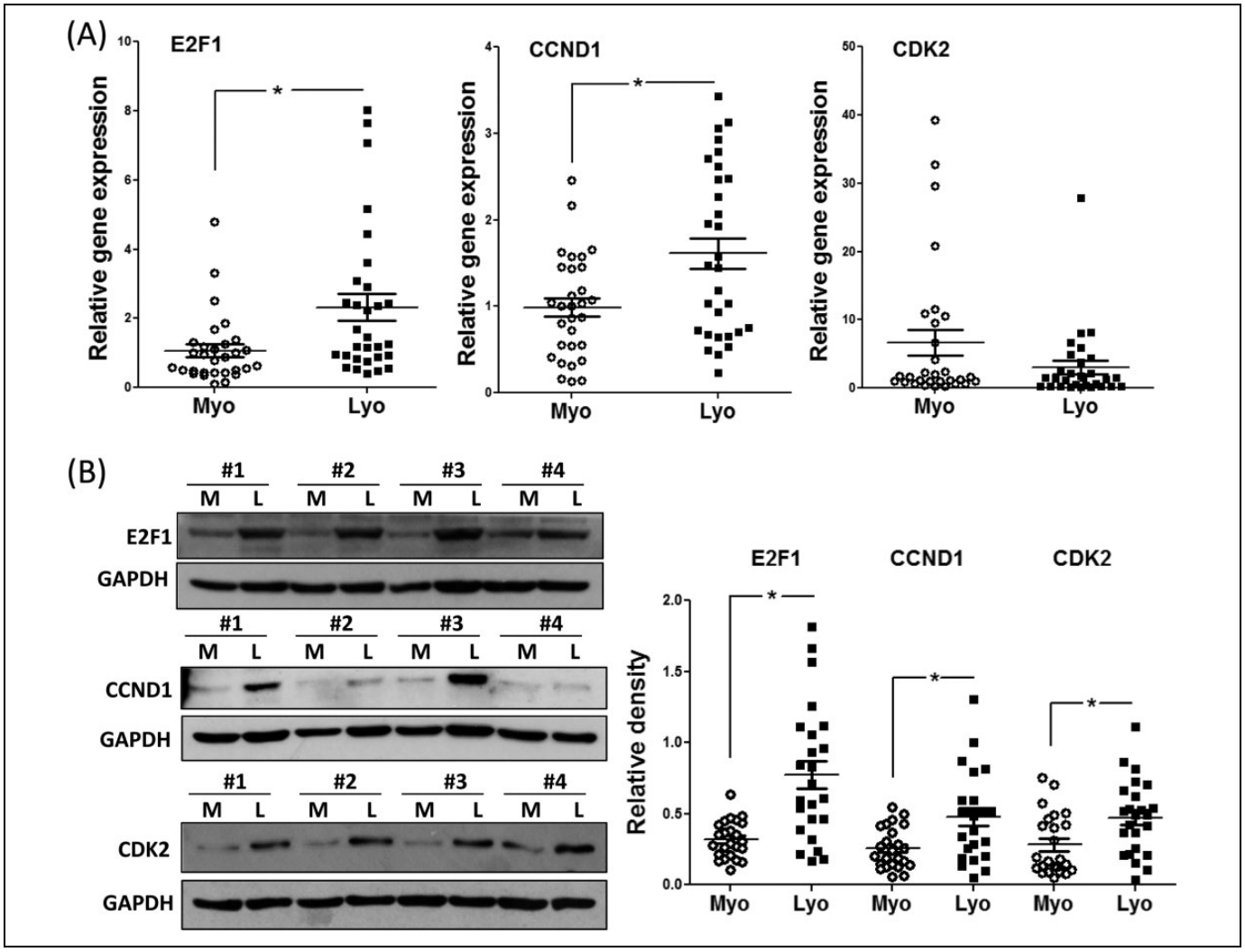

Figure 1. A, Relative expression of E2F1, CCND1, and E2F1 messenger RNA (mRNA) in leiomyoma (Lyo) and matched myometrium (Myo; n = 30). B, Representative immunoblots for E2F1, CCND1, and cyclin-dependent kinase 2 (CDK2) in paired myometrium (M) and leiomyoma (L). Bar graph shows relative protein band densities (N = 24) in myometrium (Myo) and leiomyoma (Lyo). Results are presented as mean ± SEM and analyzed using paired Student *t* test. *$P < .05$.

