Nicotinamide Adenine Dinucleotide Phosphate Oxidase Is Differentially Regulated in Normal Myometrium Versus Leiomyoma

Reproductive Sciences
2014, Vol. 21(9) 1145-1152
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DOI: 10.1177/1933719114522552
rs.sagepub.com

Nicole M. Fletcher, PhD¹, Mohammed G. Saed¹,
Suleiman Abuanzeh, MD¹, Husam M. Abu-Soud, PhD¹,
Ayman Al-Hendy, MD, PhD², Michael P. Diamond, MD³,
and Ghassan M. Saed, PhD¹

Abstract

Uterine fibroids are the most common benign tumor in women. The goal of this study was to investigate whether nicotinamide adenine dinucleotide phosphate oxidase (NOX), a major source of superoxide and subsequent oxidative stress, was differentially regulated in myometrium versus leiomyoma. Expression levels of NOXs1-5, dual oxidase (DUOX), DUOX2, NOX organizer (NOXO) 1, NOX activator 1, p47^{phox}, p67^{phox}, and p22^{phox} were determined in cells treated with hypoxia by real-time reverse transcription-polymerase chain reaction, Western blot, and immunohistochemistry in tissues. Expression of NOX4 increased in fibroid compared to myometrial tissues and cells. The NOX2, DUOX1, and p67^{phox} were higher while p22^{phox} was lower in fibroid than that in myometrial cells. Hypoxia increased NOX4, DUOX1, and NOXO1 and decreased p22^{phox} in myometrial and reduced DUOX1 in fibroid cells. The NOX1, NOX3, NOX5, and DUOX2 were undetectable. Fibroid cells are characterized by a unique NOX profile, which promotes a severe prooxidant state that may be responsible for their development. Targeting these subunits may be beneficial for future therapeutic interventions.

Keywords

NAD(P)H oxidase, leiomyoma, hypoxia, oxidative stress

Introduction

Uterine fibroids, also known as leiomyomas, are the most common tumor of the reproductive tract in women, occurring in over half of American women of reproductive age and are 3 to 4 times more common in African American women.¹⁻³ Although often asymptomatic, leiomyomas can cause severe symptoms, including pain, excessive blood loss with menstruation, other abnormal uterine bleeding patterns and obstetric complications, pelvic pressure, obstructive urinary and bowel symptoms, and reproductive problems such as infertility.⁴ Despite being common, the molecular mechanisms of fibroids remain largely unknown.

Oxidative stress has been shown to be a major player in common profibrotic gynecologic disorders such as fibroids, endometriosis, and postoperative adhesions.⁵⁻⁸ Hypoxia, a major source of oxidative stress, triggers a number of critical adaptations that enable cell survival, including apoptosis suppression, altered glucose metabolism, and an angiogenic or profibrotic phenotype.⁹ Recent investigations suggest that O₂ depletion stimulates mitochondria to further increase reactive oxygen species (ROS), with subsequent activation of signaling pathways, such as hypoxia-inducible factor-1 α , that promote

cell survival and consequently fibrotic growth.⁹ Moreover, hypoxia has been demonstrated to play an essential role in leiomyoma formation.¹⁰

The presence of the nicotinamide adenine dinucleotide phosphate (NAD(P)H) oxidase (NOX) system and its importance in mitogenic signaling pathways, as well as the necessity of NOX-derived ROS for epithelial growth factor, and platelet-derived growth factor signaling pathways leading to cell proliferation have been indicated in leiomyomas.¹¹ In addition to hypoxia-

¹ Department of Obstetrics and Gynecology, Wayne State University, Detroit, MI, USA

² Department of Obstetrics and Gynecology, Meharry Medical College, Nashville, TN, USA

³ Department of Obstetrics and Gynecology, Georgia Regents University, Augusta, GA, USA

Corresponding Author:

Ghassan M. Saed, Department of Obstetrics & Gynecology, C.S. Mott Center for Human Growth and Development, Wayne State University School of Medicine, 275 E. Hancock, Detroit, MI 48201, USA.

