MicroRNA-34a Modulates Cytoskeletal Dynamics through Regulating RhoA/Rac1 Cross-talk in Chondroblasts*

Received for publication, May 25, 2011, and in revised form, February 3, 2012. Published, JBC Papers in Press, February 20, 2012, DOI 10.1074/jbc.M111.264382

Dongyun Kim†1, Jinsoo Song‡1, Sunhyo Kim‡, Hyang Mi Park‡, Churl-Hong Chun‡, Jongkyung Sonn‡, and Eun-Jung Jin

From the †Department of Biological Sciences, College of Natural Sciences, Wonkwang University, Iksan, Chunbuk 570-749, Korea, the ‡Department of Orthopedic Surgery, Wonkwang University School of Medicine, Iksan, Chunbuk 570-749, Korea, the §Department of Biological Sciences, College of Natural Sciences, Kyungpook National University, Daegu 730-090, Korea, and ¶National Institute of Crop Science, RDA, Suwon 441-857, Korea

This article has been withdrawn by the authors. The control Alcian blue panel in Figs. 1A and 3D was reused in Fig. 3G as STSN. The GAPDH immunoblot in Fig. 1B was reused from Kim, D., et al. (2010) J. Biol. Chem. 285, 26900-26907. The GAPDH immunoblot in Fig. 3B was reused in Kim, D., et al. (2011) Biochem. Biophys. Res. Commun. 415, 551-557. In Fig. 3C, the mir-34a and RhoA/mir-34a images are the same. The RhoA/anti-mir-34a image in Fig. 3D was reused from Kim, D., et al. (2010) J. Biol. Chem. 285, 26900-26907. The control image in Fig. 3G was reused in Kim, D., et al. (2009) Exp. Mol. Med. 41, 656-664. The Type II collagen and Hsp70 immunoblots in Fig. 4B were reused in Kim, D., et al. (2011) Exp. Mol. Med. 43, 455-461.

Background: JNK signaling involved in regulation of chondrogenic differentiation contributes modulation of miR-34a.

Results: JNK signaling modulates miR-34a level and regulates stress fiber formation in chondroblasts.

Conclusion: miR-34a regulates RhoA/Rac1 cross-talk and negatively modulates the actin cytoskeleton reorganization during chondrogenesis.

Significance: This study provides new insights into understanding the regulatory role of miR-34a in the process of chondrogenesis.
The Role of miR-34a in Chondroblasts

the regulation of cartilage formation. Recently, a few studies have suggested that the JNK signaling pathway (also known as the stress-activated pathway) is involved in the differentiation of articular chondrocytes (13, 14). However, the results from these studies are contradictory. In articular chondrocytes, Wnt-3a caused dedifferentiation of chondrocytes by up-regulating c-Jun expression and the JNK-mediated phosphorylation of c-Jun, resulting in activation of the c-Jun/activator protein (14). In contrast, treatment with transforming growth factor-β superfamily members promoted cartilage-specific gene expression during in vitro chondrogenic differentiation of mesenchymal progenitor cells from bone marrow and trabecular bone through activation of p38, ERK1, and JNK (15). In addition, although JNK signaling appears to be involved in chondrogenic differentiation, the precise pathways and their effects have not yet been fully elucidated.

MicroRNAs (miRNAs)3 are evolutionarily conserved small non-coding RNAs that regulate gene expression and play important roles in diverse biological functions, including cell differentiation, tumorigenesis, apoptosis, and metabolism (16–20). For miRNA biogenesis, the miRNA-encoding genes are transcribed mainly by RNA polymerase II as long primary transcripts, which are then processed by the nuclear RNase, Drosha, to produce precursor miRNAs that are subsequently exported to the cytoplasm. The precursor miRNAs are further processed into mature miRNAs by the cytoplasmic RNase, Dicer (21). Functionally, miRNAs recognize and bind to partially complementary sites in the 3′-UTRs of their target mRNAs, resulting in either translational repression or degradation of the mRNAs (22). However, although miRNAs are involved in a wide variety of biological functions, relatively little is known regarding the regulation of miRNA expression.

Dicer, an essential enzyme in the miRNA pathway, is known to be involved in both cell proliferation and differentiation (23), suggesting a possible importance of miRNA in limb development. Recent studies have shown that miRNAs are also important for tissue morphogenesis and several miRNAs, including lin-4, lin-7, and miR-196, have been shown to play roles in limb development. In particular, miR-196 is thought to be involved in specifying hind limb development (24). However, it is not yet known which miRNA(s) could be the key player(s) in limb development. In this study, we show that miR-34a is a key modulator of cytoskeletal dynamics through interaction between RhoA and Rac1 during chondrogenesis.

