PI:	Title:	
Received: 06/16/	FOA:	Council:
Competition ID:	FOA Title:	
1 R21	Dual:	Accession Number:
IPF:	Organization:	
Former Number:	Department: Obstetrics and Gynecology	
IRG/SRG:	AIDS: N	Expedited: N
Subtotal Direct Costs (excludes consortium F&A) Year 1: Year 2:	Animals: N Humans: Y Clinical Trial: N Current HS Code: HESC: N	New Investigator: N Early Stage Investigator: N
Senior/Key Personnel:	*Organization:*	*Role Category:*
		PD/PI
		Co-Investigator

OMB Number:
Expiration Date: 06/30/

APPLICATION FOR FEDERAL ASSISTANCE
SF 424 (R&R)

3. DATE RECEIVED BY STATE	**State Application Identifier**

1. TYPE OF SUBMISSION*

❍ Pre-application ❍ Application ● Changed/Corrected Application

4.a. Federal Identifier

b. Agency Routing Number

2. DATE SUBMITTED | **Application Identifier**

c. Previous Grants.gov Tracking Number

5. APPLICANT INFORMATION **Organizational DUNS*:**

Legal Name*:
Department:
Division:
Street1*:
Street2:
City*:
County:
State*:
Province:
Country*: UNITED STATES
ZIP / Postal Code*:

Person to be contacted on matters involving this application

Prefix: First Name*: Middle Name: Last Name*: Suffix:

Position/Title:
Street1*:
Street2:
City*:
County:
State*:
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*:
Phone Number*: Fax Number: Email:

6. EMPLOYER IDENTIFICATION NUMBER *(EIN) or (TIN)**

7. TYPE OF APPLICANT* M: Nonprofit with 501C3 IRS Status (Other than Institution of Higher Education)

Other (Specify):

Small Business Organization Type ❍ Women Owned ❍ Socially and Economically Disadvantaged

8. TYPE OF APPLICATION*

● New ❍ Resubmission
❍ Renewal ❍ Continuation ❍ Revision

If Revision, mark appropriate box(es).

❍ A. Increase Award ❍ B. Decrease Award ❍ C. Increase Duration
❍ D. Decrease Duration ❍ E. Other *(specify)* :

Is this application being submitted to other agencies?* ❍Yes ●No What other Agencies?

9. NAME OF FEDERAL AGENCY*

10. CATALOG OF FEDERAL DOMESTIC ASSISTANCE NUMBER
TITLE:

11. DESCRIPTIVE TITLE OF APPLICANT'S PROJECT*

12. PROPOSED PROJECT
Start Date* Ending Date*
04/01/ 03/31/

13. CONGRESSIONAL DISTRICTS OF APPLICANT

Tracking Number: Funding Opportunity Number: Received Date:

SF 424 (R&R) APPLICATION FOR FEDERAL ASSISTANCE

14. PROJECT DIRECTOR/PRINCIPAL INVESTIGATOR CONTACT INFORMATION

Prefix: Dr. First Name*: Middle Name: Last Name*: Suffix: M.D.
Position/Title: Investigator
Organization Name*:
Department: Obstetrics and Gynecology
Division:
Street1*:
Street2:
City*:
County:
State*:
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*:
Phone Number*: Fax Number: Email*:

15. ESTIMATED PROJECT FUNDING

a. Total Federal Funds Requested*	$	
b. Total Non-Federal Funds*		$0.00
c. Total Federal & Non-Federal Funds*	$	
d. Estimated Program Income*		$0.00

16.

a. YES ❍ THIS PREAPPLICATION/APPLICATION WAS MADE AVAILABLE TO THE PROCESS FOR REVIEW ON:

DATE:

b. NO ● PROGRAM IS NOT COVERED

❍ PROGRAM HAS NOT BEEN SELECTED BY STATE FOR REVIEW

17. By signing this application, I certify (1) to the statements contained in the list of certifications* and (2) that the statements herein are true, complete and accurate to the best of my knowledge. I also provide the required assurances * and agree to comply with any resulting terms if I accept an award. I am aware that any false, fictitious, or fraudulent statements or claims may subject me to criminal, civil, or administrative penalties. (U.S. Code, Title 18, Section 1001)

● I agree*

** The list of certifications and assurances, or an Internet site where you may obtain this list, is contained in the announcement or agency specific instructions.*

18. SFLLL or OTHER EXPLANATORY DOCUMENTATION File Name:

19. AUTHORIZED REPRESENTATIVE

Prefix: Dr. First Name*: Middle Name: Last Name*: Suffix: Ph.D
Position/Title*:
Organization Name*:
Department: Obstetrics and Gynecology
Division:
Street1*:
Street2:
City*:
County:
State*:
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*:
Phone Number*: Fax Number: Email*:

Signature of Authorized Representative* **Date Signed***

20. PRE-APPLICATION File Name:

21. COVER LETTER ATTACHMENT File Name:

424 R&R and PHS-398 Specific
Table Of Contents

	Page Numbers
SF 424 R&R Cover Page	1
Table of Contents	3
Performance Sites	4
Research & Related Other Project Information	5
Project Summary/Abstract(Description)	6
Project Narrative	7
Facilities & Other Resources	8
Equipment	9
Research & Related Senior/Key Person	10
PHS398 Cover Page Supplement	19
PHS 398 Modular Budget	21
Personnel Justification	24
PHS 398 Research Plan	25
Specific Aims	26
Research Strategy	27
Human Subjects Section	33
Protection of Human Subjects	33
Planned Enrollment Report	36
Bibliography & References Cited	37
Letters Of Support	40

Project/Performance Site Location(s)

Project/Performance Site Primary Location

❍ I am submitting an application as an individual, and not on behalf of a company, state, local or tribal government, academia, or other type of organization.

Organization Name:
Duns Number:
Street1*:
Street2:
City*:
County:
State*:
Province:
Country*: USA: UNITED STATES
Zip / Postal Code*:
Project/Performance Site Congressional District*:

File Name

Additional Location(s)

OMB Number:
Expiration Date:

RESEARCH & RELATED Other Project Information

1. Are Human Subjects Involved?* ● **Yes** ❍ **No**

1.a. If YES to Human Subjects

Is the Project Exempt from Federal regulations? ● Yes ❍ No

If YES, check appropriate exemption number: _ 1 _ 2 _ 3 ✔ 4 _ 5 _ 6

If NO, is the IRB review Pending? ❍ **Yes** ❍ **No**

IRB Approval Date:

Human Subject Assurance Number

2. Are Vertebrate Animals Used?* ❍ **Yes** ● **No**

2.a. If YES to Vertebrate Animals

Is the IACUC review Pending? ❍ Yes ❍ No

IACUC Approval Date:

Animal Welfare Assurance Number

3. Is proprietary/privileged information included in the application?* ❍ **Yes** ● **No**

4.a. Does this project have an actual or potential impact - positive or negative - on the environment?* ❍ **Yes** ● **No**

4.b. If yes, please explain:

4.c. If this project has an actual or potential impact on the environment, has an exemption been authorized or an environmental assessment (EA) or environmental impact statement (EIS) been performed? ❍ Yes ❍ No

4.d. If yes, please explain:

5. Is the research performance site designated, or eligible to be designated, as a historic place?* ❍ **Yes** ● **No**

5.a. If yes, please explain:

6. Does this project involve activities outside the United States or partnership with international collaborators?* ❍ **Yes** ● **No**

