Myometrial progesterone hyper-responsiveness associated with increased risk of human uterine fibroids

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Abstract

Background: Uterine Fibroids (UFs) growth is ovarian steroid-dependent. Previous studies have shown that estrogen and progesterone play an important role in UF development. However, the mechanism underlying progesterone induced UF pathogenesis is largely unknown. In this study, we determined the expression of progesterone receptor and compared the expression level of progesterone-regulated genes (PRGs) in human myometrial cells from normal uteri (MyoN) versus uteri with UFs (MyoF) in response to progesterone.

Methods: Primary human myometrial cells were isolated from premenopausal patients with structurally normal uteri (PrMyoN). Primary human myometrial cells were also isolated from uterus with UFs (PrMyoF). Isolated tissues were excised at least 2 cm from the closest UFs lesion(s). Progesterone receptor (PR) expression was assessed using Western blot (WB). Expression levels of 15 PRGs were measured by qRT-PCR in PrMyoN and PrMyoF cells in the presence or absence of progesterone.

Results: WB analysis revealed higher expression levels of PR in PrMyoF cells as compared to PrMyoN cells. Furthermore, we compared the expression patterns of 15 UF-related PRGs in PrMyoN and PrMyoF primary cells in response to progesterone hormone treatment. Our studies demonstrated that five PRGs including Bcl2, FOXO1A, SCGB2A2, CYP26a1 and MMP11 exhibited significant progesterone-hyper-responsiveness in human PrMyoF cells as compared to PrMyoN cells (P < 0.05). Another seven PRGs, including CIDEC, CANP6, ADHL5, ALDHA1, MT1E, KIK6, HHI showed gain in repression in response to progesterone treatment (P > 0.05). Importantly, these genes play crucial roles in cell proliferation, apoptosis, cell cycle, tissue remodeling and tumorigenesis in the development of UFs.

Conclusion: These data support the idea that progesterone acts as contributing mechanism in the origin of UFs. Identification and analysis of these PRGs will help to further understand the role of progesterone in UF development.

Keywords: Uterine fibroids, Progesterone, Progesterone-regulated genes, Progesterone receptor
reduction [6–8]. In addition, LNG (levo –norgestrel) (treatment in vitro decreased UF cell viability and induced apoptosis. Similarly, a number of antiprogestin drugs and SPRM (selective progesterone receptor modulator) have been developed and tested in clinical trials for the treatment of UF’s, including Mifepristone, Asoprisnil and Ulipristal acetate. These studies provide strong evidence for the mitogenic effect of progesterone on UF pathogenesis [9–11].

The progesterone responses are mediated by two pathways, the rapid non-genomic signaling and slower genomic one. The genomic pathway can be mediated by binding of progesterone to PR to result in binding to DNA and regulate the expression of target genes. There are two types of PR, PR-A and PR-B [12–14]. PR-A and -B actions are divergent from each other. PR-B differs from PR-A in that it contains an additional 164 amino acids at the amino-terminus [15, 16]. Another mode of action are non-genomic pathway, in which the progesterone activate a variety of rapid signaling events in the cells [17].

Eker rat model carrying a germ-line defect in the tuberous sclerosis complex-2 (Tsc-2) tumor suppressor has been used to determine the interaction between genetic susceptibility and early-life environmental exposure, which contributes to the pathogenesis of UF’s [18, 19]. Developmental exposure to xenoestrogens such as diethylstilbestrol (DES) increased the tumor-suppressor gene penetrance, tumor multiplicity and size in predisposed animals, and DES exposure caused reprogramming of estrogen-response genes expressed in UF’s and resulted in alteration of these genes in UF’s tumor genesis. Recently, we demonstrated that developmental exposure to DES expands the myometrial stem cells (MMSCs), which linked to the increases risk of UF development. In human studies, the correlation between number of MMSCs with risk of UF’s was identified in women. Myometrium from Caucasian (CC) women with UF’s exhibited increased numbers of MMSCs as compared to CC women without UF’s, and myometrium from African-American (AA) women had the highest number of MMSCs: AA-with UF’s > CC with UF’s > AA—without UF’s > CC-without UF’s [20]. In addition, MMSC population expanded in African American women, is correlated with parity and UF number, and fluctuates with cyclic menstrual cycle hormone changes and age [21, 22]. These studies suggest that MyoF was primed and exhibited a distinct profiling at molecular and cellular levels as compared to MyoN, which become at risk for later tumorigenic transformation. However, how the P4 triggers the transformation is unknown.

