Integrin Upregulation and Localization to Focal Adhesion Sites in Pregnant Human Myometrium

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Abstract

Focal adhesions are integrin-rich microdomains that structurally link the cytoskeleton to the extracellular matrix and transmit mechanical signals. In the pregnant uterus, increases in integrin expression and activation are thought to be critical for the formation of the mechanical syncytium required for labor. The aim of this study was to determine which integrins are upregulated and localized to focal adhesions in pregnant human myometrium. We used quantitative polymerase chain reaction, Western blotting, and confocal microscopy to determine the expression levels and colocalization with focal adhesion proteins. We observed increases in several integrin transcripts in pregnant myometrium. At the protein level, integrins such as α_5 -integrin (ITGA5), ITGA7, ITGAV, and ITGB3 were significantly increased during pregnancy. The integrins ITGA3, ITGA5, ITGA7, and ITGB1 colocalized with focal adhesion proteins in term human myometrium. These data suggest that integrins $\alpha_3\beta_1$, $\alpha_5\beta_1$, and $\alpha_7\beta_1$ are the most likely candidates to transmit mechanical signals from the extracellular matrix through focal adhesions in pregnant human myometrium.

Keywords

myometrium, integrin, pregnancy

Introduction

The switch of uterine myometrium from a quiescent to a contractile state at the end of pregnancy is a highly regulated process that requires activation of at least 2 signal transduction pathways initiating from the fetus.¹ The first pathway is caused by mechanical distension of the uterus due to rapid fetal growth. Mechanical stretch leads to upregulation of myometrial contraction-associated² and mechanosensitivity proteins.³ Contraction-associated protein expression is believed to prime or activate the uterus to effectively respond to a second endocrine-based signal transduction pathway originating from the fetal hypothalamic–pituitary–adrenal–placental axis.¹ In a mouse model, increased expression of contractile-associated proteins appears to be responsible for increased uterine contractility at birth.⁴

The switch to increased myometrial contractility is associated with increased expression of proteins associated with focal adhesion complexes.³ Focal adhesions are membrane domains that structurally link the cell cytoskeleton to the extracellular matrix and transmit mechanical signals mediating a variety of processes, such as adhesion-dependent migration, survival, and proliferation.⁵ Focal adhesion sites are rich in integrins, a family of heterodimeric transmembrane cell surface receptors that act as mechanosensors.⁶ Integrin engagement at focal adhesion sites

results in activation and recruitment of focal adhesion kinase (FAK) to these sites.^{6,7} FAK then interacts with a host of regulatory and signaling proteins to affect changes in the actin cytoskeleton organization, protease activation, and focal adhesion stability.⁷

Research in animal models indicates change in myometrial integrin expression patterns during pregnancy. At the transcript level, expression of *ITGA1*, *ITGA3*, and *ITGB1* increases during pregnancy, although changes in the corresponding protein levels were not observed.⁸ ITGA5 transcript and protein levels increase in the rat myometrium during pregnancy⁹ and these increases appear to be due to mechanical stretch of the tissue.¹⁰ In addition, ITGA5 localizes to focal adhesion complexes in the myometrium of the pregnant rat and ewe.^{9,11} However, little is known about integrin expression and localization in the pregnant human myometrium.

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Since increases in integrin receptors and their corresponding extracellular matrix adhesion molecules are thought to be critical for focal adhesion development and formation of a mechanical syncytium,¹² we conducted a series of experiments to determine which integrins are upregulated and localized to focal adhesions in term human myometrium. The ITGA5 has been reported to be upregulated in laboring human myometrium.¹³ Although additional integrins have been identified in the uterus, their expression in term and preterm human myometrium remains largely uncharacterized. The objective of the experiments described here is to characterize integrin expression and localization in the term human myometrium.