6.a. If yes, identify countries:

6.b. Optional Explanation:

Filename

7. Project Summary/Abstract*

8. Project Narrative*

9. Bibliography & References Cited

10.Facilities & Other Resources

11.Equipment

Impact of Aberrant miRNA Expression in the Pathobiology of Uterine Leiomyomata

Uterine leiomyoma (LYO) [fibroids] are the most common neoplasms in premenopausal women, confer significant morbidity during the reproductive years, remain the leading indication for hysterectomy, and account for approximately 34 billion dollars in annual healthcare expenditures in the United States. The phenotypic signature of these sex steroid responsive uterine tumors is the *de novo* transformation of the uterine wall smooth muscle (myometrium) into LYO which demonstrate excessive formation of the extracellular matrix (ECM). The recent successful application of small noncoding small RNAs, microRNA (miRNAs or miRs), as diagnostic biomarkers and therapeutic targets in various areas of clinical medicine has garnered interest in the field of fibroid biology. Aberrant expression of several miR species in LYO (compared to native myometrium) has been confirmed by several authors as well as our group. Our recent comprehensive miR expression screening of patient matched myometrium (MYO) and LYO and preliminary *in vitro* miR 15b and miR 21-5p overexpression experiments confirmed gene targets of these miRs, which have been implicated in cell proliferation, invasion, and dysregulated ECM formation in MYO and LYO cells.

Unfortunately, investigation of the impact of these and other miRs in the genesis of LYO is in its infancy and the underlying mechanisms of LYO development and recurrence are incompletely understood.

Narrative

Aberrant expression of several microRNA (miR) species in leiomyoma (LYO) (relative to native myometrium) has been confirmed by several authors as well as our group, and has been implicated in the pathobiology of disease. Our recent comprehensive miR expression signature screening of patient matched myometrium (MYO) and LYO and preliminary *in vitro* miR 15b and miR 21-5p overexpression experiments identified gene targets of these miRs which have been implicated in cell proliferation, invasion, and dysregulated ECM formation in MYO and LYO cells.

Resources and Environment

Laboratory:

Core Facilities:

Computer: Dr. has an HP Compaq Pentium IV 2.6 GHz personal computer in his office with a laser color printer, DVD/CD-ROM and with access to external internet sources. There are 24 additional Compaq Pentium IV 2.6 GHz computers with DVD/CD-ROM and Zip drives in the VCRB. Multiple Compaq Pentium4/1GHz computers are available in the laboratory for graphics, data entry, and analysis. Online links for gene analysis, electronic communications, and library resource packages and online journals are present.

Office: Dr. office encompasses approximately 200 sq ft

The coinvestigator, Dr. has adequate office space and a computer with full internet and wireless access. All investigators have dedicated computer access and administrative support.

Clinical:

Laboratory Equipment

OMB Number:
Expiration Date:

RESEARCH & RELATED Senior/Key Person Profile (Expanded)

PROFILE - Project Director/Principal Investigator

Prefix: Dr. First Name*: Middle Name Last Name*: Suffix: M.D.

Position/Title*:
Organization Name*:
Department: Obstetrics and Gynecology
Division:
Street1*:
Street2:
City*:
County:
State*:
Province:
Country*: USA: UNITED STATES
Zip / Postal Code*:

Phone Number*: Fax Number: E-Mail*:

Credential, e.g., agency login: akstyer

Project Role*: PD/PI Other Project Role Category:

Degree Type: M.D. Degree Year:

File Name

Attach Biographical Sketch*: 1234-Biosketch -

Attach Current & Pending Support:

PROFILE - Senior/Key Person

Prefix: Dr. First Name*: Middle Name Last Name*: Suffix: Ph.D

Position/Title*:
Organization Name*:
Department:
Division:
Street1*:
Street2:
City*:
County:
State*:
Province:
Country*: USA: UNITED STATES
Zip / Postal Code*:

Phone Number*: Fax Number: E-Mail*:

Credential, e.g., agency login:

Project Role*: Co-Investigator Other Project Role Category:

Degree Type: Ph.D. Degree Year:

File Name

Attach Biographical Sketch*:

Attach Current & Pending Support:

BIOGRAPHICAL SKETCH

NAME	POSITION TITLE
eRA COMMONS USER NAME (credential, e.g., agency login)	

EDUCATION/TRAINING

INSTITUTION AND LOCATION	DEGREE	MM/YY	FIELD OF STUDY
	BS		Biology
	MD		Medicine
			Internship/Residency
			Research Fellowship in Reproductive Biology
			Clinical Fellowship in Reproductive Endocrinology and Infertility

A. Personal Statement

I have successfully maintained an appropriate balance of delivering innovative clinical care, conducting translational research, and educating clinical fellows, resident physicians, and medical students in the comprehensive medical and surgical care of patients with uterine fibroids, pelvic pain, infertility, endometriosis, endocrine disorders, and advanced minimally invasive gynecologic surgery.

Integrated within the clinical program is our translational research enterprise. I am currently a principal investigator of the interdisciplinary team which is characterizing molecular signatures (microRNA) of fibroids to gain further insight into the pathobiology of this reproductive disorder. Our preliminary work successfully delineated aberrant microRNA (miRNA) expression in leiomyomata compared to matched native myometrium and my group is planning *in vitro* functional studies in primary and immortalized fibroid and myometrial cells. In tandem with these studies, we are investigating pertinent clinical characteristics and outcomes of surgical patients from whom tissue has been banked for molecular studies. With this approach, our goal is to elucidate the underlying impact of aberrant leiomyoma miRNA expression in the genesis, phenotype, and severity of disease and to identify miRNA species with fibroid relevant gene targets which will support the development of effective targeted therapies in the future.

As a principal investigator, my clinical (ambulatory care and surgical volume) and molecular (current and prior research) experience provides the well balanced expertise and resources necessary for impactful translational investigation in this field of fibroid biology. With our recent findings and progress, comprehensive research and tissue repository infrastructure, support of our robust clinical service to provide specimens, and collaboration with my colleague and coinvestigator, Dr.
I am confident that our foundation and approach will ensure the successful organization and execution of the proposed exploratory research plan and will contribute novel insight into the underlying mechanisms of fibroid development and recurrence.

B. Positions and Honors

Positions and Employment

Clinical Fellow in Obstetrics, Gynecology, and Reproductive Biology,
Research Fellow, Reproductive Biology,

Instructor in Obstetrics, Gynecology and Reproductive Biology,
Clinical and Research Fellow in Reproductive Endocrinology and Infertility,

Assistant Professor in Obstetrics, Gynecology and Reproductive Biology

Other Experience and Professional Memberships

Professional Societies

American College of Obstetricians and Gynecologists, Fellow
American Society for Reproductive Medicine, member
Society for Reproductive Endocrinology and Infertility, member

Society for Reproductive Surgeons, member
Society for Reproductive Investigation (formerly Society for Gynecologic Investigation)
World Endometriosis Society
The Endocrine Society

Honors

C. Selected Peer-reviewed Publications

1.

2.

3.

4.

5.

6.

7.

8.

9.

10.

11.

12.

13.

14.

15.