The object of this study is to identify progesterone responsive genes in cells from human MyoF verse MyoN tissues that will help to understand the role of progesterone in UF development.

Methods
Patients and myometrium specimens
The study was approved by Augusta University’s Institutional Review Board. Myometrium were obtained from Caucasian women who underwent abdominal hysterectomy for UF’s cause or any other causes. The ages of the patients are from 33 to 48 years old and none had received hormonal therapy for at least three cycles before surgery. The case interquartile range is 6. Informed consent was obtained from each patient before surgery for the use of extirpated uterine tissues for culture experiments.

Myometrial cell isolation and cell culture
Primary human myometrial cells were generated from the adjacent myometrial tissue of human uterus with UF after hysterectomy at least 2 cm away from the closest UF’s lesion (PrMyoF). Also we isolated the primary human myometrial cells from uterus without UF’s (PrMyoN). Isolation of the primary cell population from tissues was performed as described previously [23]. Briefly, a portion (0.5 cm3) of fresh myometrial tissue was washed in culture medium to remove blood and then chopped into small pieces under sterile conditions, transferred into a 15-ml screw cap tube, and suspended in Hanks Balanced Salt Solution containing 13 antibiotic-antimycotic (Thermo Fisher Scientific) and 300 U/ml collagenase type 4 (Worthington Biochemical Corp.). Suspended tissue pieces were incubated at 37°C for at least 12 h to obtain individual cells and/or clumps of cells. The cell suspension was passed through a 100-μm pore-sized sterile nylon filter and the suspension of individual cells was plated out and incubated at 37°C, allowing the cells to attach to the 100-mm sterile tissue culture-treated plate containing smooth muscle cell basal medium (SmBM; catalog no. CC-3181; Lonza) containing 5% fetal bovine serum (FBS) and supplemented with SmBM singlequots (catalog no. CC-4149). This SmBM singlequots contains hEGF, insulin, hFGF-B, and gentamicin / amphotericin-B. These cell culture experiments were performed successfully with ten uterine tissue specimens collected from different patients, of which five were from the normal uterus and the other five were from uterus with UF’s.

Protein extraction and Western blot analysis
Pellets were lysed in lysis buffer with protease and phosphatase inhibitor cocktail (Thermo Fisher scientific, Walram, MA, USA), and protein was quantified using the Bradford method (Bio-Rad protein Assay kit, Hercules, CA, USA). Western blot was performed as described previously [24] Blots were done for two different isoforms of the progesterone receptors PR-A, PR-B. Both are polyclonal antibody used in dilution 1 : 500 (Santa Cruz sc-7208, sc-538).
Cell treatment
Primary myometrial cells were cultured in 60-mm dishes at 30–40% confluence at an approximate density of 5 x 10^5 cells/dish at 37°C in a humidified atmosphere of 5% CO₂ in the regular SmBM media. When the cells were reached at approximately 80% confluence, the cells were grown in serum-free medium for 24 h. Then the cells were treated with P4 (1.0 ng/mL) for 72 h.

Quantitative real-time PCR
RNA was isolated according to the protocol using RNase Mini Kit. Following RNA extraction, cDNA was made by reverse-transcribing 1 μg of RNA using the (RNA to cDNA Eco Dry Premix (double Primed)). Aliquots of cDNA were made for each sample and stored at −20°C until analyzed.

SYBR Green real-time PCR was performed as described previously [25]. Briefly, RNA expression of genes was detected using Sso Advanced Universal SYBR Green Supermix on a Bio-Rad CFX96 real-time PCR system. Data were analyzed using Bio-Rad CFX manager software. Each biological sample was run in triplicate for each individual experiment. All assays were carried out in 96-well format. Real-time fluorescent detection of PCR products was performed with the CF96X Real-Time PCR System (Bio-Rad) using the following thermocycling conditions: 1 cycle of 95°C for 10 min; 40 cycles of 95°C for 30 s, and 60°C for 1 min. The primer sequences for qPCR were shown in Table 1 [26].