Materials and Methods

Tissue Collection

All research was reviewed and approved by the University of Nevada Biomedical Review Committee (institutional review board) for the protection of human participants. Human uterine myometrial biopsies were obtained with written informed consent from women undergoing hysterectomy when premenopausal and without pathology involving the uterine muscle or from mothers undergoing elective Cesarean section either in labor at term or at term not in labor. Tissues were transported to the laboratory immediately by suspension in cold physiological buffer, dissected to isolate smooth muscle, snap frozen in liquid nitrogen, and stored at -80°C . The average age of disease-free nonpregnant patients was 41 ± 13 years. The average age for patients in the pregnant laboring and nonlaboring groups was 28 ± 11 and 30 ± 9 years, respectively. Pregnant patients ranged from 37 to 40 weeks of gestation, with the mean at 38.9 weeks for both laboring and nonlaboring groups. Parity ranged from 1 to 4, with the average at 2.5 for nonlaboring patients and 2.3 for laboring patients. Patients represented a range of ethnicities and were 48% Caucasian, 41% Hispanic, 7.3% African American, and 3.7% Pacific Islander. All samples were from singleton pregnancies.

Quantitative Polymerase Chain Reaction

For quantitative polymerase chain reaction PCR (qPCR), total RNA was extracted from the frozen human myometrial tissue using Trizol reagent (Invitrogen, Carlsbad, California) as described.¹⁴ The RNA was converted to complementary DNA (cDNA) using SuperScript III reverse transcriptase (Invitrogen) and random hexamers. The cDNA from 6 myometrial samples per group was pooled and subjected to qPCR on a human extracellular matrix and adhesion molecules PCR array (PAHS-013C, SABiosciences, Frederick, Maryland). The qPCR reactions were carried out in an ABI StepOne Plus machine according to manufacturer's protocol. To confirm the transcript level changes, qPCR was performed in triplicate on 6 individual human myometrial cDNA samples per group using gene-specific TaqMan gene expression assays (900 nmol/L primers, 250 nmol/L 6-carboxyfluorescein labeled TaqMan

dihydrocyclopypyrroloindole tripeptide minor groove binder probe) and $1 \times$ TaqMan Fast mix (Applied Biosystems, Foster City, California) in an ABI 2720 real-time thermocycler according to the recommended cycling parameters. Quantity means were normalized to 18S ribosomal RNA, whose expression is known to be stable during human pregnancy and labor.¹⁴

Western Blotting

For Western blotting, tissue from 9 to 15 patients per group was extracted in radioimmunoprecipitation assay buffer containing 150 mmol/L sodium chloride, 1.0% NP-40, 1 mmol/L EDTA, and 50 mmol/L Tris, pH 7.4 with Halt protease inhibitors (Thermo Scientific, Rockford, Illinois) and quantified by bicinchoninic acid assay (ThermoScientific). Equal amounts of total protein were separated on 7.5% or 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis gels and transferred to nitrocellulose membranes. Primary antibodies were added to blots in Odyssey blocking (Licor Biosciences, Lincoln, Nebraska) buffer and incubated overnight. Primary antibodies to ITGA1 (MAB1973Z, 1:500), ITGA3 (AB1920, 1:500), and ITGAV (AB1930, 1:5000) were purchased from Millipore (Billerica, Massachusetts). Primary antibodies to ITGB3 (PM6/13, 1:200), ITGB1 (1:2000), and ITGB5 (D01P, 1:500) were purchased from ABD Serotec (Raleigh, North Carolina), Abnova (Taipei City, Taiwan), and Abcam (Cambridge, Massachusetts), respectively. Antibody to ITGA7 (1:1000) was a gift from Stephen Kaufman, University of Illinois, Urbana, Illinois. Antibody to ITGA5 (1:1000) was a gift from Dr Maria Valencik, University of Nevada, Reno, Nevada. Primary antibodies were detected by incubating blots with Alexafluor680 (Molecular Probes, Eugene, Oregon) or IRDye800 (Rockland Immunochemicals, Gilbertsville, Pennsylvania) fluorescently conjugated secondary antibodies. Bands were detected and band intensities quantified with an Odyssey Infrared Imaging System (LiCor Biosciences).

