Expression of erythropoietin messenger ribonucleic acid in wild-type *MED12* uterine leiomyomas under estrogenic influence: new insights into related growth disparities

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Objective: To determine factors that impact erythropoietin (EPO) production in leiomyomas. We have previously implicated EPO production in promoting the growth of some leiomyomas.
Design: The relationship between EPO messenger RNA (mRNA) expression and *MED12* gene mutations or mRNA expression levels of high-mobility group AT-hook (HMGA) 1 and HMGA2 were analyzed. Effects of 10^{-8} M 17β-E_2 on EPO mRNA expression were evaluated using leiomyoma cells grown in primary cultures.
Setting: Graduate school of medicine.
Patient(s): Patients with leiomyoma.
Intervention(s): We used tissue samples and clinical data of 108 patients with leiomyomas to analyze the relation between EPO mRNA expression and *MED12* mutation. Tissue samples from another 10 patients with leiomyomas were collected for in vitro experimentation using primary cultures of leiomyoma and myometrial cells.
Main Outcome Measure(s): Relations between EPO mRNA expression, *MED12* exon 2 mutation, and HMGA1/HMGA2 mRNA expression levels in leiomyoma samplings, in addition to effects of estrogen (E) on EPO mRNA expression in cultures of leiomyoma cells.
Result(s): The EPO mRNA level was threefold higher in leiomyomas with wild-type (vs. mutated) *MED12* genes. There was no correlation between EPO and HMGA1 or HMGA2 mRNA expression levels. In wild-type *MED12* leiomyomas only, E_2 treatment produced a twofold increase in EPO mRNA expression, whereas mutated *MED12* leiomyomas were unaffected.
Conclusion(s): The EPO mRNA expression increased significantly after E_2 treatment only in leiomyomas lacking *MED12* mutations. In conjunction with prior evidence linking EPO mRNA expression levels and tumor size, E_2-stimulated EPO mRNA expression may explain the marked growth disparities seen in these tumors. (Fertil Steril® 2019;111:178–85. ©2018 by American Society for Reproductive Medicine.)
El resumen está disponible en Español al final del artículo.

Key Words: Uterine leiomyoma, erythropoietin, MED12, estrogen, erythrocytosis

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Worldwide, uterine leiomyoma is the most frequent tumor in women, with a cumulative incidence of >70% by the age of 50 years (1). Nevertheless, the mechanisms by which these tumors develop and grow are not fully understood. The broad spectrum of tumor growth displayed is of particular interest. Some patients may require medical intervention due to rapid, massive growth, whereas in others, these tumors may grow slowly or remained unchanged for indefinite periods of time. More insight into factors promoting growth in leiomyomas may enable gynecologists to anticipate their eventual behaviors and adopt management plans beneficial to patients.

Erythropoietin (EPO) is produced mainly in the kidneys and is an important hormone for erythrocytes. In addition to its hematopoietic activity, it has been reported that EPO is produced in tissues such as neurons, skin, and heart. In addition, increasing evidence suggests that EPO contributes to protecting tissues from ischemic damage and tissue remodeling through cell differentiation, control of apoptosis, angiogenesis, and/or vasculogenesis (2). We have previously reported that EPO is on occasion produced in uterine leiomyomas, and EPO messenger RNA (mRNA) levels seem to correlate with bulkier tumors, suggesting that EPO may promote the growth of leiomyomas in an autocrine and/or paracrine manner (3). Because EPO expression in leiomyomas varies dramatically from patient to patient, ranging from undetectable to extremely high levels, we believe that this variability in EPO expression may have bearing on the growth patterns of leiomyomas.

Recent reports have cited two major cytogenetic abnormalities specific for uterine leiomyomas. In 48%–92% of patients with leiomyomas, somatic mutations have been found to involve exon 2 of the mediator complex subunit 12 (*MED12*) gene (4). Such mutations seem deeply rooted in tumorigenesis through attenuated mediator-associated cyclin-dependent kinase (CDK) activity and overexpression of *RAD51 B* (5). It is also apparent that patients with *MED12* mutations (MUT) (vs. wild-type [WT] *MED12*) tend to develop multiple and smaller-sized leiomyomas by comparison (6). Chromosomal rearrangements likewise occur with considerable frequency (about 50%) in association with leiomyomas, regularly involving the high-mobility group AT-hook 1 and 2 (*HMGA1, HMGA2*) genes. They are therefore overexpressed in most instances of leiomyomas with chromosomal rearrangements (7). The HMGA proteins regulate transcription for different genes, and *HMGA* overexpression is known to drive the proliferation of cells in malignant and benign tumors, including leiomyomas (8).