levels (Figure 3A), whereas knockdown of miR-93 had the opposite effect (Figure 3B). Similarly, overexpression of miR-200c and miR-29c as expected resulted in decreased expression of CDK2 mRNA (Figure 3A), whereas knockdown of miR-200c and miR-29c had the opposite effect (Figure 3B). Protein data (Figure 3C) from the transfection studies were in line with mRNA data. As shown in Figure 3C, overexpression of miR-93 resulted in decreased E2F1 and CCND1 protein levels, whereas anti-miR-93 transfection induced E2F1 and CCND1 protein levels. These results were confirmed by transduction of lentivirus that contained doxycycline-inducible miR-93 insert (data not shown). Transfection of pre-miR-29c and pre-miR-200c led to reduced CDK2 protein levels, whereas anti-miR-29c and anti-miR-200c had the opposite effect (Figure 3C).

The significance of miR-29c, miR-200c, and miR-93 in regulating leiomyoma cell proliferation and motility is demonstrated in Figure 4. Transfection of pre-miR-29c, pre-miR-200c, and pre-miR-93 in primary leiomyoma cells resulted in a time-dependent inhibition of cell proliferation as measured by the MTT assay (Figure 4A), with a more profound inhibitory effect in response to miR-29c and miR-200c transfection as compared with miR-93. Upregulation of these 3 miRNAs inhibited cell motility as determined by cell motility assay as shown in Figure 4B to D.

Discussion

Our results indicate that the miRNAs miR-93, miR-200c, and miR-29c, which were previously demonstrated to be downregulated in leiomyomas, target selectively key cell cycle regulatory proteins including CDK2, CCND1, and E2F1. Using the luciferase assay, we confirmed that miR-29c and miR-200c target CDK2 and miR-93 targets E2F1 and CCND1. As a result