EXPERIMENTAL PROCEDURES

Cell Culture and Treatments—Mesenchymal cells derived from the distal tips of Hamburger-Hamilton (HH) stage 22/23 embryo leg buds of fertilized White Leghorn chicken eggs were micromass cultured as described previously (10). Briefly, the cells were suspended at a density of $2 \times 10^7$ cells/ml in Ham’s F-12 medium containing 10% fetal bovine serum (FBS), 100 IU/ml penicillin and 100 μg/ml streptomycin (Invitrogen). The cells were plated in three drops (15 μl each) dispensed into 35-mm culture dishes or 19 drops (15 μl each) into 60-mm Corning culture dishes and incubated for 1 h at 37°C under 5% CO2 to allow attachment. The cells were maintained in 1 ml of culture medium in the absence or presence of 3 μg/ml JNK inhibitor II (Calbiochem) or 10 μM staurosporine (STSN; Sigma).

For dedifferentiated chondrocyte, after 7 days of culture, the cells were dissociated with 0.25% trypsin, 1.0 mM EDTA, and collagenase and replated at a density of $1 \times 10^6$ cells/cm2. These cells were subcultured three more times at 2–4-day intervals. Then cells were seeded at 5000 cells/cm2 onto uncoated or chitosan-coated 35-mm culture dishes. The culture medium was changed 24 h after cell seeding and every 48 h thereafter.

Analysis of Cell Condensation and Differentiation—Chondrogenic differentiation was measured by Alcian blue staining of sulfated cartilage glycosaminoglycans. To demonstrate the deposition of cartilage matrix proteoglycans, representative cultures were collected at 48 h of incubation and stained with 0.5% Alcian blue solution. Alcian blue bound to sulfated glycosaminoglycans. The extraction with 6 M guanidine HCl and quantification of the Alcian blue stain (by the absorbance of the extracts at 600 nm) was used as a specific marker for cartilage formation. Briefly, cultures were washed with PBS, then methanol/acetone (1:1) was added and incubated with 100 μg/ml CellTiter-Glo reagent. Bound PA was visualized using the ChemiDoc XRS+ and DAB substrate solution kit (Vhandam Markers, Burlingame, CA).

Cell Viability—Cell viability was assayed using CellTiter-Glo luminescent cell viability assay kit (Promega) based on quantitation of ATP present in metabolically active cells or viable cells (15). An equal volume of reconstituted CellTiter-Glo reagent was added to 100 μl of cell suspension. The contents were mixed on an orbital shaker for 2 min to induce cell lysis and incubated at room temperature for 10 min to stabilize the luminescent signal, and the luminescence was recorded (15).

Western Blot Analysis—Cells were lysed in buffer (50 mM Tris-HCl, pH 7.4, containing 150 mM NaCl, 1% Nonidet P-40, 0.1% SDS, 0.1% deoxycholic acid, 10 mM NaF, 10 mM Na4P2O7, 0.4 mM Na3VO4, and protease inhibitors) for 30 min on ice. For Western blot analysis using conditioned medium, chick leg bud mesenchymal cells were incubated in serum-free medium for 24 h after drug treatment. Total protein content of the cells was determined by the bicinchoninic acid method (Pierce). Loading amounts were standardized to the middle protein concentration for all samples. Then the conditioned medium was concentrated with 10% trichloroacetic acid (TCA) precipitation. The precipitate was dissolved in 1× sample buffer (62.5 mM Tris-HCl, pH 6.8, containing 10% glycerol, 2% SDS, and 0.005% bromphenol blue). Proteins (30 μg) were separated by 10% polyacrylamide gel electrophoresis containing 0.1% SDS and transferred to nitrocellulose membrane (Schleicher and Schuell). The membranes were incubated for 1 h at room temperature in blocking buffer (20 mM Tris-HCl, 137 mM NaCl, pH 8.0, containing 0.1% Tween and 3% nonfat dry milk) and probed with antibodies against JNK and phospho-JNK (Cell Signaling

3 The abbreviations used are: miRNA, microRNA; STSN, staurosporine; PA, peanut agglutinin; ASO, antisense oligonucleotide; PNA, peptide nucleic acid.
The Role of miR-34a in Chondroblasts