It seems logical that specific gene alterations may govern the biologic divergence of leiomyomas, which prompted us to explore differing EPO expression patterns in leiomyomas due to such genetic alterations. In the present study, we investigated whether *MED12* exon 2 mutations or HMGA genetic overexpression might correlate with expression levels of EPO mRNA in leiomyomas. In addition, we examined changes in EPO mRNA expression when stimulated by estrogen (E), the master hormone contributing to the growth of uterine leiomyomas (9), using leiomyoma cells grown in primary culture to assess the mechanisms involved.

MATERIALS AND METHODS

This study was approved by the Ethics Committee of Yokohama City University Graduate School of Medicine (IRB approval #A150122017, 27 July 2012). All study subjects granted written informed consent.

We analyzed the same tissue samples and clinical data of patients investigated in our previous report (3) to explore the presumed cytogenetic basis of EPO mRNA expression, specifically *MED12* MUT and HMGA1/HMGA2 mRNA levels. Within the prior dataset of 114 cases, there were little or no tissue reserves for 6 patients. Upon their exclusion, a total of 108 remained for reanalysis. However, another 10 leiomyomas and corresponding myometrial samples were collected anew to enable primary cell cultures for in vitro experimentation. The specimens were obtained during surgery, snap frozen in liquid nitrogen, and stored (−80°C) for RNA and DNA analyses or transferred to sterile medium for primary cultures.

Isolation and Primary Culture of Leiomyoma and Myometrial Cells

The tissue samples were washed several times in sterile Dulbecco's Modified Eagle Medium (DMEM; Life Technologies) with 1% antibiotic solution (Antibiotic-Antimycotic; Life Technologies), then minced for disaggregation by 0.2% collagenase type I (Worthington Biochemical Corp.) in DMEM at 37°C. After 3–5 hours, the cells were filtered through a Falcon 70-μm cell strainer (Corning Life Sciences), washed with medium, centrifuged twice, and transferred into 90-mm Celltight C-1 dishes (Sumitomo Bakelite Co.) at 2×10^6 cells/dish, immersed in 10 mL DMEM supplemented with 10% fetal bovine serum (Life Technologies) and 1% antibiotic solution. After incubation in 5% CO_2 at 37°C, the cultures were passaged using Accutase (Innovative Cell Technologies) at 80% confluence to 6-well plates (Celltight C-1 Plate 6F; Sumitomo Bakelite Co.), dispensing 3×10^5 cells/medium-filled well (3 mL). At approximately 70% confluence, the medium was changed to DMEM, low glucose, pyruvate, no glutamine, no phenol red, supplemented with 4 mM L-glutamine and 10% charcoal-stripped fetal bovine serum (Life Technologies), then incubated for 24 hours for further experiments. All experiments were performed using first-passage primary culture cells, given that the viability of cells isolated from *MED12*-mutated leiomyomas may rapidly decline in vitro (10, 11).

Application of 17β-E_2, Fulvestrant, And/or Hypoxia

The cultured cell isolates from 10 leiomyomas and 8 corresponding myometrial samples were individually treated for 8 hours with 10^{-8} M 17β-E_2 (Millipore Sigma). Five of the 10 leiomyoma cell samples showed increased expression of EPO in response to E_2, and four of these five leiomyoma cell samples (deemed adequate cell number for use in subsequent experiments) were cultured for 8 hours with E_2 and/or 1 μM fulvestrant (ICI 182,780; Abcam), an E receptor antagonist. In hypoxia experiments, four of six *MED12* WT cell samples were cultured under 1% O_2 with 5% CO_2 for 24 hours, with or

without 10^{-8} M E_2, to assess the impact on EPO expression in leiomyoma cells. The cells were then washed twice using cold phosphate-buffered saline (PBS), collected with Sepasol-RNA I Super G (Nacalai Tesque Inc.), and stored at -80°C for real-time reverse transcription-polymerase chain reaction (PCR). All experiments were performed in triplicate.