D. Research Support

Ongoing Support

Recently Completed

BIOGRAPHICAL SKETCH

NAME	POSITION TITLE Associate Professor,
eRA COMMONS USER NAME (credential, e.g., agency login)	

EDUCATION/TRAINING

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	MM/YY	FIELD OF STUDY
	B.S. M.S. Ph.D. Postdoctoral		

A. Personal Statement

Human leiomyomata (fibroids) are benign uterine tumors of the uterus, and are the most common neoplasms in women of reproductive age. The focus of the present proposal is to define the functional significance of specific miRNA's shown to be differentially expressed leiomyoma. This research is in line with our overall goal to assess how aberrant expression of specific miRNAs contribute to the clinical presentation, progression and severity of uterine fibroids. This proposal is a direct byproduct of our shared efforts to develop the concepts, procure and bank clinically annotated samples, develop the culture systems, generate the preliminary data, interpret the results and formulate the aims for the pilot proposals as well as the present proposal.

I have the expertise, resources and access to analytical and bioinformatics cores and/or collaborators needed to initiate and successfully complete the proposed aims. Importantly, we already have an active IRB to consent patients undergoing surgical removal of uterine fibroids along with correlative blood, collect the discarded normal and diseased tissue deemed to be excess by the pathologist along with the patients annotated clinical information. The diseased tissue can be used for analysis of microRNA, mRNA, or protein or used for the establishment of in vitro cultures to define function.

B. Positions and Honors

Positions and Employment

Assistant Research Scientist,

Assistant Professor,

Senior Scientist,
Research Scientist,
Adjunct Assistant Professor,

Associate Graduate Faculty,

Associate Biologist,
Senior Scientist,

Assistant Professor of Obstetrics, Gynecology, and Reproductive Biology,

Associate Director

Associate Professor of Obstetrics, Gynecology, and Reproductive Biology,

Affiliate Faculty,

Scientific Director
Director,

Director

Other Experience and Professional Memberships

Society of the Study of Reproduction
Endocrine Society
American Society for Reproductive Medicine
Society for the Study of Reproduction and Fertility
Society for Gynecologic Investigation
American Association Cancer Research
World Endometriosis Society

Society for Gynecologic Oncologists
Scientific Program Director,

Honors

Endocrinology Editorial Board
Reproductive Biology and Endocrinology, Assistant Editor

USDA-Cooperative State Research, Education and Extension Service review panel member; Animal Reproductive Efficiency Session
Biology of Reproduction member of the Board of Reviewers

C. Selected Peer-reviewed Publications (Selected from 87 peer-reviewed publications)
Most relevant to the current application

1.

2.

3.

4.

5.

Additional recent publications of importance to the field (in chronological order)

1.

2.

3.

.

4.

5.

6.

7.

8.

9.

10.

D. Research Support

Ongoing Research Support

Completed Research Support

PHS 398 Cover Page Supplement

OMB Number:

1. Project Director / Principal Investigator (PD/PI)

Prefix: Dr.
First Name*:
Middle Name:
Last Name*:
Suffix:

2. Human Subjects

Clinical Trial? ● No ❍ Yes
Agency-Defined Phase III Clinical Trial?* ❍ No ❍ Yes

3. Permission Statement*

If this application does not result in an award, is the Government permitted to disclose the title of your proposed project, and the name, address, telephone number and e-mail address of the official signing for the applicant organization, to organizations that may be interested in contacting you for further information (e.g., possible collaborations, investment)?

● Yes ❍ No

4. Program Income*

Is program income anticipated during the periods for which the grant support is requested? ❍ Yes ● No

If you checked "yes" above (indicating that program income is anticipated), then use the format below to reflect the amount and source(s). Otherwise, leave this section blank.

Budget Period*	Anticipated Amount ($)*	Source(s)*

PHS 398 Cover Page Supplement

5. Human Embryonic Stem Cells

Does the proposed project involve human embryonic stem cells?* ● No ❍ Yes

If the proposed project involves human embryonic stem cells, list below the registration number of the specific cell line(s) from the following list: http://grants.nih.gov/stem_cells/registry/current.htm. Or, if a specific stem cell line cannot be referenced at this time, please check the box indicating that one from the registry will be used:

Cell Line(s): Specific stem cell line cannot be referenced at this time. One from the registry will be used.

6. Inventions and Patents (For renewal applications only)

Inventions and Patents*: ❍ Yes ❍ No

If the answer is "Yes" then please answer the following:

Previously Reported*: ❍ Yes ❍ No

7. Change of Investigator / Change of Institution Questions

❑ Change of principal investigator / program director

Name of former principal investigator / program director:

Prefix:

First Name*:

Middle Name:

Last Name*:

Suffix:

❑ Change of Grantee Institution

Name of former institution*:

PHS 398 Modular Budget

Budget Period: 1

Start Date: 04/01/ **End Date:** 03/31/

A. Direct Costs

	Funds Requested ($)
Direct Cost less Consortium F&A*	
Consortium F&A	
Total Direct Costs*	

B. Indirect Costs

	Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)
1.		74.00		
2.				
3.				
4.				

(Agency Name, POC Name and Phone Number)

Indirect Cost Rate Agreement Date 05/06/

Total Indirect Costs

C. Total Direct and Indirect Costs (A + B)

Funds Requested ($)

PHS 398 Modular Budget

Budget Period: 2

Start Date: 04/01/ **End Date:** 03/31/

A. Direct Costs

	Funds Requested ($)
Direct Cost less Consortium F&A*	
Consortium F&A	
Total Direct Costs*	

B. Indirect Costs

	Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)
1.		74.00		
2.				
3.				
4.				

(Agency Name, POC Name and Phone Number)

Indirect Cost Rate Agreement Date 05/06/

Total Indirect Costs

C. Total Direct and Indirect Costs (A + B)

Funds Requested ($)

PHS 398 Modular Budget

Cumulative Budget Information

1. Total Costs, Entire Project Period

Section A, Total Direct Cost less Consortium F&A for Entire Project Period ($)
Section A, Total Consortium F&A for Entire Project Period ($)
Section A, Total Direct Costs for Entire Project Period ($)
Section B, Total Indirect Costs for Entire Project Period ($)
Section C, Total Direct and Indirect Costs (A+B) for Entire Project Period ($)

2. Budget Justifications

Personnel Justification	1242-Personnel Justification.pdf
Consortium Justification	
Additional Narrative Justification	

Budget Justification

Personnel

MD., Principal Investigator, will commit 20% (2.4 calendar months) of his effort to the project in both years. Dr. will be responsible for all aspects of the proposal including budgeting, experimental design, trouble-shooting potential pitfalls, review of research data, preparing and editing of manuscripts for submission and preparation of data for presentation at local, national and international venues. Dr. will be responsible for oversight of the research personnel and will meet regularly with Dr. to review results. Dr. is ultimately responsible for maintaining the IRB allowing for future and past collection of tissues as a source of primary cell cultures for this proposal. Dr. will ultimately be responsible for submission of the annual progress reports, communication with Partners and Federal grants administrators.

Co-Investigator, will commit 10% (1.2 calendar months) of his effort to the project in both years. He will work with Dr. in aspects of experimental design, research personnel, trouble-shooting, potential pitfalls, review of research data, preparing and editing of manuscripts for submission and preparation of data for presentation at local, national and international venues. Dr. will as be actively involved in the day to day experiments. Drs. and will meet regularly to review results and discuss future directions. Dr. will work with Dr. to ensure that the use of all human tissue is conducted in accordance with institutional guidelines.