Email: gsaed@med.wayne.edu

generated superoxide ($O_2^{\bullet-}$), NOXs are another source of $O_2^{\bullet-}$ in several cell types.¹²⁻¹⁷ The NOX consists of 7 isoforms: 5 NOX, NOXs1–5, and 2 NOX homologs, dual oxidase (DUOX) 1 and DUOX2. The NAOXs are differentially activated by different binding molecules, including p22^{phox}, p40^{phox}, p47^{phox}/or its homologue NOX organizer (NOXO) 1, p67^{phox}/or its homologue NOX activator 1 (NOXA1), and Rac.¹⁸ Activation mechanisms and tissue distribution of different members of the family are markedly different. Activation of NOX involves the translocation of regulatory elements from the cytoplasm to combine with catalytic subunits in the membrane.¹⁹ Increased NOX activity contributes to a large number of pathologies, such as cardiovascular diseases and neurodegeneration.¹²

Several studies reported the relationship between NOX enzyme complex and altered oxidative stress balance.²⁰⁻²² It is believed that the role of NOX in the alteration of this balance is mainly due to the presence of multiple membrane-associated isoforms of NOX and their cytoplasmic subunits.¹² The hypothesis of this study is that fibroids are characterized by a persistent prooxidant state and manifest a specific NOX expression profile as compared to myometrium. In this study, we will examine the expression of NOX isoforms in normal myometrium and fibroid tissues as well as in immortalized cell lines. Targeting these subunits may be beneficial for future therapeutic interventions of fibroids.

Methods

Cell Culture

Human uterine cells were a kind gift from Dr Darlene Dixon, which were derived as described previously.²³ These cells were isolated directly from corresponding tissues (myometrium or fibroid, collected at the time of hysterectomy). These tissues are homogenous and are primarily populated by these cells and once in culture, these myometrial or fibroid cells dominate the culture environment. The authenticity of such cells is additionally confirmed by the expression of the appropriate smooth muscle cell markers such as α smooth muscle actin, vimentin, and F-actin as we have previously reported.^{24,25} This study was approved by the Meherry Medical College institutional review board.

Cells were immortalized through the induction of telomerase activity with the use of a retroviral vector containing human telomerase reverse transcriptase, which allows them to bypass their normal programmed senescence.²³ Cell lines, fibroid ($n = 1$) and normal myometrial ($n = 1$), were cultured and maintained with the SmGM-2 Bullet Kit (Lonza, Walkersville, Maryland), which included smooth muscle cell basal medium containing 5% fetal bovine serum, 0.1% insulin, 0.2% human fibroblast growth factor β , 0.1% gentamycin–amphotericin 1000, and 0.1% human epidermal growth factor.

Hypoxia Treatment

All hypoxic experiments were performed in an airtight modular incubator chamber (Billups-Rothenberg, Del Mar, California).

The chamber was deoxygenated by a positive infusion of 2% O_2 in a CO_2 –nitrogen gas mixture. Cells were cultured under normal (20% O_2) or hypoxic (2% O_2) conditions for 24 hours. Cultures were placed in a standard humidified tissue incubator. All experiments were performed in triplicate.

RNA Isolation

Total RNA was extracted from cells using the RNeasy Mini Kit (Qiagen, Valencia, California) according to the manufacturer's protocol.

Reverse Transcription

A 20- μ L complementary DAN (cDNA) reaction volume, utilizing 1 μ g RNA, was prepared using the QuantiTect Reverse Transcription Kit (Qiagen), according to the manufacturer's protocol.