Immunocytochemistry

To confirm the smooth muscle nature of cells grown in primary cultures, we targeted α-smooth muscle actin by immunocytochemistry. The cells were seeded onto Permanox chamber slides (Nunc Lab-Tek Chamber Slide System; Thermo Fisher Scientific), and at semiconfluence, the cells were fixed in 95% ethanol for $\geq$30 minutes. They were then washed in sequence using deionized water followed by PBS, and endogenous peroxidase activity was blocked using 3% H_2O_2 in methanol. After washing twice with PBS, the slides were incubated with 10% rabbit serum for 15 minutes to block nonspecific binding of the secondary antibody. Primary antibody (mouse anti-α-smooth muscle actin [code 712021], ready-to-use; Nichirei Biosciences Inc.) was applied and the slides were incubated for 60 minutes on a shaker at room temperature. The slides were subsequently washed with PBS and incubated for 10 minutes with biotinylated rabbit anti-mouse antibody on a shaker at room temperature, again washing with PBS for streptavidin peroxidase application for 5 minutes. 3,3′-Diaminobenzidine served as an immunoreactive chromogen and hematoxylin as a counterstain. All procedures were performed at room temperature.

Real-Time Reverse Transcription PCR

We used the same real-time reverse transcription PCR protocol described in our previous report (3). In brief, total RNA of tissue or cells grown in primary cultures was prepared using Sepasol-RNA I Super G (Nacalai Tesque) and the illustra RNAspin Mini RNA Isolation Kit (GE Healthcare). The SuperScript VILO Master Mix (Life Technologies) was used for reverse transcription.

Polymerase chain reactions were carried out on a 7900HT Fast Real-Time PCR System (Life Technologies). Expression levels of mRNA were determined from standard curves generated by proprietary software. TaqMan Gene Expression Assays (Life Technologies) were conducted for amplification of EPO (ID: Hs00171267_m1), HMGA1 (ID: Hs00852949_g1), HMGA2 (ID: Hs04397751_m1), β-actin (ID: 4326315E), and glyceraldehyde-3-phosphate dehydrogenase (ID: 4310884E). β-Actin or glyceraldehyde-3-phosphate dehydrogenase was used as an internal control.

Sanger Sequencing of *MED12*

The leiomyoma tissue samples were analyzed for *MED12* exon 2 hotspot mutations. In addition, we confirmed that primary culture cells derived from tissues with *MED12* mutations possessed the same *MED12* mutations as the original tissues. The DNA was extracted using QIAamp DNA Mini Kit (Qiagen) according to the manufacturer's protocol and quantified by NanoDrop 2000 Spectrophotometer (Thermo Fisher Scientific). Mutated samples were designated *MED12* MUT and those without mutations as *MED12* WT Primers for Sanger sequencing used herein are cited elsewhere: forward primer, GCCCTTTCACCTTGTTCCTT; reverse primer, TGTCCCTATAAGTCTTCCCAACC) (12). The PCR was performed using the AccuPrime Taq DNA Polymerase System (Thermo Fisher Scientific) with the following sequence: 94°C for 2 minutes, followed by 35 cycles of 94°C for 15 seconds, 60°C for 15 seconds, 68°C for 35 seconds, and maintained at 10°C after reactions. The PCR products were purified using ExoSAP-IT PCR Product Cleanup (Affymetrix). Sanger sequencing of PCR products was performed using BigDye Terminator v3.1 Cycle Sequencing Kit and 3130 Genetic Analyzer (Applied Biosystems) according to the manufacturer's protocol.

Statistical Analyses

All statistical computations relied on IBM SPSS Statistics v22.0. Gene expression levels were compared in univariate analysis, using Mann-Whitney *U* test, Wilcoxon signed-rank test, Spearman's rank correlation coefficient, or Fisher's exact test. To assess EPO expression levels in primary culture cells under various conditions, Dunnett's test was applied. The χ^2 test for trend served to analyze the relation between *MED12* mutation and EPO expression. Multivariate analysis was performed by multivariate regression analysis. Values of $P<.05$ were considered statistically significant.

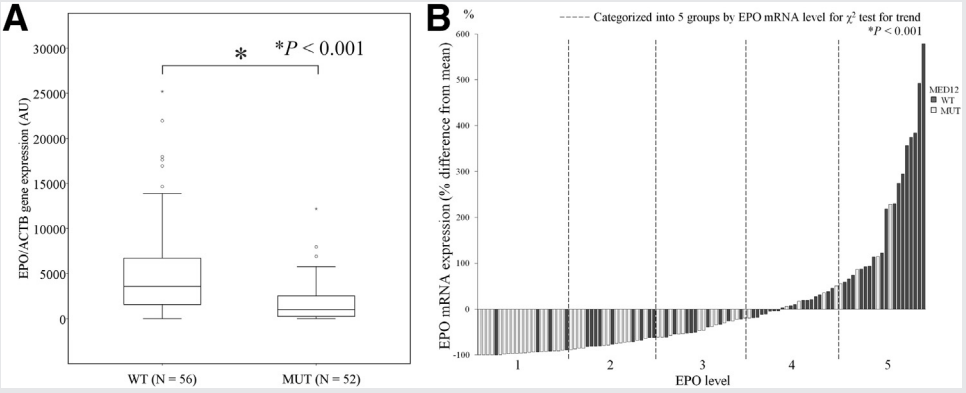
RESULTS

EPO Expression Levels in Relation to *MED12* Mutation and Expression of HMGAs in Leiomyoma Tissue Samples