Statistical analysis
All values are expressed as means ± SE. Comparisons between two groups were done using the unpaired Student t-test. Differences between groups were examined by ANOVA. Values of P<0.05 were considered statistically significant.

Results

Subject characteristics
All the samples were taken from the Caucasian women with age ranges 33–48 years old (median 41.5). Subject characteristics is shown in Table 2 (n = 10).

Altered PR expression in MyoN and MyoF
To determine if MyoN and MyoF exhibited differential gene expression pattern in response to P4 treatment, we

| Gene       | Name                          | Function                              | Forward primer (5’–3’)                        | Reverse primer (5’–3’)                        |
|------------|-------------------------------|---------------------------------------|-----------------------------------------------|-----------------------------------------------|
| FOXO1A     | Forkhead box O1A              | Transcription factor in induction of apoptosis | AAGAGCGTG6CCTACTTCAA                          | CTGTTGTTGGCCATGATGC                           |
| Bcl2       | B-cell CLL/lymphoma 2         | Block apoptotic                        | AGTTATCGGCTTCAGTGCTT                         | CTGCCCGCTCTTACGTTT                           |
| SCGB2A2    | Secretoglobin, family 2A, member 2 | A uteroglobin-related genes           | ACCATGAAGTTGCTGATGTC                          | GGCATTGTAGGGCATTGTC                           |
| CIDE       | cell death-inducing DFFA-like effector c | Control cell cycle and DNA replication |                                             |                                               |
| CAPN6      | Calpain 6                     | It is calcium-activated cysteine proteinases | GGAAGCGTCCACAGGACATT                           | TACCCCGCTGTTACCCAAA                           |
| CYP26a1    | cytochrome P450, family 26, subfamily a, polypeptide1 | RA catabolizing enzyme             | AGAGCAATCAAGACAACAGTAG                       | ATCGGAGGTTCCTCCATAAT                        |
| ALDH1a1    | Aldehyde dehydrogenase family 1, subfamily A1 | RA synthesis enzymes                | GCCAGCAGAAGCTACTTACGT                       | CTCCTACGGTTGACGATTA                         |
| ADH5       | Aldehyde dehydrogenase family5 | RA synthesis enzymes                 | ATGGGCAAGCGGTTATCAAG                        | CATGCTCAAGATCACCTGAAAA                       |
| MT1E       | Metallothionein 1E             | Metallothioneins (MTs) family that bind to heavy metal ions and minimize reactive oxygen species | GCAAGTGCAAAAAAGTGCATT                       | CACCTCTCTGACGGCCCTTT                        |
| MT2A       | Metallothionein 2A             |                                        | TCGGACAGTGCAGCTTGCT                        | CTTCCAGTTCACCTCCTC                          |
| MTG2       | Metallothionein G2             |                                        | TGGGACACACCTCTAAT                           | TGATGACCTAGGGCAGAC                          |
| KLK6       | Regulation of the inflammatory process |                                        | CCAAACCTCTGGAACCTACTACCG                      | GTGCAGGGAATACCGGCTAC                        |
| HHI ()     | Indian hedgehog               | down regulation of cellular division | AACTCGCTGGGTCTATCGGT                     | GCCCTCAATAGGACGCGGACT                       |
| Calcitonin | Reduce production of pro-inflammatory cytokines, protective factor in ischemia |                                        | CCTATCCAACATAGAGGGCAAGA                      | TGACCTCGGTCTAGCATTTGTA                      |
| MMP11      | Matrix metalloproteinases      | Regulate cell-cell interactions and release the growth factors | AGACACCAATGAGAGGCA                          | GCACCTGGAAGAACCAAT                           |
first examined the expression of both PR-A, PR-B in MyoN and MyoF primary cells. Western blot (WB) analysis showed that the expression levels of PR-A were significantly higher in PrMyoF as compared to PrMyoN (Fig. 1a). The similar result was achieved for the PR-B (Fig. 1b). We confirmed the result in other patients in our experiment ($P < 0.05$).