Real-Time Reverse Transcription-Polymerase Chain Reaction Primer Design and Controls

Optimal oligonucleotide primer pairs for real-time reverse transcription-polymerase chain reaction (RT-PCR) amplification of reverse-transcribed cDNA were selected with the aid of the software program, Beacon Designer (Premier Biosoft Int, Palo Alto, California). Human oligonucleotide primers, which amplify variable portions of the protein coding regions, are listed in Table 1. Standards with known concentrations were designed specifically for these primers using Beacon Designer software, allowing for construction of a standard curve using a 10-fold dilution series. A specific standard for each gene allows for absolute quantification of the gene in copy numbers, which can then be expressed as ng/ μ g of RNA.

Real-time RT-PCR was performed with the QuantiTect SYBR Green RT-PCR kit (Qiagen) and a Cepheid 1.2f Detection System (Cepheid, Sunnyvale, California). Each reaction was 25 μ L consisting of, 12.5 μ L of 2 $\times$ QuantiTect SYBR Green RT-PCR master mix, 1 μ L of cDNA template, and 0.2 μ mol/L, each of target-specific primer that was designed to amplify a part of the gene of interest. To quantify each target transcript, a standard curve was constructed using a 10-fold dilution series of the standard for the specific gene of interest. The PCR conditions for the primers are summarized subsequently and in Table 1. An initial cycle was performed at 95°C as indicated in Table 1, followed by 35 cycles of denaturation at 95°C for 15 seconds, annealing for 30 seconds as described in Table 1, and a final cycle at 72°C for 30 seconds to allow completion of product synthesis. Following real-time RT-PCR, a melting curve analysis was performed to demonstrate the specificity of the PCR product as a single peak. A control containing all the reaction components except for the template was included in all experiments.

Table 1. List of Oligonucleotide Primers Utilized in Real-Time RT-PCR Analysis of Myometrial and Fibroid Cells.

Accession #	Locus	Sense (5'-3')	Antisense (3'-5')	Amplicon Length	Initial PCR Cycle, seconds	Annealing, °C
AF127763	NOX1	GCTAAATCCCATCCAGTC	GCTGAAGTTACCATGAGAA	100	1200	56
NM_000397	NOX 2	GGAAACTACCTAAGATAGC	TAACATCACCACTCATA	80	900	57
AF190122	NOX3	TTCTATTACAACAAGGAG	AATTATTATTCACCACTTC	152	1100	49
AF261943	NOX 4	GTAGGAGACTGGACAGAA	ATTGAATGAAGGGCAGAAT	82	1000	54
AF325189	NOX 5	CCTTAGTCCTTCTAGTTG	CACCAATTCCAGATACAA	114	1000	53
NM_017434	DUOX1	TTCCACATCTTCTTCCTG	CTGATCTCCACCTTCTTC	83	1200	53
NM_014080	DUOX2	TTCCACATCTACTTCCTG	CTGATCTCCACCTTCTTC	83	1200	54
AY255769	NOXA1	TGGATGTTCTGTGTGAAG	GGGACTACAAAGCACTTG	88	1000	53
NM_144603	NOXO1	CATTCTTCTTCTCTCTCC	TCCTTTGCAGCTTTCTTG	104	900	55
NM_000101	p22 ^{phox}	GTACTTTGGTGCCTACTC	GGAGCCCTTCTTCTCTCTT	82	1000	54
NM_000265	p47 ^{phox}	CAGCCAGCACTATGTGTA	ACTCGTAGATCTCGGTGAA	89	1200	54
NM_000433	p67 ^{phox}	TAATAACCAGACAACAGA	TTGATAACACCAGGATTA	125	1400	51

Abbreviations: DUOX, dual oxidase; NOXA1, nicotinamide adenine dinucleotide phosphate oxidase activator 1; NOXO, nicotinamide adenine dinucleotide phosphate oxidase organizer; RT-PCR, reverse transcription-polymerase chain reaction.