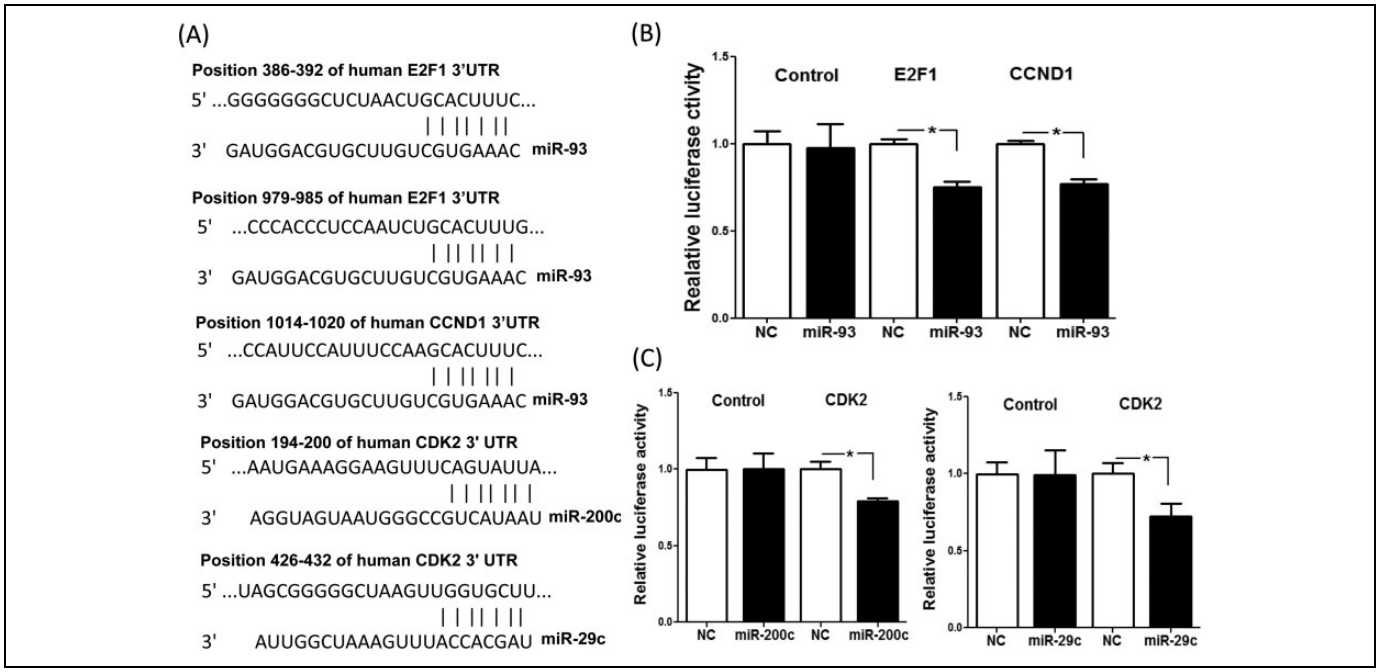

Figure 2. A, The sequence alignment of seed regions of microRNAs (miRNAs) with their target genes at the 3′-UTR region. B, Relative luciferase activity in isolated leiomyoma smooth muscle cells (LSMCs) cotransfected with Renilla and firefly luciferase reporter carrying a 3′-UTR fragment of E2F1, CCND1, cyclin-dependent kinase 2 (CDK2), pre-miR-93, pre-miR-200c, pre-miR-29c, or control oligonucleotides (NC) for 48 hours. Relative luciferase activity is presented as the ratio of Firefly:Renilla as compared to NC, which was independently set as 1. Results are presented as mean ± SEM of at least 3 independent experiments with *P* values (*$P < .05$) indicated by corresponding lines.