Confocal Microscopy

Human uterine myometrial biopsies dissected to reveal smooth muscle were flash frozen in Tissue-TEK O.C.T. compound in liquid nitrogen-cooled isopentane and stored at -80°C . Samples were cut into 10 μm sections with a cryostat and placed on coated slides (Surgipath, Buffalo Grove, Illinois). For ITGA3, ITGA5, ITGA7, ITGB1, ITGB3, and ITGB5, sections were fixed in 4% paraformaldehyde and permeabilized with 0.5% Triton X-100. For ITGAV and ITGA5 sections were fixed in -20°C acetone. The sections were blocked with 5% bovine serum albumin and incubated with polyclonal anti-ITGA3 (Millipore), anti-ITGA5 (M. Valencik, University of Nevada), anti-ITGA7 (S. Kaufman, University of Illinois), anti-ITGB1 (AbCam, Cambridge, Massachusetts), anti-ITGB3 (ABD Serotec, Raleigh, North Carolina), or anti-ITGB5 (Abnova). A fluorescein isothiocyanate-goat anti-rabbit immunoglobulin G (IgG) secondary antibody (Santa Cruz Biotech Santa Cruz, California) was used for integrin detection. Sections were counterlabeled

Table 1. Transcripts for Integrins A1, A3, A5, A7, AV, B1, B2, B3, and B5 Are Significantly Increased in Both Nonlaboring and Laboring Pregnant Human Uterine Myometrium Compared to Nonpregnant Myometrium.

	NL:NP Fold change	P Value	L:NP Fold Change	P Value
ITGA1	1.97	.008	1.33	.036
ITGA2	0.37	.007	0.26	.002
ITGA3	1.8	.019	0.67	.116
ITGA4	0.55	.019	0.61	.011
ITGA5	21.12	.001	10.47	<.001
ITGA6	0.92	.909	0.4	<.001
ITGA7	3.46	.004	2.75	<.001
ITGA8	1.91	.056	1.06	.697
ITGAL	0.26	<.001	0.17	<.001
ITGAM	1.41	.132	0.99	.944
ITGAV	1.99	.020	1.78	.001
ITGB1	1.65	.019	2.1	.002
ITGB2	1.51	.040	1.79	.002
ITGB3	4.49	<.001	2.39	<.001
ITGB4	0.67	.088	0.35	.006
ITGB5	3.66	.004	1.98	.001

Abbreviations: ITG, integrin; L, pregnant laboring; NL, nonlaboring; NP, pregnant nonpregnant.

mouse anti-vinculin (Sigma-Aldrich, St. Louis, Missouri) detected with TRITC-donkey anti-mouse IgG (Santa Cruz Biotech). Sections were mounted in Vectashield plus DAPI (Vector-Labs, Burlingame, California), and viewed using an Olympus Fluoview confocal microscope.

Data Analysis

Relative gene expression was determined using SABiosciences PCR array software and the $\Delta\Delta C_t$ was method normalized to a set of 6 control genes. TaqMan quantity means were normalized to 18S ribosomal transcript and ratios between the groups were compared by Welch corrected *t* test. Integrin protein expression was normalized to glyceraldehyde 3-phosphate dehydrogenase (GAPDH) and ratios between the groups were compared by 1-way analysis of variance followed by the Tukey-Kramer multiple comparisons test with *P* < .05 considered significant. Some data sets were log transformed in order to meet statistical test assumptions. For colocalization experiments, Pearson correlation coefficients were calculated with Fluoview software (Olympus America, Center Valley, Pennsylvania). We defined a colocalization coefficient >0.7 as a highly positive correlation, >0.5 as a positive correlation, and 0.5 or less as no colocalization.¹⁵