In tissue obtained from108 leiomyomas that we collected during our previous study (3), *MED12* exon 2 mutations were detected in 52 samples (48%), with point mutations accounting for 73% (Supplemental Table 1, available online). *MED12* WT (vs. *MED12* MUT) leiomyomas were associated with significantly higher levels of EPO mRNA expression (EPO level: WT, 5,453 ± 5,721; MUT, 1,833 ± 2m390; $P=.001$, Mann-Whitney *U* test) (Fig. 1A). As shown in the waterfall plot (Fig. 1B), more EPO mRNA expression in leiomyomas most often corresponded with *MED12* WT status. For statistical purposes, we stratified samples into five categories by EPO mRNA level, again underscoring that leiomyomas with higher expression levels of EPO mRNA were more likely to be *MED12* WT ($P<.001$, χ^2 test for trend) (Fig. 1B). In addition, *MED12* WT leiomyomas were comparatively larger in size than *MED12* MUT counterparts (tumor diameter: WT, 12.0 ± 6.7 cm; MUT, 8.9 ± 2.6 cm; $P=.037$, Mann-Whitney *U* test) (Supplemental Fig. 1, available online).

The HMGA1 and HMGA2 mRNA expression levels in leiomyoma tissue samples did not correlate with levels of EPO mRNA expression, regardless of *MED12* mutation status (Fig. 2). Analysis of the relation between EPO mRNA expression and specific patient variables showed that leiomyoma diameter and menopausal status correlated significantly with EPO mRNA expression, whereas other variables such

FIGURE 1



Relation between *MED12* exon 2 mutation status and EPO mRNA expression level. (**A**) Comparison of EPO mRNA expression levels in *MED12* WT (n=56) and *MED12* MUT (n=52) leiomyomas (*P*-value calculated via Mann-Whitney *U* test). *P<.001. (**B**) Waterfall plot of EPO mRNA expression by real-time RT-PCR in leiomyoma tissue samples. The samples were categorized into five groups by EPO mRNA expression level. Group 1: −100∼−89%, Group 2: −89∼−62%, Group 3: −62∼−19%, Group 4: −19∼+51%, Group 5: over +51% of the mean EPO mRNA level, respectively. The correlation between higher EPO mRNA level and wild-type *MED12* was calculated via χ^2 test for trend. *P<.001.

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as age, body mass index, or parity were unrelated (Supplemental Table 2, available online). In further multiple regression analysis aimed at tumor diameter, *MED12* MUT, and menopausal status, EPO mRNA expression levels showed significant correlation with *MED12* MUT and tumor diameter (Supplemental Table 3, available online), which is consistent with our previous report (3).

Effects of E_2 on EPO mRNA Expression in Primary Cultures of Leiomyoma Cells

Leiomyoma tissue samples were contributed by another 10 patients, 8 of whom also provided myometrial samples. Patient characteristics in this experimental subset are shown in Table 1. *MED12* exon 2 mutations were identified in 4 of 10 leiomyoma tissues. In all instances, with or without *MED12* MUT, further testing was limited to first-passage primary culture cells, because cell isolates from *MED12* MUT leiomyomas tend to diminish in culture (10, 11). The status of *MED12* exon 2 mutation in primary culture cells and in corresponding tissue samples proved identical. Cultured leiomyoma and myometrial cells were positive for α-smooth muscle actin in >90% in all of the samples tested (Supplemental Fig. 2, available online).

Basal levels of EPO mRNA expression in cells of the eight paired, non-E_2 treated, leiomyoma/myometrial samples indicated significantly higher levels in the leiomyomas than in the myometrium (myometrium, 4,368 ± 3,919; leiomyomas, 2,3976 ± 1,4357; *P*=.012, Wilcoxon signed-rank test) (Fig. 3A). In samples comprised of the same eight pairs and two additional leiomyomas, E_2 treatment produced significant enhancement of EPO mRNA expression in the leiomyoma samples alone, with no commensurate increase of EPO mRNA in the myometrium (median fold change: myometrium, 1.2 [range, 0.5–1.3]; *P*=.207; leiomyomas, 1.6 [range, 0.8–3.0]; *P*=.017; Wilcoxon signed-rank test) (Fig. 3B).