**Genes show gain of induction in response to progesterone (P4)**

Previous studies have identified various progesterone target genes in endometriosis or during menstrual cycle [27–29]. In this study, we selected 15 UF-related genes and determined their differential expression between PrMyoF and PrMyoN cells in the presence or absence of the P4 (1.0 ng/ml) by qRT-PCR. In MyoF primary cells, significant upregulation of five genes (Bcl2, FOXO1A, SCGB2A2, CYP26a1 and MMP11) was observed in response to P4 treatment (Fig. 2). As shown in Fig. 2a, although the FOXO1A gene showed no difference of RNA expression between MyoN and MyoF cells at basal levels, and no significant change was found in prMyoN cells in response to P4 treatment ($P = 0.5$), significant gain in induction was observed in prMyoF primary cells in response to P4 treatment ($P < 0.05$).

The basal levels of Bcl 2 gene expression between MyoN and MyoF primary cells did not reach significant difference ($P = 0.7$). However a significant increase of Bcl2 expression was observed in MyoF cells in response to P4 treatment ($P < 0.01$) (Fig. 2b), but not in MyoN cells. Similar finding was achieved for SCGB2A2 gene as MyoF cells exhibited gain in induction of SCGB2A2 gene expression ($P < 0.05$) but not MyoN cells in response to P4 treatment (Fig. 2c).

For MMP-11, a significant differential expression between MyoF and MyoN ($P < 0.05$) was observed. MyoN cells showed insignificant gain in induction in response to P4 treatment. However, MyoF cells exhibited a significant gain in induction after P4 treatment ($P < 0.01$) (Fig. 2d).

**For CYP26a1 gene a significant gain in induction in MyoF was observed in response to P4 ($P = 0.05$) (Fig. 2e). But gain in induction of CYP26a1 gene expression was not found in Pr MyoN cells.**

**Genes show gain of repression in response to progesterone (P4)**

The other seven genes showed down regulation in MyoF cells in response to P4 treatment. Three of these genes are responsible for apoptosis and cell cycle. As shown in (Fig. 3a), the expression of CIDEA gene was significantly higher in PrMyoF as compared to PrMyoN cells ($P < 0.05$). In addition, P4 treatment resulted in a significant gain of repression in MyoF cell ($P < 0.05$), but not in MyoN cells. Also the gain of repression in respond to P4 was also found for CANP6 gene (Fig. 3b) ($P < 0.05$) and HHI gene ($P < 0.05$) (Fig. 3c) in MyoF cells, but not in MyoN cells. Comparing the basal levels of CANP6 and HHI expression between PrMyoN and PrMyoF, the expression of CANP6 gene exhibited no different but the expression of HHI gene exhibited statistically significant higher in PrMyoF cells as compared to PrMyoN cells ($P < 0.001$).

The RNA expression of 2 genes (ADHLS, ALDH1A1) related to RA synthesis enzymes was also measured at basal levels as well as in P4-treated MyoN and MyoF...
cells. For ADHL5 gene (Fig. 3d), significant differential response to P4 was found between PrMyoN and PrMyoF cells. A significant gain in repression in PrMyoF cells after P4 treatment ($P < 0.05$), but not in PrMyoN cells. Similar finding was observed for ALDH1a1 expression in response to P4 in PrMyoF and PrMyoN cells (Fig. 3e).

The expression of gene KIK6 (Fig. 3f), which is the gene that associated with regulation of inflammatory process, exhibited no difference in PrMyoN and PrMyoF cells. And alteration of its expression was not observed after treatment with P4 in PrMyoN cells. However, a significant gain in repression after P4 treatment was seen in PrMyoF cells ($P < 0.05$).

Metallothionein (MT) family is responsible for binding to heavy metal ions and minimize reactive oxygen species. The response of several genes of MT family to P4 were examined (Figs. 3g and 4). MT1E (Fig. 3g) exhibited significant repression in its expression with P4 treatment in the PrMyoF cells ($P < 0.05$), but not in PrMyoN cells. However, no significant changes of other MT family genes including MT2A and MTG2 were observed between PrMyoN and PrMyoF cells at basal levels as well as in response to P4 treatment (Fig. 4a, b).

The basal level of Calcitonin expression between PrMyoN and PrMyoF cells and their response to P4 was examined. There is no significant difference of RNA
expression between MyoN versus MyoF cells. Furthermore, no significant difference of RNA expression in PrMyoN and PrMyoF cells in response to P4 treatment were found (Fig. 4c).