Results

Integrin Transcript Levels in Pregnant Human Myometrium

It has been proposed that uterine smooth muscle cells undergo a transition to a more contractile phenotype at the end of pregnancy and that this transition is associated with major changes in the expression of extracellular matrix proteins and their associated receptors.¹ To test this hypothesis, total RNA was extracted from nonpregnant, pregnant nonlaboring, and

pregnant laboring human myometrial samples. The RNA was converted to cDNA and each group of samples was pooled and subjected to qPCR on an array of primers to human extracellular matrix and adhesion molecules. Transcripts for *ITGA1*, *ITGA3*, *ITGA5*, *ITGA7*, *ITGAV*, *ITGB1*, *ITGB2*, *ITGB3*, and *ITGB5* were significantly higher in term nonlaboring human myometrial samples than in nonpregnant control samples (Table 1). The *ITGA1*, *ITGA3*, and *ITGAV* transcripts were approximately doubled in pregnant, nonlaboring myometrium compared to nonpregnant controls. Transcripts for *ITGA7*, *ITGB3*, and *ITGB5* were increased 3- to 4-fold. We observed a 21-fold increase in *ITGA5* in pregnant, nonlaboring myometrium compared to nonpregnant controls. In laboring myometrium, *ITGA1*, *ITGA3*, *ITGAV*, *ITGA7*, *ITGB3*, and *ITGB5* transcripts decreased from nonlaboring levels, but were still higher than in the nonpregnant myometrial samples. The *ITGB1* transcript was slightly increased in nonlaboring samples and increased to 2-fold in laboring tissue. Transcript levels of *ITGA2*, *ITGA4*, *ITGAL*, and *ITGB4* were significantly lower in pregnant myometrium compared to control samples. Transcript levels for *ITGA6*, *ITGA8*, and *ITGAM* were not significantly different in the term nonlaboring pregnant uterus compared to the nonpregnant controls.

In order to confirm the accuracy of the array data, TaqMan qPCR was performed in individual patient samples with primers and probes to 3 different integrin chains. Quantity means were normalized to 18S ribosomal transcript and confirmed a 3.7-fold increase in *ITGA7*, a 21-fold increase in *ITGA5*, and a 2.0-fold increase in *ITGAV* transcripts in pregnant human myometrial samples compared to nonpregnant controls (Figure 1).

Integrin Protein Levels in Pregnant Human Myometrium

In order to determine whether the observed transcript level changes corresponded to changes in expression at the protein

Figure 1. TaqMan quantitative polymerase chain reaction (qPCR) performed on individual patient samples confirmed the observed changes in integrin transcript level in the pregnant uterus. Quantity means normalized to 18S ribosomal transcript confirmed a 3.7-fold increase in α_7 -integrin (ITGA7), a 21-fold increase in ITGA5, and a 2.0-fold increase in ITGAV in pregnant human uterine samples. Graphs represent average values $\pm$ standard error of the mean (SEM) * $P < .05$, ** $P < .01$). $n = 6$ samples per group.

level, we performed semiquantitative Western blots (Figure 2). After normalization to GAPDH intensity, ITGA5, ITGA7, ITGB1, and ITGB3 protein levels were significantly higher in pregnant nonlaboring and laboring myometrial samples compared to the nonpregnant controls. The ITGAV protein was higher in pregnant myometrium compared to controls, but this was only significant in the nonlaboring group. The ITGA1, ITGA3, and ITGB5 protein levels did not increase during pregnancy (Figure 3).

Integrin Colocalization With Focal Adhesion Proteins in Pregnant Human Myometrium

We used confocal microscopy to determine which of the upregulated integrins localized to focal adhesions in term myometrium. All of the integrin chains tested could be detected

Figure 2. Representative Western blots depicting the expression of integrin protein subunits in nonpregnant (NP), pregnant laboring (L), and pregnant nonlaboring (NL) human uterine myometrial samples.