Research Technician will devote 75% (9.0 calendar months per annum) of their time to the proposed aims. He/she will continue to use tissue samples already procured f

His/her responsibilities will vary, but will include tissue processing for histological analyses, day to day maintenance of monolayer and three dimensional cell cultures, lentiviral infection of primary and immortalized myometrial and leiomyoma cells, *in vitro* migration and invasion assays, real-time PCR, Western blots, FACS, cell sorting/isolation, Flow Jo analysis. He/she will be under the direction of Dr. Furthermore, he/she will actively interact with to ensure the collection of relevant coded IRB approved specimens. Aside from conducting daily experiments, he/she will be actively involved in review of research data, and preparation of manuscripts for submission in peer-reviewed journals.

She will devote 10% (1.2 calendar months) to the proposed aims. In her role as CRC, she will be responsible for educating and consenting patients, collecting tissue from pathology, transporting it to the VCRB, and compiling coded patient information in preparation for the use of primary tissue in cell culture experiments.

PHS 398 Research Plan

Please attach applicable sections of the research plan, below.

1. Introduction to Application (for RESUBMISSION or REVISION only)	
2. Specific Aims	1243-Specific Aims.pdf
3. Research Strategy*	1244-Research Strategy.pdf
4. Progress Report Publication List	
Human Subjects Sections	
5. Protection of Human Subjects	1245-Protection of Human Subjects.pdf
6. Inclusion of Women and Minorities	
7. Inclusion of Children	
Other Research Plan Sections	
8. Vertebrate Animals	
9. Select Agent Research	
10. Multiple PD/PI Leadership Plan	
11. Consortium/Contractual Arrangements	
12. Letters of Support	1246-Letter of Support.pdf
13. Resource Sharing Plan(s)	
Appendix (if applicable)	
14. Appendix	

Specific Aims

Human leiomyomata (fibroids) are benign tumors of the uterus, and represent the most common neoplasms in women of reproductive age. The lifetime incidence of fibroids is approximately 60% in the general population.[1] A significant degree of morbidity is associated with uterine fibroids, and removal of the uterus (hysterectomy) is the only option for definitive cure. Unfortunately, symptomatic fibroids remain the leading indication for hysterectomy in the United States (US) with approximately 200,000 of these procedures performed annually.[2] The phenotypic signature of this reproductive disorder is the *de novo* transformation of the uterine wall smooth muscle (myometrium) into fibroids which demonstrate excessive formation of a collagen based extracellular matrix (ECM).[3] Aberrant mechanotransduction and dysfunction of several cell signaling pathways (e.g. TGF-β, PI3K-AKT, MAPK) have been most commonly implicated in ECM dysregulation.[4] However, the underlying mechanisms of cellular transition, proliferation, and excessive ECM formation that lead to clinically evident disease are incompletely understood.

Our long-term objective is to develop novel and more effective treatment strategies for uterine fibroids. We recently identified specific microRNA (miRNA or miR) species that are aberrantly expressed in leiomyomata compared to patient matched myometrium and confirmed the differential pattern of expression. miRNAs can potentially target multiple genes and signaling pathways which influence fibroid development and progression. to determine the functional role of specific aberrantly expressed miRNAs in the underlying pathobiology of fibroid development. Our ultimate goal is to expand our understanding of uterine fibroid pathogenesis and determine if differential miRNA expression signatures can be utilized as diagnostic biomarkers and therapeutic targets for future innovative treatment strategies.

Our central hypothesis is that aberrant expression of miR-15b and miR-21-5p negatively impact the balance of critical signaling pathways resulting in *de novo* myometrial cell transformation that leads to the formation of leiomyomata and/or worsening pathology of existing leiomyoma cells. This hypothesis is supported by evidence demonstrating that several of aberrantly expressed miRNA species (miR-15b and miR-21-5p) in fibroids target genes involved in processes relevant to the uterine fibroid phenotype including cell proliferation, ECM regulation, and tissue remodeling[5]. We have determined that miR-21 targets TGFβ family members and their associated pathways whereas miR-15b targets reversion-inducing cysteine-rich protein with Kazal motifs (*RECK*), a membrane associated matrix metalloproteinase (MMP) inhibitor which is suppressed in several malignancies, and has the potential to mediate vascular smooth muscle proliferation, ECM deposition, and collagen remodeling.[6,7] Collectively, these miRNA species may represent crucial mediators of dysregulated ECM formation and tumorigenesis in uterine leiomyomata. The following **specific aims** will test our central hypothesis and accomplish the objectives of this application:

Aim 1: Determine the functional consequence of miR-15b and miR-21-5p overexpression on myometrial and leiomyoma cells, including but not limited to, proliferation, invasion, and ECM formation.

Aim 2: Delineate the *in vitro* effect of miR-15b and miR-21-5p overexpression on cell signaling pathways implicated in myometrial cell transformation and leiomyoma pathology.

SIGNIFICANCE: The proposed focused research will expand our understanding of the functional role of specific miRNAs in the genesis and or progression of uterine fibroids. This information has the potential to identify novel therapeutic targets allowing for the development of new strategies to improve clinical management of this disease.

The lifetime incidence of fibroids is approximately 60% in the general population and the common symptoms include: heavy menstrual bleeding, anemia (secondary to excessive menstrual blood loss), pelvic pain, painful menses, urinary incontinence, constipation, infertility, and recurrent pregnancy loss. Contemporary medical treatment options (GnRH agonists, progestins, oral contraceptive pills, levonorgestrel intrauterine device, selective progesterone receptor modulators) and procedural interventions (myomectomy [uterine preservation with removal of uterine fibroids], uterine artery embolization, MR guided focused ultrasound) have variable side effect profiles and may not consistently relieve symptoms or prevent disease recurrence. Due to the significant degree of morbidity associated with fibroids, removal of the uterus (hysterectomy) may be the only option for definitive cure.

This gynecologic disorder has become a major public health issue in reproductive-aged women over the past decade. In the US, leiomyomata remain the leading indication for hysterectomy[1], and confer an estimated total annual societal cost (medical/surgical care and loss of work days) of approximately $34.4 billion.[2] Although it is one of the most commonly treated gynecologic disorders, the underlying mechanisms of development and recurrence are incompletely understood. The most commonly accepted etiology is the transformation of myometrial smooth muscle (comprises majority of uterine muscle mass) fibroblasts and dysfunctional extracellular matrix (ECM) formation [3]. However, the specific functional role of growth factors, chemokines, cytokines, and genetic mediators in cellular transformation, proliferation, apoptosis, neovascularization, and cellular hypertrophy have been difficult to characterize with certainty.[3] Most prominently, transforming growth factor beta (TGF-β) and its cognate receptor (TGF-βR), have been previously implicated by many investigators as a critical mediator of cell signaling contributing to the genesis of uterine fibroids.[3,4] The intrinsic biological function of TGF-β, which includes cellular hypertrophy and ECM turnover are central to various fibrotic disorders, and may be essential to fibroid development and progression.[3] Recently, investigators have provided additional insight into the possible roles (independent and synergistic) of several other molecules including REST (RE1 suppressing transcription factor), G protein receptor 10 (GPR10), platelet derived growth factor (PDGF), epidermal growth factor (EGF), matrix metalloproteinases (MMPs) in the alteration of several genes (e.g. *MED12*, *HMGA2*, *HMGA1*, *CYP1A1*, *CYP1B1*), proto-oncogenes (e.g. p27, p53) and related signaling pathways (e.g. PI3K-AKT-MTOR, RAS-ERK-MAP Kinase), and gene silencing via microRNA (miRNA or miR) postranscriptional inhibition in the pathobiology of fibroids.[5,6,7-9] Unfortunately, it has been difficult to correlate findings of molecular studies to disease phenotype.