**Discussion**

P4 is a key hormone, which contributes to the UF pathogenesis. However, the molecular mechanism by which P4 promotes the UF development is largely unknown. In this study, we used PrMyoN and PrMyoF cell model system and characterized the expression pattern of P4 response genes in response to P4 treatment, which may contribute to increased risk of UF development.

Previous study showed that cultured UF cells had an increased response to P4 compared to cultured normal myometrial cells [30]. This study also showed that P4 receptor mRNA is highly expressed in UF cells as compared the cells from adjacent myometrium. In our study, we focused on P4 response in primary cells from normal myometrium (MyoN) and at-risk myometrium (MyoF). We demonstrated that the expression of PR was higher in PrMyoF as compared to PrMyoN cells. The differential response of PrMyoN and PrMyoF to P4 seems to be attractive. Among the genes we detected, we found two types of changes in response to P4 treatment, gain in induction and gain of repression respectively. These results suggested that the network of P4/PR signaling was varied between PrMyoN and PrMyoF and the primed PrMyoF turned out to be hyper-sensitive to P4, which might lead to increased risk of UF development.

In this study, the expression of 15 P4-responsive genes was examined in PrMyoF and PrMyoN cells using q-
PCR analyses. Five of these genes including FOXO1A, CYP26a1, SCGB2A2, MMP11 and Bcl 2 showed significant up regulation in response to P4 treatment. The other seven genes exhibited a significant down regulation, these genes include CANP6, MT1E, ADHL5, Aldh1a1, KIK6, HHI, CIDEc. However, the expression of MT2A, MTG2 and calcitonin was not altered in response to P4 treatment.

Expression of genes control the apoptosis in response to progesterone

Apoptosis is a morphologic pattern of cell death [31]. There are multiple genes responsible for regulating this process. Korsmeye [32], reported that the Bcl-2 proto-oncogene has the ability to block apoptotic cell death in multiple contexts. Increase in expression of Bcl-2 in transgenic models will result in evasion of normal cell death mechanisms leading to accumulation of cells and tumor formation [33].

Previous studies showed that Bcl-2 protein expression was predominant in UF cells compared to that in normal myometrium cells [34]. The expression of Bcl-2 protein in normal myometrium cells was very low that raised the possibility that normal myometrium cells may be more susceptible to apoptotic cell death. In addition, UF cells exhibited increased expression of Bcl-2 protein in response to P4 treatment. But the expression of Bcl-2 protein in cultured normal myometrium cells was not affected by P4 treatment. Here in our study Bcl-2 gene expression in at-risk myometrium tissues from the uterus with UF's was remarkably augmented by P4 treatment and this change was not found in normal myometrial cells.

Another gene that responsible also for apoptosis is FOXO1A, it is a member of the FOXO subfamily of Forkhead transcription factors [35]. According to the previous study, activated FOXO proteins induced
expression of genes that encode for proteins involved in cell cycle inhibition [36]. Our study showed that this gene exhibited hyper-response in PrMyoF cells after P4 treatment. Another study determined the progestin effect in FOXO1 expression and its activity in the endometrium during endometrium menstrual cycle. They showed that progestin enhanced FOXO1 mRNA levels in mid- and late-secretory endometrium [37]. In addition, FOXO1A was considered as a key transcription factor responsible for mediating apoptosis of decasualized human endometrial stromal cells (HESC) in response to progesterone withdrawal during the menstrual cycle by inducing the cell death. Moreover, this study explains the effect of admission of medroxyprogesterone acetate (MPA, a synthetic progestin) in enhancing the expression of FOXO1A in differentiating human endometrial cells. MP also simultaneously induced cytoplasmic retention and inactivation of this gene. Withdrawal of the MPA from decidualized HESCs resulted in rapid nuclear accumulation of FOXO1A, therefore leading to activation of apoptosis and cell death [37]. Similar finding was observed in PrMyoF cells, where the expression of FOXO1 was markedly increased in response to progesterone treatment, which provide a favorable condition for the pathogenesis of UFs.