in myometrium except ITGA1 and ITGB3, which were predominantly found in cells of the vasculature. The ITGB5 was predominantly expressed in vascular and interstitial cells. We observed colocalization of ITGA3, ITGA5, ITGA7, and ITGB1 with the focal adhesion-associated protein vinculin (Figure 4), with Pearson coefficients averaging $R = .52$, $R = .8$, $R = .58$, and $R = .66$, respectively (Figure 5). The ITGA5 also showed high levels of colocalization with another focal adhesion protein (talin $R = .85$, Figure 6). We did not observe colocalization between ITGB3 ($R = .25$) or ITGB5 ($R = .45$) and vinculin in our pregnant myometrial samples (Figures 4 and 5). Because a different fixative was required for ITGAV, this integrin was observed in combination with talin as another focal adhesion protein and high levels of colocalization were not observed ($R = 0.45$, Figure 6). No significant differences in localization were observed between nonlaboring and laboring samples. Finally, although integrin expression levels were lower in nonpregnant myometrial samples, colocalization values were not significantly different between pregnant and nonpregnant myometrial samples (not shown). The discrete, punctate labeling pattern we observed around the myometrial cell membranes with anti-ITGA3, -ITGA5, -ITGA7, and -vinculin at term (Figure 4, arrows) was not detected in the nonpregnant samples.

Discussion

Integrin-rich membrane domains known as focal adhesions (or dense plaques) transmit mechanical signals from the extracellular matrix to activate signaling pathways inside the cell.¹⁶ Signaling through integrins and focal adhesions has been implicated in regulating cell morphology and contractility.^{16,17} During pregnancy, myometrial extracellular matrix and focal adhesions undergo substantial reorganization and this may contribute to development of the contractile state of this tissue at term.¹⁸ At term, focal adhesion sites may function as intercellular connection sites to coordinate contraction

Figure 3. Western blot quantitation indicates some integrin chains are more abundant in pregnant human myometrial samples compared to nonpregnant myometrium. The α_5 -integrin (ITGA5), ITGA7, ITGAV, and ITGB3 are significantly increased in the pregnant laboring (L) and nonlaboring (NL) human uterus compared to nonpregnant controls (NP), $n = 9$ to 15 per group, $P < .05$. Graphs represent average values $\pm$ standard error of the mean (SEM; * $P < .05$, ** $P < .01$, and *** $P < .001$). $n = 9$ to 15 samples per group.

throughout the myometrium.³ We hypothesized the myometrial integrin expression profile would change during pregnancy to complement the changes occurring in the extracellular matrix. We performed a series of experiments to compare integrin chain expression in nonpregnant and term human myometrium.

Transcripts for *ITGA1*, *ITGA3*, *ITGA5*, *ITGA7*, *ITGAV*, *ITGB1*, *ITGB2*, *ITGB3*, and *ITGB5* were significantly higher in term myometrial samples than in nonpregnant control samples. Changes in gene expression were highly reproducible, as evidenced by highly consistent results obtained between the SABiosciences array and Invitrogen Taqman qPCR assays. In

Figure 4. α_3 -Integrin (ITGA3), ITGA5, ITGA7, and ITGB1 (green) colocalize with the focal adhesion complex protein vinculin (red) in term, nonlaboring human myometrium by confocal microscopy. Regions of overlapping signal appear yellow. Arrows highlight regions of integrin/vinculin localization to discrete membrane regions of myometrial cells. The ITGB3 localized to cells surrounding blood vessels (BV) and not myometrial cells (M).

addition, we observed significant increases in transcript level in term myometrium of many genes encoding extracellular matrix proteins (collagens, laminins, and fibronectin; Supplemental Table 1) that have previously shown to increase during pregnancy in other species.^{10,12,19,20}

We observed downregulation of a large number of cell adhesion molecule transcripts in the laboring myometrium compared to nonlaboring myometrium, with only a single molecule exhibiting increased transcript levels. It is possible this reflects a general transcriptional shutdown of genes

Figure 5. Average Pearson coefficients were $R = .52$ for α_3 -integrin (ITGA3), $R = .8$ for ITGA5, $R = .56$ for ITGA7, and $R = .66$ for ITGB1 (B). Correlation coefficient bars represent average values $\pm$ standard error of the mean (SEM).

encoding extracellular matrix proteins that occurs with the onset of labor in preparation for remodeling associated with uterine involution.