Small noncoding RNAs have garnered mounting interest as biomarkers of disease and as therapeutic targets.[10] Novel approaches to develop miRNA as diagnostic and predictive biomarkers in human diseases such as amyotrophic lateral sclerosis and neuroblastoma have raised promise for their use to improve clinical diagnosis and surveillance.[11,12] The successful use of a miR-122 inhibitor to effectively treat active hepatitis C infection in the first ever human multicenter randomized placebo controlled trial has propelled the therapeutic application of miRNAs to the forefront of clinical medicine.[13] Sentinel studies by Marsh *et al.* and Wang *et al.* which demonstrated aberrant miRNA expression in leiomyomata (compared to native myometrium) provided the foundation for recent *in vitro* miRNA functional studies in fibroid biology.[5,14] Although several functional studies have provided preliminary insight into the possible mechanistic role of some miRNAs, more comprehensive investigations of the functional significance of specific miRNAs will be necessary to delineate their contribution to this disease.[15]

INNOVATIONS:

As mentioned, the most distinctive phenotypic feature of leiomyomata is the excessive synthesis of ECM proteins, namely rigid collagen type I and III.[16] Notably, several profiling studies have also shown that ECM genes encoding collagen proteins are differentially expressed in fibroids compared to native myometrium.[17] In addition to excessive ECM formation, dysregulated remodeling and tissue turnover may contribute to the pathology of this disorder. Specifically, aberrant expression of several matrix metalloproteinases (MMPs) (e.g. MMP2 and MMP11) and alteration in mechanotransduction have been confirmed and implicated in the activation of specific signaling mediators and pathways (e.g. Rho GTPase, PI3AKT, and MAPK) during leiomyoma proliferation and ECM organization.[11,18,19] Potential mechanisms for the propagation of collagen deposition may be due in part to complex interactions of ECM collagen proteins with integrins (primary ECM receptors) and growth factor mediated mitogenic pathways.[16] Since ECM components have been shown to support cellular morphology and serve as an interface between external stimuli and native myometrium/fibroid site by regulating proliferation, migration, and survival, this may represent a prime target to assess the functional role of specific miRNAs in uterine fibroids.

miRNA regulation of cell proliferation, apoptosis, inflammation, angiogenesis, tissue turnover, and tumor suppressor/oncogene modification, which are thought to be crucial processes in the pathobiology of human malignancy, have also been hypothesized to play significant roles in the development of uterine fibroids.[20] To this end, the field of fibroid biology has adopted similar approaches (e.g. *in vitro* models, molecular indices) for inferring the biological function of miRNAs. Several studies over the past decade have assessed the *in vitro* impact of gain (miRNA "mimics" or upregulation) or loss (miRNA inhibition, "antagomirs", "sponges") of function on endpoints which are relevant to the fibroid phenotype. **Prior functional miRNA studies in fibroids have provided preliminary insight into their potential contribution to proliferation, ECM formation, tissue turnover, and angiogenesis**. With global pattern analyses (CGH) in primary fibroid and myometrial samples, Zavadil *et al.* reported patterns of inverse association of miRNA expression with mRNA (miRNA predicted targets) expression in leiomyomata involved signaling pathways including MAP kinase, WNT, JAK-STAT and TGF-β, as well as dysregulation of genes involved in cell proliferation, cell adhesion, cell matrix formation, and transcriptional reprogramming.[15] Subsequently, investigators demonstrated that *F3* and *IL8* are direct targets of miR93/106b cluster, and implicated regulation of *IL8*, connective tissue growth factor (CTGF), plasminogen activator inhibitor (PAI-1), pentraxin related protein (PTX3) through F3 via this miR cluster.[21] Chuang *et al.* reported miR 200c as a regulator of epithelial to mesenchymal transition and tumorigenesis (*ZEB1/2* [zinc finger E-box-binding homeobox], *FBLN5* [fibulin 5]), cellular transition (E-cadherin), angiogenesis (*VEGF-A* [vascular endothelial growth factor A]), and matrix remodeling (*TIMP2* [tissue inhibitor of metalloproteinase 2] and *FBLN5*).[22] The findings of this study revealed the complexity of miR gene dysregulation and implicated miR200c as a possible regulator of several cellular processes contributing to the genesis of fibroids.[22]

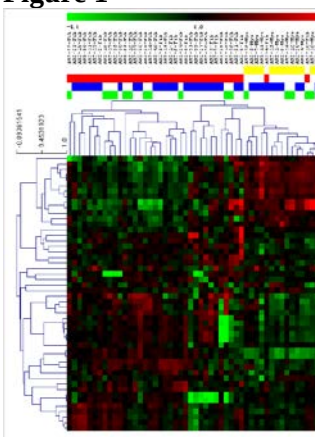
After preparing an invited review of the literature concerning miRNAs in the pathobiology of fibroids, **it became overwhelmingly evident that investigation of the functional role of aberrant fibroid miRNA expression is in its infancy and requires more comprehensive study.[23] What is known is that specific** miRNAs are possible indirect or direct regulators of gene targets which contribute to the pathology of disease and miRs such as let-7, 200a, 200c, 93, 106b, and 21 and have been implicated in cellular proliferation, apoptosis, extracellular matrix turnover, angiogenesis, and inflammation by several investigators[23]. Preliminary data also suggests that *in vitro* miRNA expression in leiomyomata and myometrium can be regulated by sex steroids.[3] Unfortunately, the recent retraction of one of the most prominent and comprehensive functional studies of miR-21-5p in leiomyomata[24] has conveyed significant uncertainty of the impact of this widely studied miR in TGF*β* signaling and its regulation by sex steroids. Moreover, recent investigation of the function role of miR-21-5p in leiomyoma by Fitzgerald *et al.* did not provide evidence of regulation of Programmed Cell Death 4 (*PDCD 4*) as anticipated.[25] Elevated miR-21-5p has been observed in myocardial, renal and pulmonary fibrosis models.[26] It is considered a "profibrogenic miR" since it has been shown to target the TGFβ downstream signaling inhibitor, SMAD7, and accentuates the profibrotic function of TGF signaling mediators in hepatic stellate cells.[26] Inhibition of miR-21-5p has been shown to increase RECK and Metalloproteinase inhibitor 3 (TIMP3) [both inhibitors of MMPs], reduce MMP 2 expression, and decreased tumor migration and invasion in an *in vitro* and *in vivo* glioblastoma model.[27] Although RECK, TIMPs, and members of the TGFβ pathway have been validated, targets of miR-21-5p and the functional significant of miR-21-5p has been delineated in various cancer models, the impact of miR-21-5p on

these and other related mediators of tumor proliferation, invasion, and ECM formation has not been confirmed in fibroids. **In light of the known role of miR-21-5p in targeting tumor suppressors in several malignancies and consistent reports of aberrant expression in fibroids in several studies, further investigation has great potential to elucidate the functional role of this miR**.