SCGB2A2 (Secretoglobin family 2A member 2) was considered as uteroglobin-related protein, which controls cell cycle and DNA replication. It was originally detected by differential RNA expression levels in Breast Cancer biopsies [38]. Previous studies demonstrated the effect of SCGB overexpression on cell proliferation in other human diseases and ovarian carcinoma. The role of SCGB2A2 in patho-physiology of the ovarian tumor was identified [39]. The overexpression of SCGB2A2 is positively correlated with the FIGO stage, the tumor grade and the mitotic index of the ovarian cancer [40]. In our study, although no significant expression of SCGB2A2 was observed in ProMyoF and PrMyoN cells, ProMyoF cells was remarkably augmented by P4, which was not the same with ProMyoN cells. This study suggests that P4 might promote the UF development by increased cell proliferation and enhancement of the DNA replication via SCGB2A2.

The cell death-inducing DFF45-like effector (CIDE) family includes CIDEa, CIDEb, and CIDEc. It has been reported that the CIDE family plays an important role in lipid and fat metabolism [40–42]. Previous studies have reported that CIDEa, CIDEc were highly expressed in adipose tissue, and in skeletal muscle. ICIDEc is capable of inducing apoptosis in mammalian cells [43]. DFF45 is a subunit of the DNA fragmentation factor which is cleaved by active caspase-3 during apoptosis. The main function of CIDEc is energy homeostasis, and its absence may result in insulin resistance, and resistance to diet-induced obesity [44]. Here in our study this gene showed gain of repression in response to P4 as a marker of decrease in the apoptosis that might be involved in UF development.

Another gene that showed gain in regression in our study was CANP6. It is calcium-activated cysteine proteinases. Calpains have been involved in many biological events including regulation of the cell cycle, apoptosis, cell adhesion and motility [45, 46]. So the regression of this gene will decrease the apoptosis as well as down regulation of cell cycle all together will favor the development of UFs.

**Expression of genes control the retinoic acid in response to progesterone**

RA, is the natural metabolite of vitamin A. Previous studies showed that RA signaling played an important role in the female reproductive trace function [47]. ADH5 and ALDH1a1 are RA synthesis enzymes and CYP26a1 (cytochrome P450, family 26, subfamily a, polypeptide1) is a RA catabolizing enzyme. Previous studies demonstrated that the expression of these are altered during preganyc which may be related to progesterone signaling. ADH5 expression was increased by 2.5 folds during pregnancy. The expression of ALDH1a1 in the endometrial glandular compartment was increased on gestational early days until the implantation phase. The expression of CYP26a1 was strongly detected in the uterine epithelium. Moreover, these studies indicated that early pregnancy needed the synthesis and degradation of RA to be balanced to allow RA signaling to prepare for implantation without harmful effects on the embryo [48]. Our result has demonstrated that RA synthesis genes (ADH5, ALDH1a1) show gain in repression in response to P4, and RA catabolic enzyme (CYP26a1) were rapidly gain induction by the P4-PR axis. This might result in increasing retinoic acid catabolism and decrease in Vitamin A in the myometrium tissue. All this together will favorable the expression of the myometrium, which provide pro-fibroid condition to increase the risk of UF development.

**Expression of other genes in response to progesterone**

In human, over 20 functional Matrix metalloproteinases MMPs have been identified [48]. MMPs are zinc endopeptidases capable of releasing the growth factors that are bound to the extracellular matrix (ECM) [49] regulating cell-matrix and cell-cell interactions. Matrix metalloproteinase 11(MMP11) is responsible of serpins cleavage and so it stimulate the development of tumor [50, 51]. Our study showed that MMP-11 mRNA was significantly increased in myometrium cells from uterus with UFs compared with myometrium cells of normal one. Also gene expression of MMP11 showed significant gain in induction after P4 treatment in PrMyoF cells.
Previous study demonstrated increased expression of MMP-11 in UFs as compared to myometrium.  

KLK6 belonging to kallikreins gene family is a serine protease [52–54]. It is down-regulated by the P4-PR axis. KLK6 is responsible for regulation of the inflammatory process and vascular permeability, and edema [55]. Previous studies in mouse graved uterus showed that this gene was upregulated by the P4-PR axis signaling suggesting the important role of this gene in the implantation of the embryo in the uterus [27]. In our study, the repression of this gene in response to P4 may result in the formation of the UFs by losing the regulation of the inflammatory process.  