The EPO mRNA expression levels in leiomyoma cells of *MED12* WT were robustly heightened by E_2 treatment in five of six samples, whereas responses to E_2 in the four samples of *MED12* MUT were marginal (Fig. 3C). Namely, responsiveness to E_2 treatment by *MED12* WT leiomyomas proved significantly more than that of leiomyomas with *MED12* mutations (*P*=.048, Fisher's exact test).

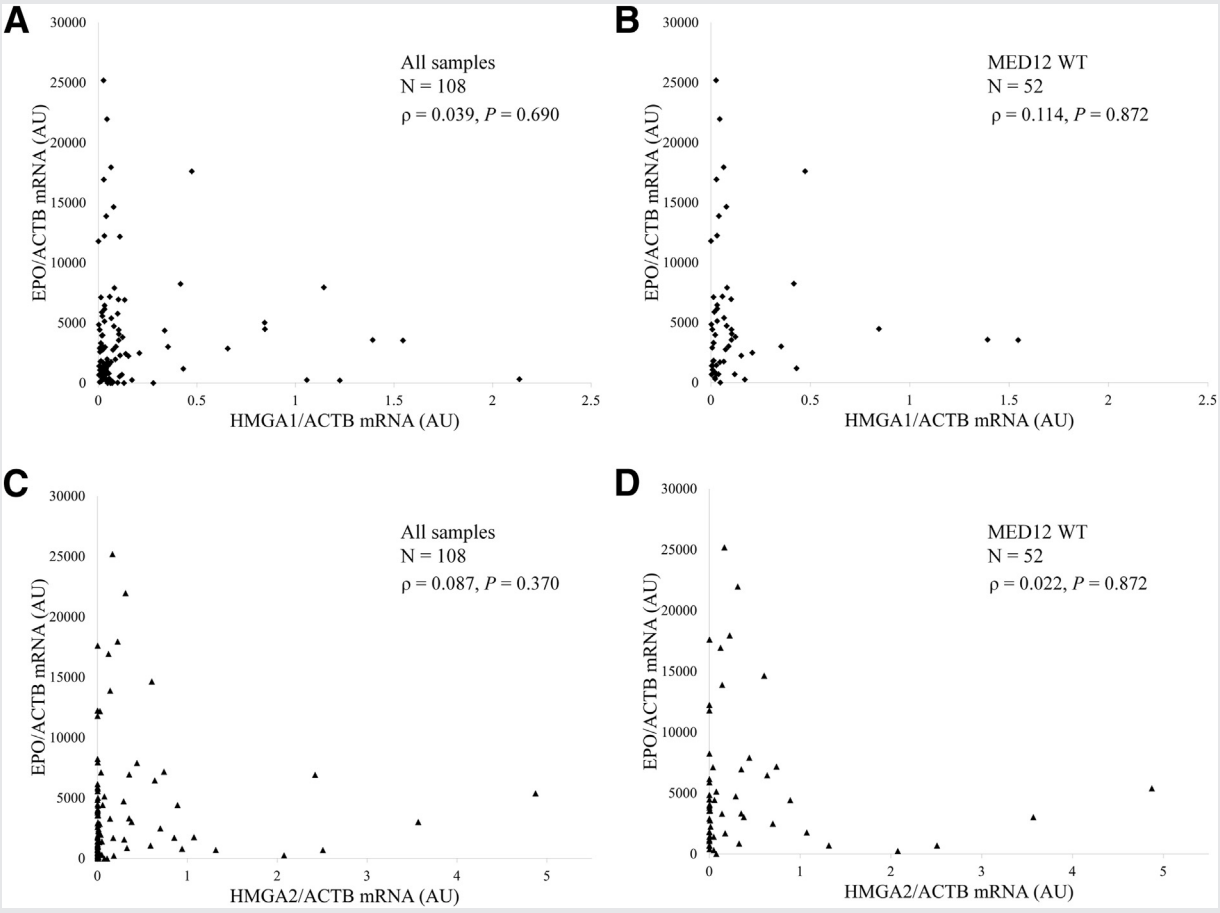
To evaluate whether the effect of E_2 on EPO mRNA expression occurred through E receptor (ER) signaling, leiomyoma cells grown in primary cultures and originating from four of five leiomyoma samples showing robust EPO mRNA expression in response to E_2 were treated with E_2 and the ICI E receptor blocker. Sample 10 was excluded from this experiment, lacking a sufficient number of isolated leiomyoma cells. As a result, the expected surge in EPO mRNA expression after E2 treatment was significantly inhibited (Fig. 3D).

