CANDIDATE’S BACKGROUND
I am a broadly trained pediatric gastroenterologist with clinical and research interests and expertise in eosinophilic esophagitis (EoE). I received a Bachelor of Science degree in chemical engineering with a bioengineering focus from Rice University. I completed my medical school education, pediatric residency, and gastroenterology fellowship at the University of Texas Southwestern (UTSW) Medical Center. I developed a passion for basic science research during my second year in fellowship. My research during fellowship has been recognized and funded by the American Gastroenterological Association (AGA) Fellowship to Faculty Award and the National Institutes of Health Child Health Research Career Development Award Program (institutional K12), and I have successfully published a first-authored manuscript entitled “Omeprazole blocks eotaxin-3 expression by oesophageal squamous cells from patients with eosinophilic oesophagitis and GORD” in Gut 2012. Since September 2011, I joined the faculty of UT Southwestern as an instructor with 80% protected research time that I intend to dedicate to this proposal.
CAREER GOALS AND OBJECTIVES
My long term career goal is to be an independent investigator and a leader in the field of EoE and tissue remodeling. I plan to develop a translational research program dedicated to basic and clinical studies that will impact the management of patients with EoE. Furthermore, I hope to train young physicians in the field of pediatric gastroenterology and encourage their participation in research.

My specific career goals and research objectives during the award period are the following:

- Through the specific aims proposed in this application, I will define the roles of periostin and Th2 cytokines in EoE fibrogenesis. I will also delineate the signaling mechanisms involved in periostin expression, and the importance of epithelial to mesenchymal signaling in fibrogenesis.
- With the experience and data achieved from this project, I will be in a unique position to advance the field of EoE. Future projects that should emerge from this research will include the use of in vivo models to demonstrate the benefits of targeting periostin-mediated fibrogenesis. In addition, the current project will provide the groundwork for future studies aimed at defining the importance of epithelial-mesenchymal cross-talk in the esophageal mucosa.
- I will expand my network of mentors and collaborators for the development of future projects, acquire further expertise in manuscript and grant writing, assemble the skills to establish a thriving laboratory, and enhance other aspects of academic career development.

I will pursue a rigorous training program to acquire the skills necessary to become a successful independent physician scientist through “hands-on” training with Dr. Rhonda Souza and Dr. Anil Rustgi, and by participating in formal intramural (UTSW Department of Clinical Sciences and Graduate School of Biomedical Sciences) and extramural course work.
CAREER DEVELOPMENT/TRAINING ACTIVITIES DURING AWARD PERIOD

The K08 career development award will provide the necessary funding and protected time to complete my research proposals and facilitate my career development into an independent investigator. The following distinguished group of accomplished researchers with an impressive track record in basic science and translational research has agreed to either mentor me or participate on my advisory committee during this career award:

1. **Rhonda Souza MD**: Dr. Souza, my co-mentor, is a Professor of Medicine at UT Southwestern Medical Center and the Dallas VA Medical Center. She is an international authority on molecular mechanisms underlying gastroesophageal reflux disease (GERD) and Barrett’s esophagus. Her laboratory consists of excellent scientists whose caliber reflects the breadth of Dr. Souza’s scientific intellect, and she has a strong track record of mentoring and training productive scientists. Dr. Souza has demonstrated her deep commitment to fostering my intellectual and scientific development, and she maintains a strong interest in mentoring my transition to independence. I have laboratory space adjacent to her laboratory and she has agreed to support expenses related to my research. Along with Dr. Stuart Spechler, we will continue to meet informally on a daily basis and formally on a weekly basis to discuss my research projects and career goals. I will also participate in our weekly laboratory meetings and journal club. With her expertise and guidance, I will advance my skills in formal presentations, grant writing, manuscript writing, research study design, and data analysis.