Induction of PNA-based miRNA Inhibitor or miR-34a Precursor Oligonucleotides—The PNA-based antisense oligonucleotides (ASOs) contained an O-linker at the N terminus of the PNA to improve solubility were purchased from Panogen. 200 nM PNA-based ASO (PNA34a, UGGCAGUGUCUAAAGCGGUGUUG) or 50 nM miR-34a precursor oligonucleotides (UGGCCAGUGUCUAAAGCGGUGUUG; Ambion) were electroporated into isolated mesenchymal cells using a square wave generator (BTX-830, Genentronics (San Diego, CA)) with 20-ms, 200 square pulses. A scrambled PNA-based ASO or scrambled miRNA was used as a negative control.

Reporter Vectors and DNA Constructs—The 3’-UTR of Rac1 (2341 bp) was PCR-amplified using primers 5’-GCCAGTTTCCCCGACATAAC3’ and 5’-GGCGGTTTAAAGAGGGT-CAG-3’ (nucleotides 514–1259) and cloned downstream of the CMV-driven firefly luciferase cassette in the pMIR-Report vector (Ambion). A reporter vector containing a directly matched miRNA-binding site oligonucleotide (~51 bp) for miR-34a was used as the positive control. A miRNA target validation, limb mesenchymal cells were electroporated using a square wave generator (20-ms, 200 square pulses; BTX-830, Genentronics (San Diego, CA)) with 20-ms, 200 square pulses. A scrambled PNA-based ASO or scrambled miRNA were used as a negative control.

RESULTS

A JNK Inhibitor Suppresses Chondrogenesis of Chick Limb Mesenchymal Cells and Stimulates miR-34a Expression—Previously, our laboratory demonstrated the involvement of JNK signaling in chondrogenesis (57). That is, the phosphorylation of JNK was increased during the early stages of normal chondrogenic differentiation. Precartilage condensation and chondrogenic differentiation were analyzed by PA and Alcian blue staining, respectively. The PA and Alcian blue staining intensities (Fig. 1A); transcription of type II collagen protein, a marker for chondrogenic differentiation; transcription of the two major proteoglycans, aggrecan and versican; and the translational level of type II collagen and condensation-related mole-
molecules, such as Sox-9 and N-cadherin, were decreased in JNK inhibitor-treated cells versus untreated controls and were suppressed by blockage of JNK signaling.

miRNAs are known to modulate a variety of cellular pathways related to cell development and differentiation (16–18, 25). To identify miRNAs that could be differentially expressed in JNK inhibitor-treated cells versus untreated controls, we performed a miRNA array screening with 200 unique miRNAs in their mature forms. From this array, we identified 14 up-regulated miRNAs and 12 down-regulated miRNAs following treatment with a JNK inhibitor, and we choose highly conserved miRNAs between human and chicken by comparing their mature sequence (supplemental Table 1). Among them, miR-34a was substantially induced by JNK signaling (determined using a p value of 0.01 as a cut-off for significance). During in vitro culture, the induction of miR-34a was decreased with the progression of chondrogenic differentiation (Fig. 1C), suggesting that miR-34a plays a role in a later stage of chondrogenesis as prechondrification condensation and chondrocyte commitment, possibly not in proliferation of chondrogenesis in this study. Treatment with the JNK inhibitor significantly increased the expression level of miR-34a as confirmed by RT-PCR (Fig. 1D). In order to examine the possible involvement of miR-34a in chondrogenic differentiation of chick limb mesenchymal cells, we exposed mesenchymal cells to 200 nM PNA-based ASOs against miR-34a, which confirmed a significant decrease in the expression level of miR-34a by RT-PCR (Fig. 1D); treated the cells with or without the JNK inhibitor; and assessed chondrogenesis by Alcian blue staining on day 5 post-treatment and by expression level of type II collagen (Fig. 1E). The decreased intensities and expression of PA and Alcian blue staining and type II collagen by JNK inhibitor were recovered by co-treatment with anti-miR-34a oligonucleotides (Fig. 1E). Conversely, overexpression of the miR-34a precursor oligonucleotides leads to a suppression of prechondrification condensation (Fig. 2A, top) and chondrogenic differentiation (Fig. 2A, bottom) without affecting cell death (Fig. 2B).