Immunohistochemical Staining of Human Myometrial and Fibroid Tissue Sections

Tissue sections (7 per condition) were deparaffinized and subjected to immunohistochemical staining, with standard streptavidin-biotin-peroxidase techniques, with diaminobenzidine as the chromogen as previously described.²⁶ Briefly, 4- to 5- μ m thick sections were antigen retrieved with citrate buffer, quenched for 10 minutes with 3% hydrogen peroxide (H₂O₂), and preincubated with blocking serum at 1:20 in 2% bovine serum albumin (BSA)/phosphate-buffered saline (PBS) solution for 15 minutes at room temperature. After incubation with primary antibody for NOX (1:20 dilution, NOX4, HPA015475; Sigma-Aldrich, St Louis, Missouri), the slides were rinsed with PBS, followed by incubation with the biotinylated secondary antibody (1:500 in PBS, 30 minutes, room temperature). After rinsing with PBS for 30 seconds, the slides were incubated with streptavidin-peroxidase at 1:500 in PBS for 30 minutes at room temperature, then rinsed with PBS and incubated for 15 minutes with 0.06% diaminobenzidine, and counterstained with Harris-modified hematoxylin (Fisher Healthcare, Hanover Park, Illinois).

For the assessment of expression levels of NOX4, the staining intensity and the percentage of stained cells were analyzed. The NOX4 was scored in the cell membrane and cytoplasm. Staining intensity was scored as 0 (negative), 1+ (weak), 2+ (medium), or 3+ (strong). For the final score, low expression was defined as an intensity of 0, 1, 2, or 3 and <10% stained cells or an intensity of 0 or 1 and <50% stained cells. High expression was defined as an intensity of 2 or 3 and >10% stained cells or an intensity of 1, 2, or 3 and >50% stained cells.

Western Blot Analysis

Protein extraction. Sections (5 per condition) were cut from the paraffin-embedded blocks of tissues, deparaffinized in xlenes, and rehydrated in graded ethanol as described previously.²⁷

Tissues were treated with extraction buffer (200 μ L; 1 mol/L sodium dihydrogen phosphate, 10 mmol/L disodium hydrogen phosphate, 154 mmol/L sodium chloride, 1% Triton X-100, 12 mmol/L sodium deoxycholate, 0.2% sodium azide, 0.95 mmol/L fluoride, 2 mmol/L phenylmethylsulfonyl fluoride, 50 mg/ml aprotinin, and 50 mmol/L leupeptin, pH 7.6) and incubated at 100°C for 20 minutes, followed by incubation at 60°C for 2 hours. After incubation, the tissue lysates were centrifuged at 15 000g for 20 minutes at 4°C. The supernatants were collected and stored at -80°C until use for Western blot analysis.

Protein assay. The protein concentration of the lysates was determined with the Bio-Rad Protein Assay per the manufacturer's protocol (Bio-Rad, Hercules, California). The protein concentration was determined based on the standard curve using BSA and absorbance at 750 nm.

Western blot and analysis. Lysates from cells or tissues were fractionated with sodium dodecyl sulfate polyacrylamide gel electrophoresis, 4% to 20% Tris-glycine gel (Invitrogen, Carlsbad, CA) at 130 V for 180 minutes against a molecular weight ladder. Proteins were transferred from the gel to a nitrocellulose membrane with the use of an electroblotting apparatus at 40 V for 2 hours. Primary antibodies diluted in 1% nonfat milk solution for polyclonal rabbit anti-NOX4 (sc-30141; Santa Cruz Biotechnology, Santa Cruz, California) and rabbit antitubulin (sc-135659; Santa Cruz) were incubated for 24 hours at 4°C. Membranes were washed and developed as described previously.²⁸ Protein bands were scanned and analyzed by NIH Image J 3.0.

Statistical Analysis

Data were analyzed using SPSS 15.0 for Windows with independent and dependent sample *t* tests by cell type on each treatment. Statistical significance of *P* < .05 was considered significant for all analyses.