The ITGA1 pairs with ITGB1 and the heterodimer binds collagens I, IV, and IX and laminin. Williams et al observed large increases in *ITGA1* transcript in the pregnant rat uterus throughout pregnancy and levels decreased again postpartum. However, uterine ITGA1 protein levels did not change in the pregnant versus nonpregnant rat uterus.⁸ Consistent with these observations, we observed a modest increase in *ITGA1* at the transcript level, but no corresponding increases were observed at the protein level. In our immunofluorescence experiments, ITGA1 localized predominantly to uterine blood vessels (not shown).

The ITGA3 pairs with ITGB1 to bind collagen and laminins.²¹ The *ITGA3* transcript is increased in the rat uterus during pregnancy and then decreased to nonpregnant levels after delivery.⁸ The ITGA3 protein tended to decrease in abundance during pregnancy in the rat, although this decrease was only significant on day 22 at the very end of pregnancy.⁸ We observed statistically significant increases in *ITGA3* transcript, but no corresponding increase in ITGA3 protein in our pregnant human myometrial samples. The ITGA3 was detected at human myometrial focal adhesion sites, as determined by colocalization with an antibody against vinculin.

The ITGA5 chain pairs exclusively with the ITGB1 chain to act as a fibronectin receptor. We observed a significant 14-fold increase in fibronectin transcript in the pregnant uterus in qPCR array experiment (Supplemental Table 1), consistent with the previous reports.^{10,19,20} In addition, we detected a dramatic 21-fold increase in *ITGA5* transcript in our term nonlaboring uterine samples compared to nonpregnant controls and a corresponding 6-fold increase in ITGA5 protein. Both ITGA5 transcript and protein levels were lower in laboring samples compared to term, nonlaboring myometrium. These results are consistent with the previous observations that ITGA5 transcript

Figure 6. α_5 -Integrin (ITGA5; green) colocalizes with the focal adhesion complex protein talin (red) in term human myometrium by confocal microscopy. In contrast, little colocalization was observed between ITGAV (green) and talin (red) (A). Regions of overlapping signal appear yellow. Average Pearson coefficients were $R = .85$ for ITGA5 and talin and $R = .45$ for ITGV and talin (B). Correlation coefficient bars represent average values $\pm$ standard error of the mean (SEM).

and protein levels are lower in laboring human myometrium compared to nonlaboring myometrium.¹³ Regulation of ITGA5 expression has been extensively studied in the rat model and both ITGA5 transcript and protein are upregulated in rat uterus during pregnancy.⁹ In the rat, ITGA5 expression is regulated at least in part by mechanical stretch of uterine tissue. Shynlova et al observed a 4- to 5-fold increase in *ITGA5* transcript and a modest increase in ITGA5 protein in the gravid horn versus the empty horn. In this animal model, *ITGA5* transcript doubled in response to the RU486, suggesting that mechanical tension rather than rising progesterone levels of pregnancy are likely to be responsible for the observed expression changes.¹⁰ We

observed high levels of colocalization between ITGA5 and the focal adhesion complex proteins vinculin and talin in our term pregnant samples, in agreement with reports in other species.^{9,11}

The ITGA7 pairs with ITGB1 to form a laminin receptor.²² The ITGA7 expression has been shown to increase in differentiated skeletal muscle²³ and is required for expression and development of the contractile phenotype in airway smooth muscle cells.²⁴ We observed significant increases in both ITGA7 transcript and protein in both laboring and nonlaboring term human myometria compared to nonpregnant controls. We also observed colocalization between ITGA7 and the focal

adhesion protein vinculin in our term human myometrial samples.

The ITGAV can pair with several β chains to form integrins avb1, avb3, avb5, avb6, and avb8. These integrin heterodimers bind to fibronectin, vitronectin, fibrinogen, or tumor necrosis factor β -latency-associated protein, many at RGD (Arg-Gly-Asp) sites. We observed a 2-fold increase in *ITGAV* transcript and ITGAV protein in the term nonlaboring uterus compared to nonpregnant uterine controls. Although present in uterine myometrium, we did not observe high levels of colocalization between ITGAV and the focal adhesion protein talin.