The cytoskeleton plays fundamental roles in cell survival, migration, division, and chromosome and organelle movement. Cytochalasin, a cytoskeleton-interrupting reagent, has varying effects on different types of cells, suggesting that the cytoskeleton can have critical yet cell type-dependent functions (26, 27). To examine the possible interaction between miR-34a and cytoskeletal reorganization, we used phalloidin staining to observe actin distribution in chick limb mesenchymal cells (Fig. 2C). Actin stress fibers were intensified in JNK inhibitor-treated cells versus untreated controls, but this effect was at least partially rescued by co-treatment with the anti-miR-34a oligonucleotides.

miR-34a Modulates Rearrangement of Actin Cytoskeleton by Regulating RhoA during Chondrogenic Differentiation of Chick Limb Mesenchymal Cells—Rho GTPases, a family of small GTP-binding proteins, is known to be involved in cell cytoskel-
The role of miR-34a in chondroblasts

miR-34a is involved in Rac1-RhoA cross-talk in chondrogenic progenitors—previously, Rac-Rho cross-talk has been suggested in several studies (33, 34). This coordinated and
The Role of miR-34a in Chondroblasts

FIGURE 4. Redifferentiation of dedifferentiated chondrocytes on chitosan membrane forms the cortical actin ring possibly through miR-34-modulated RhoA level. Dedifferentiated chondrocytes were cultured on chitosan membrane or were treated with anti-miR-34a. Scrambled PNA-based ASOs were used as a control (Con). A, cells were stained with Alexa488-conjugated phalloidin. B, the expression of miR-34a was measured with real-time PCR (left), and change in protein levels of type II collagen and RhoA was examined by Western blotting (right). The data shown are representative of at least four independent experiments. The mean is plotted, and the error bars represent 95% confidence interval (lower/upper limit). *, statistically different from control cells (p < 0.005).

FIGURE 3. miR-34a alters stress fiber formation in limb mesenchymal cells through modulation of RhoA expression. A, cells were with 5 μM JNK inhibitor. Change in protein level of RhoA was determined by Western blotting at 2 days of culture. B, cells were treated with 100 nM anti-miR-34a oligonucleotides (anti-mir-34a) or 50 nM miR-34a precursors (mir-34a). Cells were electroporated with wild type RhoA expression vector (RhoA) and cultured with 100 nM anti-miR-34a oligonucleotides (anti-mir-34a) or 50 nM miR-34a precursors (mir-34a). C, wild-type RhoA was introduced by electroporation, and the level of RhoA after electroporation was confirmed by Western blotting with anti-HA antibody (top). Cells without treatment of anti-miR-34a or miR-34a precursor (Con, —, RhoA) were treated with scrambled PNA-based ASOs (bottom). D, cells were stained with Alcian blue at day 5 of culture, and chondrogenesis was quantified by measuring the absorbance of bound Alcian blue at 600 nm. E, the expression of miR-34a was measured with real-time PCR (left), and RNA level of RhoA was measured by reverse transcription PCR (right). GAPDH was used as a loading control. Cells were electroporated with wild type RhoA expression vector (RhoA) and cultured with or without 10 μM STSN. Cells without treatment of anti-miR-34a or miR-34a (Con, STSN, STSN/RhoA) were treated with scrambled PNA-based ASOs. F, cells were stained with Alexa488-conjugated phalloidin. G, cells were stained with Alcian blue at day 5 of culture, and chondrogenesis was quantified by measuring the absorbance of bound Alcian blue at 600 nm. The data shown are representative of at least four independent experiments. The mean is plotted, and the error bars represent 95% confidence interval (lower/upper limit). *, statistically different from control cells (p < 0.005). The diameter of standard culture is 5 mm.
opposed activity of Rac1 and RhoA is known to be crucial to cellular dynamics, the former promoting membrane protrusion and cell polarity and spreading and the second promoting cytoskeleton contractility and tail retraction. Therefore, we examined whether JNK signaling is involved in regulation of Rac1. Treatment of JNK inhibitor blocked the translational level of Rac1 (Fig. 5A). To verify whether Rac1 is one of targets for miR-34a, we checked the expression level of Rac1 by anti-miR-34a or miR-34a precursor oligonucleotides (Fig. 5B). We observed a significant reduction in Rac1 protein expression among miR-34a precursor-treated chondroprogenitor cells, whereas these levels were recovered in cells that had been treated with anti-miR-34a oligonucleotides. To further confirm that Rac1 is a target of miR-34a, we cloned the entire 3'-UTR of Rac1 into a luciferase reporter vector,electrooporated the vector into chondrogenic progenitor cells along with miR-34a precursor or a cognate non-targeting negative control, and assayed cell lysates for luciferase expression. We found that cells transfected with the Rac1 3'-UTR-driven vector plus miR-34a precursor exhibited significantly less luciferase activity compared with cells that received the reporter plus the non-targeting negative control (Fig. 5C and supplemental Figs. 1 and 2), suggesting that miR-34a targets Rac1 and represses its expression during JNK-mediated signaling.