As evidenced by our preliminary data, miR-15b also represents a promising miR species for further investigation. Irregular miR-15b expression has been observed in hepatitis as well as a diversity of malignancies. Recently, investigators have demonstrated PDGF induced miR-15b expression as a requisite event in vascular smooth muscle cell proliferation in the pulmonary system[28]. *RECK* is a confirmed gene target of miR-15b by our group (please refer to Approach section), and inhibits the enzymatic activities of specific MMPs.[29] This membrane associated MMP inhibitor is suppressed in many tumor types, impacts collagen matrix remodeling, and is a direct inhibitor of MMP9.[29] Furthermore, investigators have confirmed that *RECK* is also a target of miR-21-5p in prostate cancer and is associated with the rate of tumor invasion.[30] Notably, miR-15b has also been confirmed to target SMAD 7, a downstream inhibitor of the TGF β signaling pathway.[31] RECK mutants lacking target sites for this miR demonstrate significantly reduced tumor invasion and metastases.[32] **As a confirmed miR associated with human malignancy and as a suppressor of *RECK*, miR-15b represents an intriguing target to expand our understanding of the potential miR regulation of proliferation, invasion, and ECM remodeling.**

Our understanding of the putative functional role of miRNAs in leiomyomata has been guided by a limited number of studies with gene targets and endpoints adapted from other disease models. Therefore, the field's limited knowledge of the impact of these small noncoding RNAs on the pathology of leiomyomata provides a prime opportunity to expand prevailing understanding of their role in the genesis and progression of disease. **We will direct our attention to miR-15b and miR-21-5p, which have known roles as oncomirs and have validated gene targets that impact cell proliferation, ECM formation and tissue remodeling. To this end, the studies proposed in this R21 application will primarily focus on miR-15b and miR-21-5p gain of function *in vitro* studies to delineate their contribution to the pathobiology of the disease.**

Figure 1



APPROACH:

I. Rationale: Matched specimens (fibroid and myometrium) from 37 women undergoing either myomectomy or hysterectomy for symptomatic uterine fibroids from 2010-2012
were analyzed (miRCURY LNA™ Universal RT microRNA qPCR [Exiqon Inc.]) for expression of miRNA species. Relative fibroid (LYO) miRNA expression (Benjamin Hochberg Adjustment ($p < 0.01$ statistically significant) were assessed relative to matched native myometrium (NMYO). Approximately 360/742 unique miRNA species were detected per sample and 142/742 miRNA species were common to all specimens analyzed. One hundred and eight differentially expressed miRNAs were observed in all LYO samples compared to all NMYO. Representative hierarchical heat map [red represents upregulated miRNAs and green represents down regulated miRNA relative expression in LYO compared to NMYO (Figure 1)].

In summary, these initial miRNA studies independently confirmed aberrant expression of several miRNA species in LYO, as supported by two prior sentinel studies [14,33] and provided invaluable guidance to assess potential miRNA candidates for functional studies.

Validated gene targets of the miRNA were identified by two independent investigators in our group with the use of several commonly cited miRNA data bases (http://microrna.sanger.ac.uk/; http://www.target scan.org/; /; http://www.umm.uni-heidelberg.de/apps/zmf/mirwalk). This analysis provided guidance to select miRNA-gene target cohorts which we felt relevant for miRNA functional studies. There were a number of select miRNA species and their gene targets with statistically significant expression in LYO relative to NMYO ($p<0.001$) (Fold change of 1.5 or greater). Real time PCR was performed to confirm initial miRNA analyses of the primary banked specimens. Many of these have validated gene targets reported in tissue systems other than fibroids.

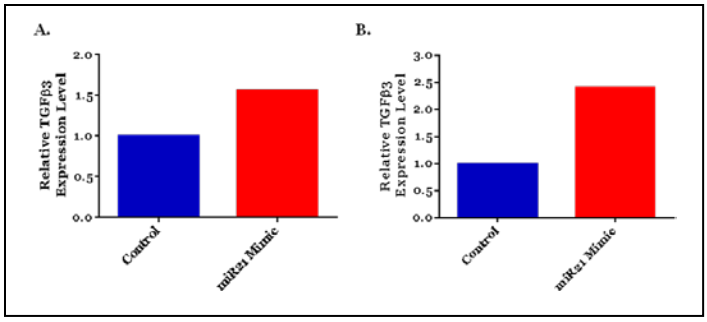


Figure 2. Real time PCR analysis of *TGFβ3* expression following miR-21-5p mimic transfection in primary human myometrial (A) and fibroid cells (B).

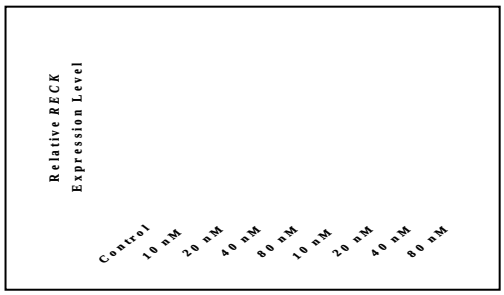


Figure 3. Real time PCR analysis of *RECK* expression following transfection with a miR-15b mimic (red) or inhibitor (green).

Since the respective gene targets of miR-15b and miR-21-5p mediate processes which are biologically relevant to the proliferation and the dysregulated ECM formation of fibroids, these two species were identified as promising candidates for *in vitro* functional studies.

In preliminary experiments, our group confirmed that upregulation of miRs 15b and 21-5p in myometrial and fibroid cell cultures (obtained from patient matched surgical specimens) and commercial myometrial (Promocell HutSMC C 12575) and fibroid cells (Coriell Institute GM 10964) with transient transfection of their respective mimics (Sigma Aldrich) is feasible (data not shown). miR gain of function experiments with short term miR mimic transfection resulted in a change in expression of the gene targets for miR-15b (*RECK*) and miR 21 (*TGFβ3*) miR 21 mimic transfection in myometrial cells (Promocell HutSMC C 12575) resulted in upregulation of *TGFβ3* expression at 24 hrs (Figure 2). Reduced and increased expression of *RECK* following transfection of myometrial cells with miR-15b mimic and inhibitor respectively was observed via real time PCR at 48 hrs (Figure 3). Similar respective trends of RECK protein expression change (Western blot and Immunofluorescence) were observed following transfection experiments (data not shown). **For the first time, these studies have confirmed potential *in vitro* gene targets of miR-15b and miR-21-5p in fibroids. <u>These preliminary results provide justification for future comprehensive functional studies.</u>**

II. Proposed Studies:

Aberrant expression of both species of miRNA have been implicated in the onset or progression of other disease via mediators of cell invasiveness (e.g. RECK, MMPs) and ECM remodeling (TGFβ), so it is reasonable to ascertain their involvement in the development and/or progression of uterine fibroids. An increase in miR-15b and miR-21-5p expression in fibroids relative to the myometrium has been confirmed by either our preliminary studies and/or in the case of miR-21-5p, by prior investigators as well. Moreover, our preliminary data supports the concept that transient over expression of miR-15b and/or miR-21-5p will negatively impact the expression of RECK and TGFβ3 which we hypothesize will result in myometrial cells gaining molecular and phenotypic characteristics associated with leiomyoma cells. Furthermore, we hypothesize that over expression of miR-15b and/or miR-21-5p in leiomyoma cells that either express little to no miR-15b and/or miR-21-5p or a moderate expression of miR-15b and/or miR-21-5p will 'worsen' the molecular or phenotypic characteristics associated with leiomyoma cells. The endpoints would include but are not be limited to alteration of proliferation, invasiveness, migratory potential, and collagen levels.