Figure 1. Immunohistochemistry and Western blot analysis of NAD(P)H oxidase (represented by the NOX4 isoform) in myometrial and fibroid tissues. **A**, Immunohistochemical staining for NAD(P)H oxidase demonstrates high positivity in fibroid tissue ($n = 7$) with no detectable reactivity in myometrium peritoneal tissues ($n = 7$). **B**, Western blot analysis of NAD(P)H oxidase demonstrates higher expression of NOX4 in fibroids ($n = 5$) than that in normal myometrial tissues ($n = 5$). NAD(P)H indicates nicotinamide adenine dinucleotide phosphate; NOX, NAD(P)H oxidase.

Results

Nicotinamide Adenine Dinucleotide Phosphate Oxidase Is Overexpressed in Human Fibroid Tissues and Cells

We utilized immunohistochemistry using the NOX4 antibody to demonstrate the expression of NOX in myometrial and fibroid tissues ($n = 7$). There was high positive detectable staining for NOX (as represented by NOX4) in fibroid tissues and no detectable staining in myometrial tissues (Figure 1A). The immunohistochemical detection of the NOX4 subunit was further confirmed by Western blot analysis (Figure 1B).

Expression of NOX Isoforms

There was no detectable messenger RNA (mRNA) for NOX1, NOX3, and NOX5 in myometrial or fibroid cells by real-time RT-PCR.

Expression of NOX2

Levels of NOX2 mRNA were significantly higher in fibroid cells (115.6 ± 1.2 fg/ μ g RNA) than that in myometrial cells (64.2 ± 7.3 fg/ μ g RNA, $P = .001$, Figure 2A). Hypoxia had no significant effect on NOX2 mRNA levels in myometrial or fibroid cells.

Expression of NOX4

Levels of NOX4 mRNA were significantly higher in fibroid cells (113.5 ± 4.7 fg/ μ g RNA) than that in myometrial cells (74.5 ± 4.8 fg/ μ g RNA, $P < .02$, Figure 2B). Hypoxia further significantly increased NOX4 mRNA levels in myometrial cells (to 118.3 ± 8.7 fg/ μ g RNA, $P < .05$) and had no significant effect on NOX4 in fibroid cells.

Expression of p22^{phox}

The mRNA levels for p22^{phox} were significantly lower in fibroid cells (1.5 ± 0.4 fg/ μ g RNA) than that in myometrial cells (31.8 ± 2.4 fg/ μ g RNA, $P < .004$, Figure 1C). Hypoxia significantly decreased p22^{phox} mRNA levels in myometrial (to 23.8 ± 2.0 fg/ μ g RNA, $P < .03$) cells while having no effect on fibroid cells.

Expression of DUOX1 and DUOX2

Levels of DUOX1 mRNA were significantly higher in fibroid cells (498.8 ± 17.7 fg/ μ g RNA) than that in myometrial cells (267.3 ± 19.8 fg/ μ g RNA, $P < .007$; Figure 1D). Hypoxia significantly increased DUOX1 mRNA levels to 821.0 ± 53.2 fg/ μ g RNA in myometrial cells ($P < .03$) while it decreased DUOX1 mRNA levels to 233.1 ± 1.3 fg/ μ g RNA in fibroid cells ($P < .04$). There was no detectable mRNA for DUOX2 in either cell line.

Expression of p67^{phox} and NOXA1

The mRNA levels for p67^{phox} were significantly higher in fibroid cells (68.4 ± 1.5 fg/ μ g RNA) than that in myometrial cells (15.1 ± 0.1 fg/ μ g RNA, $P < .0005$; Figure 3A). Hypoxia increased p67^{phox} mRNA levels, although not quite significantly, in myometrial cells (to 28.4 ± 1.7 fg/ μ g RNA, $P = .06$) while having no significant effect on fibroid cells. There were no significant differences in NOXA1, a homolog of p67^{phox} mRNA levels between the cell lines or in response to hypoxia (Figure 3B).