To confirm the correlation among Rac1, RhoA, and miR-34a during chondrogenic differentiation, the expression level of Rac1 and RhoA was examined. The expression of Rac1 was increased with the progression of chondrogenic differentiation (Fig. 5D) along with a decrease in the induction of miR-34a (Fig.

The Role of miR-34a in Chondroblasts

miRNAs play key roles in diverse regulatory pathways, including development (25–30, 35), cell proliferation, differentiation (16–18), apoptosis (19, 20), tumorigenesis (36, 37), and many other physiological or pathological processes (38, 39). Recent studies have indicated that microRNAs are also important for tissue morphogenesis (40, 41). However, the precise roles of miRNAs in cartilage biology are largely unknown, and many of the individual miRNA targets that are likely to be important within chondroblasts remain yet unidentified. Here, we characterized one specific miRNA involved in the morphological transition of chondroprogenitors from fibrillar organization to cortical organization, which is a necessary step for chondrogenic differentiation (42, 43). More specifically, we showed that miR-34a was up-regulated by chondro-inhibition, whereas miR-34a knockdown stimulated the transition of cells from a fibroblastoid morphology to a rounded or polygonal morphology. In addition, we found that this occurred at least in part through regulating RhoA, which is known to modulate the organization of stress fibers and focal adhesions (30, 44). Previously, miR-143 and miR-145 were shown to act as integral components of the network that regulates cytoskeletal remodeling and phenotypic switching of smooth muscle cells during vascular disease (45). The miR-200 family of miRNAs has been shown to directly target WAVE3 to regulate remodeling of the.
**The Role of miR-34a in Chondroblasts**

Actin cytoskeleton and the epithelial-mesenchymal transition in cancer cells (46). Previously, miR-34 had been shown to help regulate the cell cycle and cell growth by targeting proteins, such as Cdk, cyclin E2, c-Met, and Bcl-2 (47, 48). miR-34a inhibits the invasiveness of cervical carcinoma and choriocarcinoma cells through targeting Notch1 and Jagged1 (49) and migration of hepatocellular carcinoma cells by down-regulation of c-Met (50). During human monocyte-derived dendritic cell differentiation, miR-34a targets WNT1 and JAG1, factors involved in stalling monocyte-derived dendritic cell differentiation (51).

Adding to these previous findings, we herein show for the first time that miR-34a regulates rearrangement of the actin cytoskeleton by regulating RhoA/Rac1 cross-talk during the chondrogenic differentiation of chick limb mesenchymal cells. Actin filaments are associated with the plasma membrane at sites where the cells form connections with the extracellular matrix or other cells. Well spread cells exert tension on the adhering extracellular matrix via fully extended stress fibers (52, 53). Stress fibers directly terminate at focal adhesions, amid accumulations of numerous structural proteins that are responsible for connecting the cell membrane to the underlying substrates. Chondrogenesis is characterized by a drastic change in cell shape, which transitions from a fibroblastoid shape to a round or polygonal morphology. We previously showed the activation of matrix metalloprotease-2 (MMP-2) is required for reorganization of the actin cytoskeleton to a cortical pattern, with cell rounding occurring in parallel (10). We also previously showed that dedifferentiated chondrocytes are capable of regaining their differentiated phenotype on a chitosan membrane and that this re-expression is associated with decreased RhoA protein levels and a loss of stress fibers, suggesting that miR-34 may trigger the transition are largely unknown. Studies of stress filaments in undifferentiated mesenchymal cells treated with a JNK inhibitor may be seen bundled as stress fibers. However, when these cells were treated with anti-miR-34a oligonucleotides, they regained a rounded shape and re-expressed sulfated proteoglycans, suggesting that miR-34 may trigger the disassembly of the actin stress fibers, followed by their replacement with cortically distributed microfilaments. Rho proteins are small GTases that are involved in modulating the organization of stress fibers and focal adhesions (30, 34, 43, 44). Blocking the Rho signaling pathway leads to enhanced chondrogenesis among mesenchymal cells, suggesting that Rho plays a negative role in chondrocytic differentiation (29). Rac1 signaling is a required signaling pathway to generate normal N-cadherin-dependent cellular junctions that are known to be essential for chondrogenesis (55). Culture of differentiated chondrocytes in alginate gel has been shown to be associated with decreased RhoA protein levels and a loss of stress fibers, comitochondria the re-expression of the chondrocytic differentiation program. A mutual antagonism between the Rac and Rho GTases has been observed in several cellular settings, suggesting reciprocal control of Rac and Rho small GTases. Coordinated and opposed activity of Rac1 and RhoA is crucial to cellular dynamics, the former promoting membrane protrusion, cell polarity, and spreading (33, 34). Here, we report for the first time that the forced overexpression of miR-34a in chick limb mesenchymal cells can induce intensified stress fibers, whereas blockade of miR-34a induced morphological transition to chondrocyte-specific morphotype through modulation of Rhoa/Rac1 cross-talk. Based on our findings, it seems possible that intensified stress fibers seen in JNK inhibitor-treated cells may be due to suppression of miR-34-targeted Rac1 expression. Our data suggest that miR-34a regulates RhoA/Rac1 cross-talk and negatively modulates reorganization of the actin cytoskeleton, which is one of the essential processes for establishing chondrocyte-specific morphology.