<u>Techniques</u>: To test our hypotheses we will utilize matched primary myometrium and fibroid derived cells retrospectively or prospectively collected from consented patients. The samples are de-identified

In addition, we will use immortalized matched myometrial and fibroid cell lines

The cells will be infected with lentiviral vector microRNA mimic system (either miR-15b or 21-5p mimics) to achieve miRNA overexpression with the appropriate controls for all experiments of this specific aim. Each experiment herein will be performed in triplicate per cell type. Monolayer culture systems will be utilized for assessment of cell proliferation and migration as previously

described[16,35]. Concurrent flow cytometric analysis of matched samples (derived from same cell source for proliferation assay) will be analyzed for cell cycle phase distributions with 3-laser LSR II instrument and analyzed using FlowJo 7.6.3 in support of the cell counts. A three dimensional culture system will be used for assessing colony forming and invasion parameters[36]. Note** Prior to initiation of proposed experiments, we will conduct preliminary experiments to determine the appropriate MOI for a minimum of a two fold increase in the targeted proteins, RECK and TGFβ3, as observed in the transient transfection experiments

Real Time PCR and Western blot analysis of the impact of miR-15b mimic and miR-21-5p mimic on the gene and conjugate protein of RECK and TGFβ3 respectively will be performed at 0, 24, 48, 72, and 120 hrs following lentiviral infection. In addition, baseline smooth muscle markers including SMC-specific α-actin, desmin, and vimentin of all respective miR mimic treatments and cell types cultured in both conventional plastic *in vitro* culture systems and the 3D system will be assessed at 0, 24, and 48 hrs. Additionally, markers of ECM formation will be assessed (0, 24, 48, 72 & 120 hrs) for all respective miR mimic treatments and cell types cultured in conventional monolayer and 3D cultures. ECM endpoints will include Western blot expression of fibronectin, versican variant (V0), fibromodulin, dermatopoetin, thrombospondin, COL1A1, active RhoA as previously described by others.[19,34] To gain further insight into the *in vivo* interplay of native myometrium and fibroid during excessive ECM formation, the same ECM markers will be assessed in dual tissue cultures systems (myometrial and fibroids cell types) as previously described in this aim. Concurrent expression of MMP2 and MMP11 (previously implicated in dysregulated tissue turnover in fibroids) and MMP9 (inhibited by the miR-15 b target *RECK*) will be assessed by Western blot at similar intervals described above.

Expected Results: Since our preliminary experiments and prior published studies with miR-15b and miR-21-5p have confirmed gene targets such as the TGFβ and RECK, and these genes mediate proliferation, tissue remodeling and ECM formation in several organ systems, it is anticipated that increased miR expression via lentiviral miR mimic infection will confirm the functional role of these miRs in the pathobiology of disease. Since both miR-15b and miR-21-5p were expressed at greater levels in leiomyoma compared to native myometrium, a differential effect of increased miR expression following lentiviral mimic infection may be dependent upon cell type (e.g. myometrial versus leiomyoma and primary tissue cell culture versus immortalized cell line). Specifically, proliferation, invasion, and ECM indices observed in gain of function studies in cells derived from myometrium may be similar to control treatments of cultured cells derived from leiomyomata.

Individual miRNAs are known to have multiple targets that could impact individual or multiple cell signaling pathways. Thus, preliminary evidence supporting miRNA induced gene and protein changes does not necessarily imply a direct effect. By example, the aberrant expression of miR-15b and/or 21-5p could have a far reaching impact. Notably, investigators have demonstrated that TGFβ stimulated miR 21 utilizes PTEN to facilitate AKT/mTORC1 signaling for cellular hypertrophy and matrix expansion glomerular mesangial cells.[37] Likewise, miR 21 is shown to be regulated by β catenin and promotes invasion of glioma cells direct targeting of RECK [38]. Hypoxia and RAS signaling pathways down regulate RECK protein via various miR[32]. These examples provide evidence to support the concept that, the addition or deletion of miR-15b or 21-5p could affect anywhere upstream of the changes we observed at the gene and protein levels. To better define how expression (or lack thereof) of the miRs affect RECK and TGFβ and their applicable cell signaling pathways, we will utilize next generation RNA quantification strategy to provide better insight as to how these miRs might be impacting the genes of interest but also provide a comprehensive global analysis of potential upstream and downstream mediators which may be involved with miR-15b and miR-21-5p expression or consequence thereof in myometrial and leiomyoma cells.

Techniques: Cultured fibroid and myometrial cells (matched myometrial and fibroid cells from our repository and immortalized matched myometrial and fibroid cell lines [34]) will be infected with lentiviral vector microRNA mimic system (either miR-15b or 21-5p mimics) to achieve miRNA overexpression with the appropriate controls. Cultures will be harvested at 0, 48, 72, 96, and 168 hrs post

infection. RNA will be extracted from each sample (n = 3 replicates per cell type cultured and miR treatment) and subjected to next generation RNA quantification using Illumina HiSEQ 2000 to determine genome level changes. Differentially expressed genes will be identified in supervised analysis using absolute fold change (FC>2) and P value (P value <0.05). To further understand the biological impact of miRNA overexpression, we will perform pattern, functional, and pathways analysis in each respective cells type. Significant gene changes that occurred exclusively as a result of introduction of either lentiviral mimic when compared to the non infected and control infected cells will be identified and then stratified by the number of gene interactions, number of initiating molecules and known interactions. The focus of our analyses will be on fibroid relevant signaling pathways including TGFβ associated pathways such as MAPK and SMAD, ERK 1/2, PI3AKT (for miR-15b and miR-21-5p), STAT3, RAS pathways (miR15b/*RECK*).[39]

Expected Results: We anticipate the development of a signature comprising genes and gene signaling pathways whose expression significantly changes with the increase expression of the miR-15b and miR-21-5p respectively. This signature is expected to provide insight as to where in the pathway and on what gene(s), miR-15b and miR-21-5p might affect its direct action allowing for follow up confirmatory experiments including but not limited to promoter analysis. We expect to demonstrate pathway regulation post change in mimic, will confirm the impact of the proposed miR targets, and will reveal potential novel areas for functional miRNA/gene interaction studies in leiomyomata.