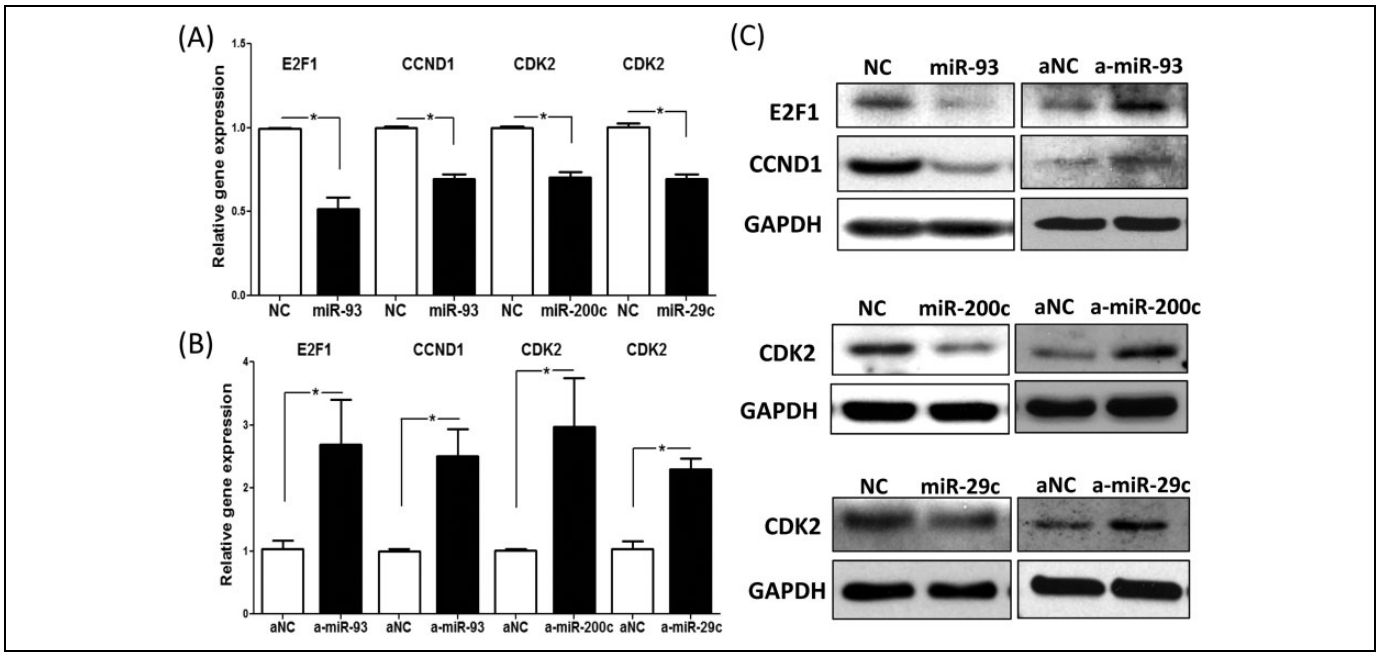

Figure 3. Quantitative RT-PCR analysis of E2F1, CCND1, and cyclin-dependent kinase 2 (CDK2) messenger RNA (mRNA) expression in leiomyoma smooth muscle cells (LSMCs) following transfection with pre-miR-93 (miR-93, for E2F1 and CCND1), pre-miR-200c (miR-200c, for CDK2), pre-miR-29c (miR-29c, for CDK2), and control pre-miR oligonucleotides (NC) (A) or anti-miR-93 (a-miR-93, for E2F1 and CCND1), anti-miR-200c (a-miR-200c, for CDK2), anti-miR-29c (a-miR-29c, for CDK2), and control anti-miR oligonucleotides (aNC) (B) for 72 hours. Results are presented as mean ± SEM of at least 3 independent experiments with *P* values (*$P < .05$) indicated by corresponding lines. C, Western blot analysis of E2F1, CCND1, and CDK2 following transfection of LSMCs with control pre-miR oligonucleotides (NC), pre-miR-93 (miR-93, for E2F1 and CCND1), pre-miR-200c (miR-200c, for CDK2), pre-miR-29c (miR-29c, for CDK2) or control anti-miR oligonucleotides (aNC), anti-miR-93 (a-miR-93, for E2F1 and CCND1), anti-miR-200c (a-miR-200c, for CDK2), anti-miR-29c (a-miR-29c, for CDK2) for 96 hours. Results are representative of at least 3 independent cell preparations.