Indian hedgehog (HHI), one of the Hedgehog family of ligands, is a P4-regulated gene in the uterus [56, 57]. It plays a role in down regulation of cellular division [58]. In the human endometrium, the role of Hedgehog signaling in UFs is largely unknown [59]. HHI gene shows a significant decrease in expression s between the early secretory to the mid secretory phase. It also plays a role in embryo implantation by regulation of stromal cell proliferation and inhibition of epithelial E2 signaling. In addition, Hedgehog signaling was involved in the women with endometriosis [60–62]. and in endometrial cancer [63]. Here in our study this gene showed a gain of repression in the PrMyoF cells in response to P4, suggesting that this change may be involved in the increased risk of UF pathogenesis.  

Metallothioneins (MTs) comprise a family of genes clustered on chromosome 16q that bind to heavy metal ions and minimize reactive oxygen species. Previous studies demonstrated low MT expression in endometrium of women with endometriosis [29]. In our study MTIE is the only one we detected that showed significant repression with P4 treatment in the PrMyoF cells.  

The location of the Calcitonin gene is in non-neuronal tissues. Its define function remains unclear, but previous studies identified their role in cardiovascular system as it exhibited a potent vasodilator effect [64]. There are many other researches done on calcitonin effect on the heart. These researchers show its role in prevention of ischemia as well as endotoxic shock [65]. These shock can be done by the suppressor effect of calcitonin on some pro-inflammatory cytokines production [e.g., macrophage inflammatory protein-2 (MIP-2) and keratinocyte chemotactrant (KC)] [66, 67]. Moreover, calcitonin has a protective effect against ischemia [68]. So the decrease in its expression may result in ischemia stimulation of the inflammatory reaction. However, in our study it showed gain in repression suggesting the complex role of this gene in response to P4 treatment.  

Conclusion  
Our studies demonstrate for the first time that PrMyo cells and PrMyoN from either at risk myometrium or normal myometrium exhibit a differential response to P4, the key hormone for UF development. P4-responsive genes in PrMyoF cells exhibit a P4-hyper-responsiveness, suggesting that myometrium from uterus with UFs is primed and become at risk for later tumorigenic transformation. However, due to sample size (n = 10) and race limitation (all from Caucasian), further investigating the role of P4 in alteration of normal myometrium in a large sample size as well as using tissues from at high risk populations such as African American are needed. Moreover, evaluating of P4 response in relevant animal model or 3D system is also highly needed for understanding the pathogenesis of UFs.

Abbreviations  
DFF: DNA fragmentation factor; ECM: Extracellular matrix; FBS: Fetal bovine serum; GnRH agonists: Gonadotropin releasing hormone agonist; HESC: Human endometrial stromal cells; KC: Keratinocyte chemoattractant; LNG IUS: Levo –norgestrie intra-uterine device; MMSC: Myometrial stem cell; MPA: Medroxyprogesterone acetate; MyoF: Human myometrial cells from uterus with fibroids; MyoN: Human myometrial cells from normal uterus; P4: Progesterone; PR: Progesterone receptor; PRGs: Progesterone-regulated genes; PrMyoF: Primary human myometrial cells from uterus with fibroids; PrMyoN: Primary human myometrial cells from normal uterus; qPCR: Quantitative real-time polymerase chain reaction; RA: Retinoic acid; SmBMI: Smooth muscle cell basal medium; SPRM: Selective progesterone receptor modulator; UFs: Uterine Fibroids; WB: Western blot.

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Authors’ contributions  
AA and QY designed the research. MO, AK performed the experiments and analyzing the data. MO wrote the manuscript. QY edited the manuscript. All authors read and approved the final manuscript.  

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Availability of data and materials  
The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate  
The study was approved by Augusta University’s Institutional Review Board. Myometrium were obtained from Caucasian women who underwent abdominal hysterectomy. Informed written consent was obtained from each patient before surgery for the use of extirpated uterine tissues for myometrial cell isolation.

Consent for publication  
Not applicable.

Competing interests  
The authors declare that they have no competing interests.

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