DISCUSSION

Previously we reported that some leiomyomas are inclined to produce more EPO than surrounding myometrium and that the overall size of a leiomyoma correlates with EPO expression level, thus implicating aberrant expression of EPO in the growth of leiomyomas (3). During the present study, we further characterized leiomyomas that are capable of high-level EPO mRNA expression, documenting an association between such augmented production and *MED12* WT status, whereas low-level EPO mRNA expression prevailed in *MED12* MUT leiomyomas. In addition, we have shown that in EPO-producing *MED12* WT leiomyomas, levels of EPO mRNA are enhanced significantly by E_2 administration. Consequently, it seems that a specific phenotype of leiomyoma imparts an advantage in terms of tumor growth through expression of EPO and responsiveness to E.

Given our former observation that >50% of leiomyomas express higher levels of EPO than the myometrium they

FIGURE 2



Relation between HMGA and EPO mRNA expression levels. Spearman correlation coefficients (ρ) reflecting correlations between expression levels of HMGA1 or HMGA2 mRNA and EPO mRNA. (**A**, **B**) HMGA1 and EPO mRNA expression levels; and (**C**, **D**) HMGA2 and EPO mRNA expression levels (**A**, **C**: all samples; **B**, **D**: *MED12* WT samples).

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inhabit (3), we initially speculated that *MED12* mutations, the major genetic alterations occurring in leiomyomas, accounted for aberrant EPO expression by leiomyoma cells. Surprisingly, however, we found significant elevations of EPO mRNA expression levels in *MED12* WT tumors only (Fig. 1B), indicating that a normal function of *MED12* mutation might be crucial in EPO production. We then explored potential links between EPO and HMGA1 or HMGA2 mRNA expression levels. Overexpression of HMGA1 and HMGA2 due to chromosomal aberrations have been observed in 6% and 20% of patients with leiomyomas, respectively (13, 14); and *HMGA* genes are known to be the second-most influential in leiomyoma development. Still, no significant correlation emerged in this regard (Fig. 2), indicating that EPO expression in leiomyoma cells is independent of HMGA protein overexpression. Because the two chief cytogenetic factors implicated in tumorigenesis of uterine smooth muscle may not be the reason for elevated EPO, we are currently conducting a comprehensive search to pinpoint the genetic origins of aberrant EPO production in leiomyoma cells.

Our experiments using leiomyoma cells grown in primary culture have likewise demonstrated that E, a master hormone regulating the growth of leiomyomas, enhances EPO mRNA expression in *MED12* WT leiomyoma cells. On the other hand, EPO mRNA expression in leiomyoma cells cultured under hypoxic condition was not altered (Supplemental Fig. 3, available online). It is well known that EPO expression is largely stimulated by hypoxia through a cascade of hypoxia-inducible factors (HIFs) (15). Unlike other bodily tissues/organs, leiomyomas express low levels of hypoxia-related markers (including HIFs), despite inherently low internal oxygen pressure, signifying a probable loss of responsiveness to hypoxia (16, 17). Thus, the limited hypoxia-induced EPO expression demonstrated in our experiment is aligned with previous evidence establishing a loss of response to hypoxia by leiomyoma cells.

We have instead confirmed that E_2 increases EPO production in *MED12* WT leiomyomas. It has been reported by other investigators that E_2 inhibits hypoxia-induced EPO production in the kidney (18). At the same time, other studies have

TABLE 1

Patients' characteristics of primary culture samples (n = 10).

Characteristics	Data
Mean age (range)	43.3 y (37–48)
Mean BMI (range)	23.0 kg/m^2 (19.4–30.8)
Parity	
0	3
≥1	7
Menstrual status	
Premenopausal	10
Postmenopausal	0
GnRH-a therapy before surgery	4
Mean size of sampled leiomyoma (range)	9.4 cm (6.5–15.8)
Mean hemoglobin (range)	11.9 g/dL (11.6–12.5)

Note: BMI = body mass index; GnRH-a = gonadotropin-releasing hormone agonist.