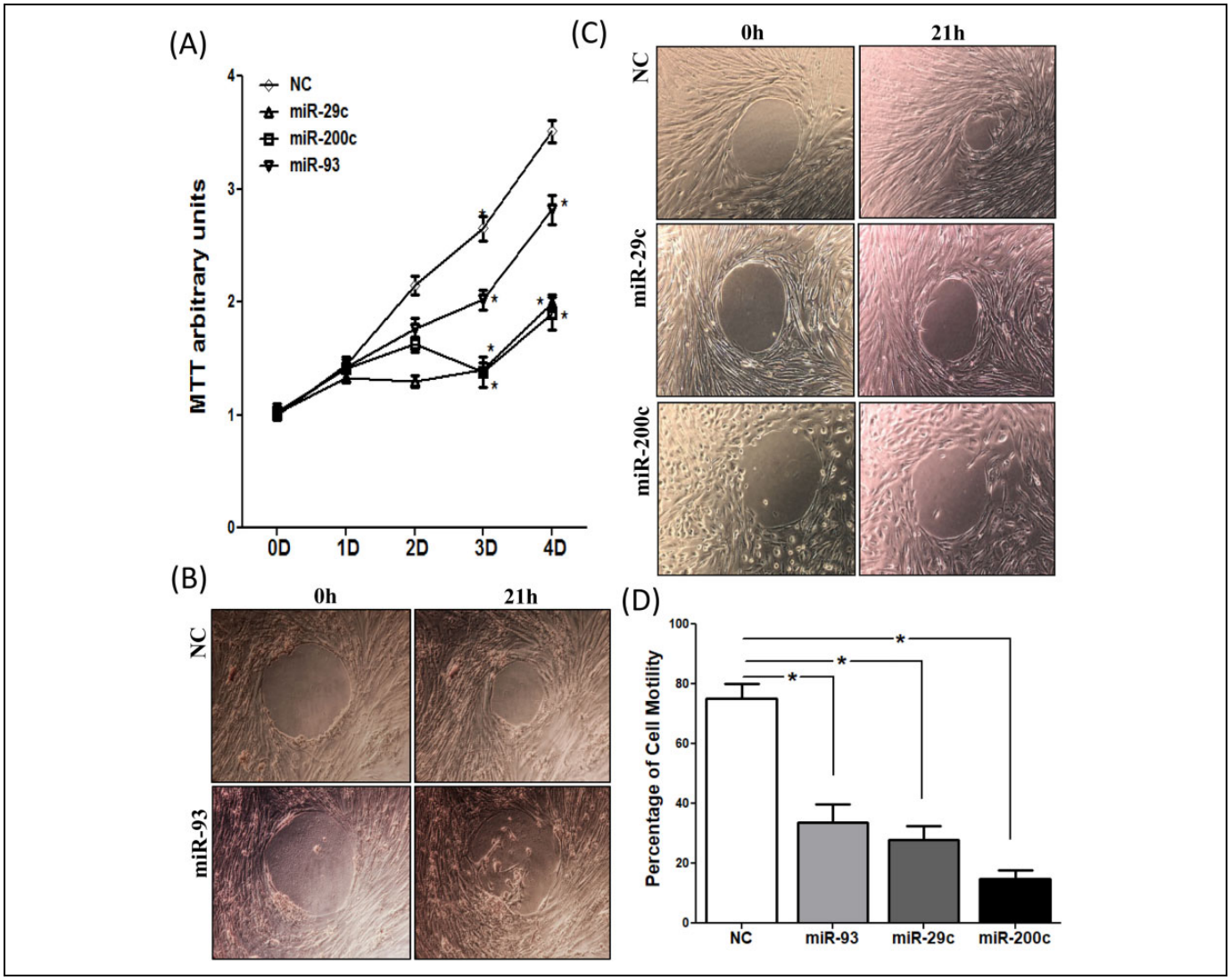

Figure 4. A, The rate of cell proliferation was determined after 4 days transfection of pre-miR-93, pre-miR-200c, pre-miR-29c, and control pre-miR oligonucleotides (NC) in leiomyoma smooth muscle cells (LSMCs) by MTT assay on the indicated days, with culture media changed every 2 days. Results are presented as mean ± SEM of 6 independent experiments. *$P < .05$. B and C, Photomicrographs of LSMCs transfected with pre-miR-93 (B), pre-miR-29c (C), pre-miR-200c (C) or the corresponding negative controls (NC) for 72 hours. The LSMC motility was determined after the biocompatible gels were removed for 21 hours. Motility assays were performed, in triplicates, using 3 independent cell preparations. D, Quantification of cell motility was analyzed by ImageJ and shown as mean ± SEM with *P* values (*$P < .05$) as compared to NC.

of downregulation of these miRNAs in leiomyoma, their target proteins, that is, CDK2, CCND1, and E2F1, were upregulated as demonstrated in this study. The significance of this group of miRNAs in leiomyoma cell proliferation was demonstrated through transfection of these miRNAs in isolated leiomyoma cells that resulted in a time-dependent decrease in cell proliferation and inhibition of cell motility as would be expected.

The regulation of cell cycle is highly complex and it is regulated through interaction of cyclins that form the regulatory subunit and CDKs, which are the catalytic subunits of an activated heterodimers.[40] CDKs, through phosphorylation of target proteins, can activate or deactivate target proteins that control entry of cell into the next phase of cell cycle.[40] In response to growth factors, cyclin D1 is produced, which binds to CDK4 or CDK6. Then, this complex phosphorylates the retinoblastoma susceptibility protein (Rb) that dissociates from the E2F/Rb complex, resulting in activation of E2F. The E2F family are transcription factors that are crucial in control of cell cycle.[41] Activation of E2F induces transcription of genes, such as Cyclin E, Cyclin A, DNA polymerase, and thymidine kinase.[42] Cyclin E binds to CDK2 enabling transition of cell from the G1 to S phase.[40] G1 to S phase transition point is also known as a restriction point in the cell cycle and is a rate-limiting step in the cell cycle.[40] Our results indicating increased expression of CDK2, E2F1, and cyclin D1 protein in fibroid tumors would suggest that there is an enhanced transition of fibroid cells from the G1 to S phase, which is consistent with the cell proliferation data presented. In contrast to EDF1 and

CCND1, in case of CDK2, we did not find any changes in mRNA expression. This may be because the miRNAs targeting CDK2 may exert their effect through inhibition of protein translation rather than mRNA degradation.[43]