Expression of p47^{phox} and NOXO1

The mRNA levels for p47^{phox} were significantly higher in fibroid cells (0.55 ± 0.02 fg/ μ g RNA) than that in myometrial cells (0.29 ± 0.002 fg/ μ g RNA, $P < .0007$; Figure 3C). Hypoxia increased p47^{phox} mRNA levels, although not quite significantly, in myometrial cells (to 0.54 ± 0.06 fg/ μ g RNA, $P = .07$) while it was significantly increased in fibroid cells (0.73 ± 0.09 fg/ μ g RNA, $P < .05$). There was no difference in mRNA levels for NOXO1 between cell lines (Figure 3D). Hypoxia resulted in a significant increase in NOXO1 mRNA levels (from 369.0 ± 77.3 to 1581.3 ± 187.3 fg/ μ g RNA, $P < .05$; Figure 3D) in fibroid cells while mRNA levels also increased in myometrial cells but not quite significantly (from 304.4 ± 21.4 to 626.4 ± 90.6 fg/ μ g RNA, $P = .09$).

Figure 2. Quantitative real-time RT-PCR analysis of nicotinamide adenine dinucleotide phosphate (NAD(P)H) oxidase isoforms NOX2, NOX4, p22^{phox}, and DUOX1. Real-time RT-PCR was utilized to measure messenger RNA (mRNA) levels of NOX4 (A), NOX4 (B), p22^{phox} (C), and DUOX1 (D) in myometrial cells (n = 1) and fibroid cells (n = 1) treated with and without hypoxia (2% O₂). Expression is depicted as the mean with error bars representing standard deviation. *P < .05 as compared to untreated myometrial cells and **P < .05 as compared to untreated fibroid cells. DUOX indicates dual oxidase; NOX, NAD(P)H oxidase; RT-PCR, reverse transcription-polymerase chain reaction.

Discussion

Oxidative stress has been shown to be a major player in common profibrotic gynecologic disorders such as endometriosis, postoperative adhesions, and fibroids.⁵⁻⁸ This study gathered further evidence to support a role of oxidative stress in the pathophysiology of fibroids. We have demonstrated the overexpression of NOX in fibroid as compared to normal myometrial tissues and cells. Furthermore, we demonstrated that NOX isoforms and their activation molecules are differentially expressed in normal myometrial as compared to fibroid cells creating a prooxidant state, which may contribute to their mechanism of development. In this study, hypoxia was found to increase both NOX4 and DUOX1 in normal myometrial cells and p47^{phox}, NOX4, and NOXO1 in fibroid cells. Therefore, we believe hypoxia is unlikely to stimulate NOX-generated O₂^{•-} but rather directly generates O₂^{•-} in cells.

The origins of fibroids remain unknown but accumulating evidence suggests that hypoxia is likely implicated in early cellular events, which lead to myometrial smooth muscle cell transformation into leiomyoma.^{10,29} Fibroid cells are severely hypoxic and an oxygen-limited microenvironment has been demonstrated to protect fibroid cells against apoptosis and maintain a proliferative state.²⁹ Also, recent experimental results have documented fibroid tissues to be severely and uniformly hypoxic.²⁹ Hypoxia is known to acutely promote the generation of O₂^{•-} from various intracellular enzyme systems.^{9,30,31}

The NOX is a major source of ROS, generating O₂^{•-} from NAD(P)H and molecular oxygen, in various cell types and plays a crucial role in various physiological and pathological processes.^{32,33} The NOX enzymes are essential for the control of many cellular functions including differentiation, proliferation, and cell death, as well as signal transduction.³⁴

Figure 3. Quantitative real-time RT-PCR analysis of nicotinamide adenine dinucleotide phosphate (NAD(P)H) oxidase subunits p67^{phox}, NOXA1, and p47^{phox}. Real-time RT-PCR was utilized to measure messenger RNA (mRNA) levels of p67^{phox} (A) and its homolog, NOXA1 (B), and p47^{phox} (C) and its homolog, NOX organizer (NOXO) 1 in myometrial cells (n = 1) and fibroid cells (n = 1) treated with and without hypoxia (2% O₂). Expression is depicted as the mean with error bars representing standard deviation. *P < .05 as compared to untreated myometrial cells and **P < .05 as compared to untreated fibroid cells. NOX indicates NAD(P)H oxidase; NOXA1, NOX activator 1; RT-PCR, reverse transcription-polymerase chain reaction.