Interpretation, Potential Problems, and Future Directions: Since a particular miR species may have several concurrent gene targets and/or tissue or organ specific biologic function(s), the results of miRNA gain of function may have variable effects of our selected endpoints in primary cell cultures compared to cultured immortalized cell lines. To this end, we plan to utilize different types of myometrial and leiomyoma pairs (derived from patient matched primary surgical specimens and immortalized cell lines) to provide a comprehensive assessment of *in vitro* miR effects following introduction of lenti-miRs. We appreciate that we are focused on over expression via mimics and not antagomiRs. Although their effectiveness is often challenged, we have the option to utilize this strategy and test the concept that reduction of miR-15b and miR-21-5p could reverse the anticipated molecular and phenotypic changes observed in these cells. Alternatively, once we identify the possible molecular epicenter we can employ other strategies to better define their direct effects (i.e. promoter reporter, down stream add back, etc). It is also important that we appreciate the appropriate time interval from initial miR introduction to an observed *in vitro* change in our selected endpoints may also be variable. Time points for proliferation, migration, invasion, and ECM experiments may require modification. Since dysregulated ECM formation is characteristic of leiomyomata cells *in vivo* and *in vitro*, results of some experiments utilizing 3D culture systems (contain collagen) may demonstrate differential results than conventional cell cultures. We will assess similar ECM endpoints in both conventional and 3D cultures to account for any intrinsic culture system confounders. Since aberrant overexpression of miRs in native myometrium may account for its *de novo* development into fibroids, gain of function of miR-15b and/or miR-21 in cells derived from leiomyoma may result in an exaggeration of the fibroid phenotype or may not result in an observed change in our selected endpoints compared to myometrial cells. As proof of concept, we may need to utilize loss of function studies with miR-15b and miR-21-5p inhibitors to assess reversion of leiomyoma cells towards myometrial phenotype. It should be noted that all antibodies and ELISA proposed are commercially available. Moreover we have extensive experience in conducting these and all other assays proposed. The next generation RNA quantification on the Illumina HiSEQ 2000 platform will be conducted by a core facility and supported by their bioinformatics personnel. We don't anticipate any difficulties with this aspect of the proposal since recently performed similar analyses. Given the extensive number of signaling pathways involved in any biological organ/tissue system and disease process, we anticipate that additional changes in gene signatures and gene signaling pathways other than those previously observed in fibroids will be observed. To this end, supplementary confirmatory studies may be required. Since gene transcription, activation, and inhibition in gene signaling pathways may not occur at predictable time intervals following miR gain of function, time points may need to be modified to capture the potential effects.

Human Subjects

In accordance with Exemption 4 - the research described herein involves the use of primary tissue and corresponding clinical information derived from the .
All tissue and clinical information provided (patient age, tissue histophenotype) will be coded by the so the investigators cannot directly or through identifiers link the specimens back to the subjects.

1. Risks to the Subjects

a. Human subject involvement

A experiment of the research described in this proposal includes the establishment of cell cultures derived from primary myometrium or fibroid samples (via hysterectomy or myomectomy) collected from patients cared for by the gynecology clinical team at from purchased commercial cells, or from previously established deidentified immortalized cell lines which were graciously provided by an outside institution. Benign and malignant gynecologic tissues/cells are collected under trial protocol for deposit into and for immediate use in IRB approved research projects as proposed herein.

Once a candidate patient is identified, the surgeon will typically contact a Clinical Research Coordinator (CRC) who educates the patient and provides a consent form to the patient for review and the option to sign. If the patient consents, the CRC coordinates appropriate tissue handling with the Pathologist. Patients are asked at the time of consent for permission to bank excess myometrium, fibroids, serum, and urine. All information related to patient history, diagnosis, treatment, outcome and samples is stored on a secure data base for which only IRB approved personnel have access. For the proposed study, only coded tissue/cells and annotated clinical information will be provided via the Banking Manager.

b. Characteristics of the subject population

The patients are identified by Minimally Invasive Gynecologic Surgery, and General Gynecology Teams For the project proposed, we will be focusing on adult females preparing for surgical intervention following a diagnosis of symptomatic uterine fibroids. Based on the records to date we anticipate the age of the patients to range from 20 to 50 years old.

c. Criteria for inclusion and exclusion.

We will utilize tissue/cells from any consented adult female following a diagnosis of symptomatic uterine fibroids. It is extremely rare that a female child would develop symptomatic uterine fibroids. Regardless, our IRB does not allow for inclusion of children.

d. Involvement of vulnerable populations.

We anticipate that we will have more than sufficient amounts of available tissue and have no reason to include any vulnerable populations. Moreover, this population would not be eligible for consent

2. Sources of Materials

We will be receiving the tissue/cells and clinical information from the The samples will be coded prior to delivery. has tumor banking technicians that collect fresh myometrium and fibroids from pathology after review by the pathologist or pathology technologist. Although not to be used for this proposal, serum, urine, and plasma are typically collected preoperatively after the patient has signed consent. Under protocol , it is also possible that discarded tissue can also be collected from non-consented patients. However, no patient identifiers are allowed to follow these samples and they are required to remain anonymous.

3. Potential Risks

Only excess surgically discarded material that has been cleared by our institutional pathologist is collected for banking and ongoing studies. Hence, the risks to the patient are minimal. Tissue holding identifier links are kept by the appropriate administrative component When Drs. are involved in the research, all sample requests have to processed by a third party not directly involved in the project. The links are stored on the server under appropriate electronic safeguards and requires user IDs, passwords and has the ability to track usage. When necessary, secondary linking numbers are generated to separate unique identifiers from the material.

4. Adequacy of Protection Against Risks

a. Recruitment and informed consent

Drs. have completed the CITI Course in the Protection of Human Subjects. Some tissue and primary cells utilized in proposed experiment o in Dr. laboratory will be derived from the infrastructure.

Patients are allowed to withdraw consent and have their tissues destroyed at any time.

b. Protection against risks

Tissue banking trials collecting excess tissue from clinically indicated procedures entails very minimal risks to participants. Tissues are distributed to collaborators, are delivered without unique identifiers. Drs (and or another banking administrator delegate when Drs. are involved in the research) have the ability and permission to distribute non-identifying information, such as histology, or treatment outcome (e.g. response to primary therapy).

5. Potential Benefits of the Proposed Research to the Subjects and Others

It is very unlikely that the knowledge gained from the study of these tissues will directly benefit the subjects who donated the tissue. It is possible that molecular characterization of some of the material may provide therapeutic clues for their treatment but this is thought to be unlikely. It is possible that if this research proves that specific microRNAs (miRNAs) are differentially expressed and have a functional role in the pathobiology of uterine fibroids, then this information could be used to develop new strategies to reduce the risk of recurrence of uterine fibroids and benefit women in the future.

6. Importance of Knowledge to be Gained

Early detection and prevention of the development and/or recurrence is a major obstacle in the diagnosis and treatment of uterine fibroids. Hence, identifying and characterizing miRNAs that support the future development of diagnostic biomarkers and and/or therapeutic targets has the potential to greatly improve the medical management of uterine fibroids.

7. Inclusion of Women and Minorities

A portion of the research activity described in this grant will be focused in part on tissue cultures derived from surgical specimens (myometrium and fibroids) obtained from adult women undergoing surgery for symptomatic uterine fibroids under the auspices of our approved departmental . We have no control over what cases are scheduled or which cases yield sufficient tissue Nevertheless, the principal investigator appreciates the importance of increasing the accrual of underrepresented groups

The specific break down is provided in the Target enrollment report. Please note that the departmental tissue repository will bank tissue and biologicals from women of Caucasian, African American, Asian, Hispanic, and Native American descent. For the purposes of the proposed grant aims, we will utilize samples of women from all ethnicities. This has been reported as such in the target enrollment report.

8. Inclusion of Children

All clinical research activity described in this grant will be focused entirely on adult women. Since ovarian cancer does not typically occur in children, the study of children in such a setting is neither feasible nor appropriate and children will not be included in these studies.

Planned Enrollment Report

Study Title:

Domestic/Foreign: Domestic

Comments:

Racial Categories	Ethnic Categories				Total
	Not Hispanic or Latino		Hispanic or Latino		
	Female	Male	Female	Male	
American Indian/Alaska Native	5	0	0	0	**5**
Asian	0	0	0	0	**0**
Native Hawaiian or Other Pacific Islander	5	0	0	0	**5**
Black or African American	30	0	25	0	**55**
White	215	0	20	0	**235**
More than One Race	0	0	0	0	**0**
Total	**255**	**0**	**45**	**0**	**300**

Study 1 of 1

References

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