The miR-29 family, which consists of miR-29a, miR-29b, and miR-29c, share a common seed sequence with largely overlapping sets of predicted target genes; however, their differential expression and regulation suggest unique functional activities.[44] This family of miRNAs is downregulated in various fibrotic disorders, and key ECM genes, including collagen subtypes and elastin, are targets of miR-29 regulatory function.[37,45] Similar to our findings with miR-29c in fibroids,[12] a recent report demonstrated reduced expression of miR-29b in leiomyoma as compared with matched myometrium, and restoring miR-29b expression in isolated LSMCs implanted in subrenal xenograft in a mouse model resulted in inhibition of ECM accumulation and rate of cell proliferation.[15] MiR-29 family, as in fibroid tumors, is also downregulated in a number of malignancies including gastric,[46,47] breast,[48] osteosarcoma,[49,50] and lymphomas.[51,52] In gastric cancer cells, similar to our results in leiomyoma cells, enforced expression of miR-29a-3p inhibited the expression of CDK2, CDK4, and CDK6 and inhibited cell proliferation and cell migration.[47] MiR-29b was shown to regulate cyclin D1 expression in adipocytes, and it is critical for adipogenic differentiation.[53] Similarly, miR-29b was reported to inhibit intestinal mucosal growth by repressing CDK2 translation[27] and targeted CDK6 in mantle cell lymphoma.[52]

The miR-200 family is normally expressed in cells of epithelial origin and not in fibroblasts of mesenchymal origin.[54,55] This family of miRNAs suppresses tumors by inhibiting epithelial to mesenchymal transition.[19] The role of miR-200 in EMT and tumor progression has been linked to bladder,[24] breast,[23] ovarian,[22] pancreatic,[21] melanoma,[56] and stomach.[57] Our own work showed downregulation of this miR-200c in leiomyomas, which was race dependent, with tumors from African Americans expressing lower levels of miR-200c in leiomyomas compared to Caucasians.[14] In comparison with isolated leiomyoma cells, the expression of miR-200c was also lower in SKLMS-1, a leiomyosarcoma cell line.[18] In this cell line, miR-200c was shown to target CDK2 and CCNE2, and its overexpression led to increased caspase 3/7 activity, thereby increasing apoptosis and reducing cell proliferation and migration, similar to our findings here with primary leiomyomas cells. Similar to leiomyoma cells, others have reported that miR-200c targets CDK2 and suppresses tumorigenesis in renal cell carcinoma[26] and that miR-200b inhibits cell migration and invasion in non-small cell lung carcinoma cells by targeting FSCN1.[58]

As with miR-29c and miR-200c, the expression of miR-93 is reduced in leiomyomas; however, its host gene MCM7, a member of DNA helicases with a central role in initiation of DNA replication and cell cycle progression, is significantly increased in leiomyomas.[13] Here, we demonstrate that miR-93 directly targets E2F1 and CCND1. The role of miR-93 in regulation of cell cycle–associated proteins and cell proliferation is cell and context dependent because, in contrast to leiomyoma cells, overexpression of miR-93 in gastric cancer cells activated CDK2 and aided in G1/S cell transition.[59] In contrast to our findings in leiomyomas cells, Hazarika et al showed that overexpression of miR-93 in human umbilical vein cells (HUVEC) cell and CD2C12, a skeletal cell line, stimulated cell proliferation, and miR-93 and E2F1 levels were inversely related.[60]

In summary, leiomyomas are characterized by downregulation of miR-29c, miR-200c, and miR-93, as a result of which there is upregulation of their target cell cycle proteins, including CCND1, CDK2, and E2F1. Here, we show that miR-93 directly targets E2F1 and CCND1, and miR-29c and miR-200c target CDK2. These cell cycle proteins are key regulators of G1/S transition, which is a rate-limiting step of the cell cycle. The physiological significance of our findings is underscored by our data showing upregulation of miR-29c, miR-200c, and miR-93 in leiomyoma cells decreases cell proliferation and motility. Potential therapies that could correct the dysregulation of miR-29c, miR-200c, and miR-93 in leiomyomas restoring them to normal levels could potentially hold promise in inhibiting leiomyoma cell proliferation and fibroid progression.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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