Activation of NOX enzymes 1, 2, and 3 is dependent on different combinations of their binding molecules whereas NOX5 requires activation by calcium.^{18,35} The NOX4 is activated through the binding of p22^{phox} and is known to control the expression of the other NOX family members.³⁵ Lack of NOX4 expression, that is, a dominant negative NOX4 phenotype, results in a lack of NOX2 expression, while NOX4 overexpression is known to increase the generation of ROS in kidney fibroblast cells.³⁵⁻³⁸ The NOX4 is also known to be localized to the endoplasmic reticulum and the nucleus of many cell types, including endothelial cells, adipocytes, and fibroblasts.³⁹ In fact, NOX4 has been demonstrated to be expressed in several tumor types and is involved in cellular senescence, resistance to apoptosis, tumorigenic transformation, cell proliferation, cell survival, and radiation resistance.³³ The NOX4 and p22^{phox} are also known to function as active O₂ sensors, while DUOX1 and DUOX2 are the sole proteins directly generating H₂O₂ outside of the cell.⁴⁰⁻⁴²

Expression of the different NOX isoforms as well as the regulatory subunits is known to affect the levels of the core protein, which can ultimately affect the enzyme activity level. Thus, observed increase in expression and activity of NOX in fibroid as compared to myometrium tissues and cells could be attributed to a unique pattern of expression of NOX isoforms and their binding molecules in fibroids. It is known that the binding between either one of the NOX isoforms and p22^{phox} is essential for the production of O₂^{•-}.^{32,43} The binding of NOX4 with p22^{phox} produces O₂^{•-} constitutively without combining with other subunits, whereas the binding of NOX1/NOX2 and p22^{phox} requires the addition of cytosolic regulatory subunits, such as p47^{phox} or the GTPase Rac.^{42,44} Our results showed a significant basal level increase in NOX4 and a decrease in p22^{phox} in fibroid cells than that in myometrial cells (Figure 2).

Therefore, it is interesting that many of the other subunits described in this study, such as NOX2, DUOX1, p47^{phox}, and

p67^{phox}, are found to be increased in fibroids as compared to myometrial cells and may serve as a potential compensatory mechanism for overproduction of O₂^{•−} in fibroid cells. Accordingly, fibroid cells manifest constitutively higher oxidants levels than normal myometrium cells. An additional factor that modulates activation of NOX is the translocation of regulatory subunits from the cytoplasm to combine with catalytic core in the membrane. There is an increase in the membrane-bound p47^{phox} and p67^{phox} in fibroid but not in normal myometrium cells, indicating that the translocation of p47^{phox} and p67^{phox} may contribute to enhanced oxidase activity in fibroid cells, whereas the lack of this translocation may explain the attenuated oxidase activity in normal myometrium.

Parallel preliminary results from our laboratory have indicated NAOX to be overexpressed in both endometriosis and postoperative adhesions as compared to their respective counterparts. These results further support a role for NOX in promoting a prooxidant state in benign conditions. In this study, we have demonstrated the upregulation of NOX4 in fibroid as compared to myometrial cells and tissues. Additionally, we have shown that fibroid cells are characterized by a unique NOX profile, which promotes a severe prooxidant state that may contribute to the development of fibroids. Therefore, targeting NOX family members may be beneficial for future therapeutic interventions.

Declaration of Conflicting Interests

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

Funding

The author(s) received no financial support for the research, authorship, and/or publication of this article.

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