The ITGB3 pairs with ITGAV and is a marker for uterine receptivity during the implantation window of early pregnancy. In uterine endometrium and Ishikawa cells (uterine epithelial), *ITGB3* transcription is increased by epidermal growth factor and inhibited by estrogen and progesterone.^{25,26} We observed 4-fold more ITGB3 protein in the term, nonlaboring pregnant uterine tissue (when circulating progesterone levels are high 160) compared to the nonpregnant uterus (when circulating progesterone levels are low) suggesting other factors are important for the upregulation of ITGB3 in the pregnant myometrium. However, it is important to note that *ITGB3* was predominantly expressed in the uterine vasculature and not the myometrial cells.

The *ITGB1* transcript is increased ($\sim 10\times$) in the pregnant mouse uterus throughout pregnancy and returns to nonpregnant levels by 4 days postpartum. Although Williams et al did not observe changes in ITGB1 protein abundance by Western blot, they did observe increased membrane localization.⁸ We observed a modest (1.65-fold) increase in *ITGB1* transcript in the pregnant, nonlaboring uterus compared to nonpregnant controls, but did not detect changes in total ITGB1 protein during pregnancy. The differences in the magnitude of transcript change between our observations and those of Williams et al may represent genuine species differences or differences in primers used. We observed colocalization between ITGB1 and the focal adhesion protein vinculin, consistent with the role of ITGB1 as a binding partner to ITGA3, ITGA5, and ITGA7.

It is interesting to note that the increased transcript levels of ITGA5, ITGA7, ITGAV, and ITGB3 corresponded to increased protein levels, while increases in ITGA1, ITGA3, ITGB1, and ITGB5 did not correspond to increased protein levels. Cells can control molecular expression at both the levels of transcription and translation. Indeed, for many genes transcript levels display little correlation with the final protein levels.²⁷ Possible explanations for these observations might include variations in posttranscriptional processing and differences in protein stability.²⁷

We observed a discrete, punctate integrin (ITGA3, ITGA5, ITGA7, and ITGB1) and vinculin labeling pattern around myometrial cells in our term human samples, but not in our nonpregnant myometrial samples. In the pregnant rat myometrium, organized focal adhesion complexes form relatively late in pregnancy in response to both hormonal and mechanical cues.⁹ In contrast, in the sheep model, the ITGA5

integrin chain localizes to myometrial focal adhesion complexes that begin to form during the first trimester.¹¹ Since focal adhesion signaling has been implicated in myometrial contractility and in coordinating contraction throughout the tissue,^{3,28} it will be interesting to examine preterm human samples to determine whether myometrial integrins localize to discrete focal adhesions in early or late pregnancy and whether their presence at these sites is associated with preterm labor.

Among the myometrial genes that were downregulated during pregnancy are E-cadherin (*CDH1*/cadherin) and catenin/cadherin-associated protein delta 2 (*CTNND2*; Supplemental Table 1). The CDH1 is a cell-cell adhesion molecule that regulates uterine formation, cell proliferation, and apoptosis.²⁹ Loss of *Cadhl* leads to loss of adherens and tight junctions, abnormal cell proliferation, and apoptosis in murine uterine epithelia, but does not cause obvious myometrial defects.²⁹ When CTNND2 is low, cadherin is targeted for degradation and junctions are destabilized.³⁰ Together with our observation that myometrial focal adhesions form during pregnancy, these data suggest the possibility that myometrial cell adhesion structures may undergo a switch from cadherin-rich adhesions junctions in the nonpregnant state to integrin-rich focal adhesions at term.

In summary, we observed increases in several integrin chains at both the messenger RNA and protein levels in human myometrium during pregnancy. Colocalization of the ITGA3, ITGA5, ITGA7, and ITGB1 chains with focal adhesion proteins at term suggests a potential role for $\alpha 5\beta 1$, $\alpha 3\beta 1$, and $\alpha 7\beta 1$ in regulating myometrial contractility. Crosstalk between these integrins may also play a role in mechanotransduction and coordination of myometrial contraction during pregnancy.

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Authors' Note

Supplemental Table 1 is available online at <http://rsx.sagepub.com/supplemental>.

Declaration of Conflicting Interests

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