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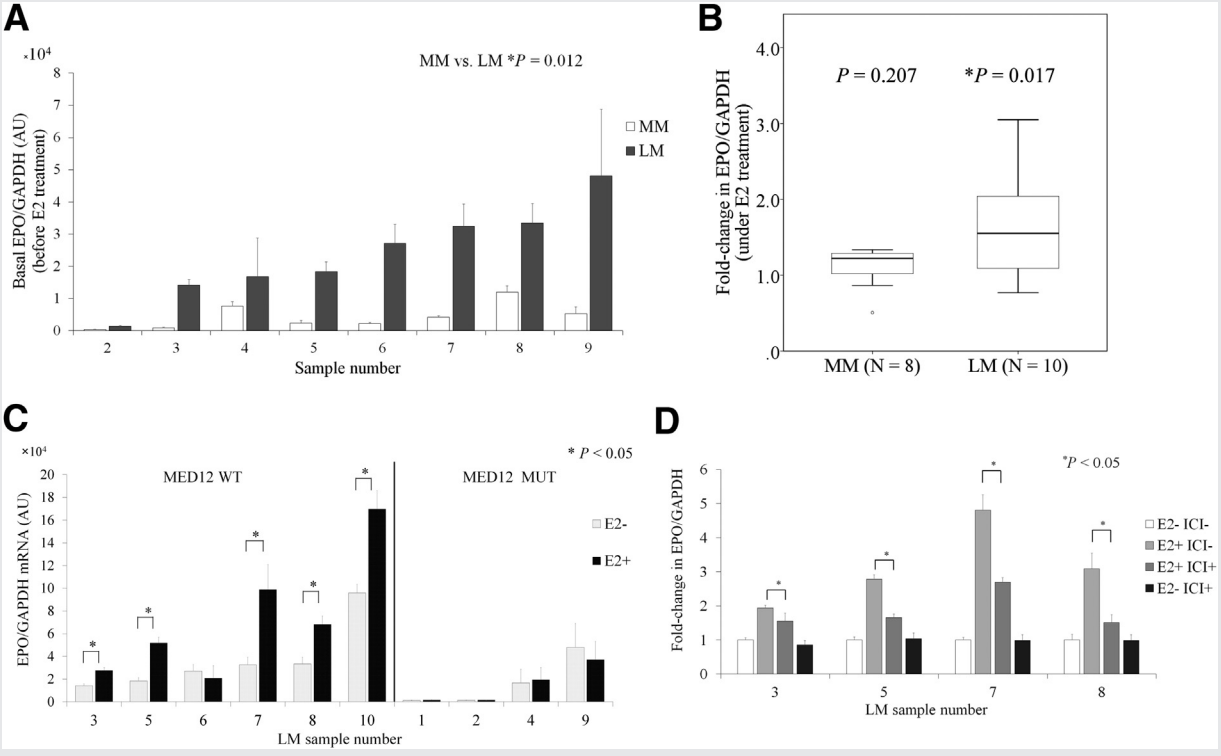
indicated that E_2 increased EPO production in murine uteri independently or in combination with hypoxia (19–21). Because our data also demonstrated that induction of EPO mRNA by E_2 in *MED12* WT leiomyoma was abolished by ICI ER blocker, it is likely that EPO mRNA expression is subject to an E-linked receptor pathway. Regulation of EPO production in the uterus or in leiomyomas seems distinctive from that of other organs and is intriguing as a means of exploring precise mechanisms of EPO production in E_2-responsive leiomyomas.

Myomatous erythrocytosis syndrome is a rare disease entity in which erythrocytosis develops as a consequence of EPO secretion by leiomyoma cells. Reported tumor sizes in instances of myomatous erythrocytosis syndrome are generally of gigantic proportions (22), but even in the absence of myomatous erythrocytosis syndrome, our previous study illustrates that EPO mRNA expression levels in leiomyomas correlate with tumor size (3). Therefore, it is likely that EPO produced by these tumors is influential in their growth. The present study further reveals that *MED12* MUT (vs. *MED12* WT) leiomyomas express comparatively lower levels of EPO mRNA (Fig. 1B). This finding caught our attention, because *MED12* MUT leiomyomas have been reported as smaller-sized (compared with *MED12* WT tumors) (6), just as the tumors studied herein (Supplemental Fig. 1).

FIGURE 3



Expression levels of EPO mRNA in primary cultures of normal myometrium and leiomyomas. (**A**) Comparison of EPO mRNA expression levels in non-estrogen treated leiomyoma (LM) and myometrial (MM) paired cellular samples (n=8). In all samples, EPO mRNA expression levels of LM exceeded those of MM; (**B**) Fold changes in EPO mRNA expression after E2 treatment in cultures of MM (n=8) and LM (n=10) samples (*P*-value calculated by Wilcoxon signed-rank test); (**C**) Changes in EPO mRNA expression levels after E2 treatment in *MED12* WT and *MED12* MUT leiomyomas; (**D**) Effect of E2 on EPO mRNA expression with or without ICI blocking (LM samples used for experiment clearly showing induction of EPO by E2). *$P<.05$ (Dunnett's test). Error bar, SD.