**REFERENCES**

1. Goldring, M. B., Tsuchimochi, K., and Ijiri, K. (2006) The control of chondrogenesis. J. Cell. Biochem. 97, 33–44
2. Olsen, B. R., Reginato, A. M., and Wang, W. (2000) Bone development. Annu. Rev. Cell Dev. Biol. 16, 191–220
3. Karsenty, G., and Wagner, E. F. (2002) Reaching a genetic and molecular understanding of skeletal development. Dev. Cell. 2, 389–406
4. Shum, L., and Nusse, R. (2003) The life cycle of chondrocytes in the developing skeleton. Cell 114, 1041–1064
5. von der Mark, K. (1977) The role of three genetically distinct metalloproteinases in classification and calcification of the cartilage matrix. Calcif. Tissue Int. 20, 249–254
6. Bennett, C. F., Lollo, B., and Griffey, R. (2004) MicroRNA-143 regulates inhibition of c-met expression in chondrogenesis by the Wnt/β-catenin signaling pathway, which is associated with the control of apoptosis in chondrocytes. J. Cell. Biochem. 91, 796–804
7. Kawahara, K., and Carthew, R. W. (2004) Expanding roles for miRNAs and the regulatory molecules involved in this sort of morphological transition are largely unknown. Studies of stress filaments in undifferentiated mesenchymal cells treated with a JNK inhibitor may be seen bundled as stress fibers. However, when these cells were treated with anti-miR-34a oligonucleotides, they regained a rounded shape and re-expressed sulfated proteoglycans, suggesting that miR-34 may trigger the disassembly of the actin stress fibers, followed by their replacement with cortically distributed microfilaments. Rho proteins are small GTases that are involved in modulating the organization of stress fibers and focal adhesions (30, 34, 43, 44). Blocking the Rho signaling pathway leads to enhanced chondrogenesis among mesenchymal cells, suggesting that Rho plays a negative role in chondrocytic differentiation (29). Rac1 signaling is a required signaling pathway to generate normal N-cadherin-dependent cellular junctions that are known to be essential for chondrogenesis (55). Culture of differentiated chondrocytes in alginate gel has been shown to be associated with decreased RhoA protein levels and a loss of stress fibers, comitochondria the re-expression of the chondrocytic differentiation program. A mutual antagonism between the Rac and Rho GTases has been observed in several cellular settings, suggesting reciprocal control of Rac and Rho small GTases. Coordinated and opposed activity of Rac1 and RhoA is crucial to cellular dynamics, the former promoting membrane protrusion, cell polarity, and spreading (33, 34). Here, we report for the first time that the forced overexpression of miR-34a in chick limb mesenchymal cells can induce intensified stress fibers, whereas blockade of miR-34a induced morphological transition to chondrocyte-specific morphotype through modulation of Rhoa/Rac1 cross-talk. Based on our findings, it seems possible that intensified stress fibers seen in JNK inhibitor-treated cells may be due to suppression of miR-34-targeted Rac1 expression. Our data suggest that miR-34a regulates RhoA/Rac1 cross-talk and negatively modulates reorganization of the actin cytoskeleton, which is one of the essential processes for establishing chondrocyte-specific morphology.