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Mediator complex subunit 12 is one of the RNA polymerase II transcriptional mediator complex subunits, assembling with MED13, cyclin C, and CDK8 (or CDK19, isoform of CDK8) (23) and playing important roles in the transcription of various genes. The *MED12* mutations in leiomyomas result in disruption of CDK8/CDK19 activity and thus attenuate of cyclin C-dependent activity (24, 25). In addition, inhibition of CDK8 in ER-positive breast cancer cells has resulted in suppression of E-induced transcription and diminished cell growth (26). It is therefore conceivable that *MED12* mutations inhibit E-induced transcription due to disrupted CDK8 activity, and attenuated EPO mRNA expression in response to E_2 may be one reason why *MED12* MUT tumors are generally smaller than *MED12* WT tumors. In our experiments, we focused solely on the relation between EPO expression and *MED12* exon 2 mutations, representing most of *MED12* mutations in leiomyomas (6, 27). However, there is mounting evidence that *MED12* exon 1 mutation is involved in the tumorigenesis of leiomyomas (5, 11). We cannot as yet confirm this premise, which clearly remains a topic of our future study.

Finally, it is quite possible that leiomyomas under stable hypoxic states may achieve only limited growth due to HIF nonresponse. Our previous efforts have divulged a proclivity for more mature blood vessels in high-level EPO-producing leiomyomas (3). Coupled with present findings, this observation indicates that some leiomyomas, lacking *MED12* mutations, very likely produce high levels of EPO under estrogenic influence, independent of hypoxia. Hence, the prolific growth of EPO-producing leiomyomas may well be promoted by extraordinarily favorable vascularity and circulation in tumors prone to hypoxia. Although the regulatory mechanisms for EPO expression in *MED12* WT leiomyomas and their functional ramifications have yet to be fully elucidated, the data herein support a novel phenotype capable of sizeable growth in response to E.

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Expresión de ARNm de eritropoyetina en los leiomiomas uterinos MED 12 de tipo salvaje bajo la influencia de estrógenos: nuevos aspectos relacionados con disparidades de crecimiento

Objetivo: Determinar qué factores tienen efecto en la producción de eritropoyetina (EPO) en los leiomiomas. La implicación en la producción de EPO sobre el crecimiento de algunos leiomiomas ha sido previamente descrita por nosotros.

Diseño: Análisis de la relación entre la expresión de ARNm de EPO y las mutaciones en el gen de MED 12 y los niveles de expresión de ARNm del grupo gancho AT de alta movilid (HMGA) tipo 1 y tipo 2 (HMGA2). Los efectos de 10^8 M 17b-E_2 en la expresión de ARNm de EPO fueron evaluados en cultivos primarios de células de leiomiomas.

Lugar: Escuela superior de medicina.

Pacientes: Pacientes con leiomiomas.

Intervenciones: Se utilizó tejido y datos clínicos de un total de 108 pacientes con leiomiomas y se estudió la relación entre los niveles de ARNm de EPO y las mutaciones en el gen MED12. Para los experimentos in vitro con cultivos primarios de células de leiomiomas y células del miometrio se utilizaron un total de 10 muestras más procedentes de 10 pacientes con leiomiomas.

Medidas principales: Relación entre la expresión de ARNm de EPO, las mutaciones en el exón 2 del gen MED12 y los niveles de expresión de HMGA1/HMGA2. Además se estudiaron los efectos de los estrógenos (E) en la expresión de ARNm de EPO en cultivos de células de leiomiomas.

Resultados: Los niveles de expresión de ARNm de EPO fue tres veces mayor en los leiomiomas portadores del gen salvaje MED 12 con respecto a las muestras portadores del gen mutado MED 12. No hubo correlación entre los niveles de EPO y los niveles de expresión de HMGA1 o HMGA2. En los leiomimas con MED 12 de tipo salvaje el tratamiento con estradiol incrementó dos veces la expresión de EPO mientras que en los casos con el gen MED12 mutado no se observó ningún efecto.

Conclusiones: La expresión de ARNm de EPO se incrementa significativamente tras el tratamiento con estradiol solo en las muestras no portadoras de la mutación en el gen MED 12. En conjunto con las evidencias previas que mostraban relación entre ARNm de EPO y el tamaño del tumor, la expresión de ARNm por estimulación con estrógenos puede explicar las disparidades de crecimiento en estos tumores.