2. **Stuart Spechler MD**: Dr. Spechler, my co-mentor, is Professor of Medicine and the Berta M. and Cecil O. Patterson Chair in Gastroenterology at UT Southwestern Medical Center, and the Chief of Gastroenterology at the Dallas VA Medical Center. Drs. Spechler and Souza have been an extraordinary collaborative team for over 14 years. Dr. Spechler is an internationally-recognized expert on esophageal diseases including EoE, GERD, Barrett’s esophagus, and esophageal cancer. Along with Dr. Souza, we will continue to meet informally on a daily basis and formally on a weekly basis to discuss my research and career. In particular, Dr. Spechler will guide me in statistical analysis and in highlighting the clinical relevance of my studies.

3. **Glenn Furuta MD**: Dr. Furuta, a Professor of Pediatrics at the University of Colorado with widely-recognized expertise in eosinophilic gastrointestinal diseases, has functioned as my content mentor in the past and has agreed to be on my advisory committee. As an advisor, he will meet with me twice yearly to discuss my career development, and we will continue to hold our bi-monthly teleconferences regarding research progress and clinical relevance to pediatrics. In addition, we will meet face-to-face at the national conferences for AGA and North American Society for Pediatric Gastroenterology, Hepatology, and Nutrition.

4. **Frederick Grinnell PhD**: Dr. Grinnell is a Professor of Cell Biology as UT Southwestern Medical Center, and is best known for his work and contribution in tissue fibrosis, including the discovery of fibronectin. He will provide guidance in my study design involving fibroblast biology on an ongoing basis. As a member of my advisory committee, he will also meet with me formally twice a year to assess my career development and progress.

I will receive “hands-on” training in the following research techniques/protocols:

1. **Site-directed mutagenesis** to introduce single or double mutations into a promoter sequence under Dr. Souza's supervision.

2. **Chromatin immunoprecipitation assay** to assess relevant binding sites on a promoter sequence under Dr. Souza's supervision.

3. **Organotypic 3D culture protocol** to create novel, tissue-like constructs under Dr. Anil Rustgi’s supervision. Dr. Rustgi, Professor of Medicine at the University of Pennsylvania School of Medicine, has developed a successful protocol for 3D esophageal cultures, and he has agreed to provide me training on this technique at his institution (see letter of collaboration). I will evaluate the role of epithelial to mesenchymal signaling in fibrogenesis using these constructs.

4. **Immunohistochemical staining** of human histology specimens to evaluate relevant markers of fibrosis under Dr. Souza’s supervision.

I will be participating in the following formal course work and didactics:

1. **Fibrosis: Translation of Basic Research to Human Disease and Novel Therapeutic** – Five-day workshop offered by Keystone Symposia every 2-3 years to provide an integrated perspective of basic disease mechanisms and clinical development of anti-fibrotic drugs.
2. **Ethics in Clinical Science (Course # DCS 5105)** – A formal course organized by the UTSW Department of Clinical Sciences (description under Training in Responsible Conduct of Research).

3. **Responsible Conduct of Research (Course # DCS 5107)** – A formal course organized by the UTSW Department of Clinical Sciences (description under Training in Responsible Conduct of Research).

4. **Grant writing and Funding Strategies (Course # DCS 5106)** – A formal course organized by the UTSW Department of Clinical Sciences. This course will review the different types of federal grant mechanisms as well as grants or contracts from research foundations, advocacy organizations and industry. How to write a persuasive, well-reasoned application will be the main focus of the course including the budget, resources and environment, preliminary data, and the research plan.

5. **Conceptual Biostatistics for the Clinical Investigator (Course # DCS 5309)** – A formal course organized by the Division of Biostatistics, UTSW Department of Clinical Sciences. This course offers a conceptual approach to statistical analysis of biomedical data. It provides a review of fundamental statistical principles focusing on explanation of the appropriate scientific interpretation of statistical tests rather than the mathematical calculation of the tests themselves.

6. **Pathology of Mouse Models for Human Disease** – An annual workshop offered by Jackson Laboratory that provides a week of intensive training sessions in basic mouse genetics, concepts of mouse model generation, approaches to working up mutant mice, and models of various disease areas.

7. **University Lecture Series** - UTSW Graduate School of Biomedical Sciences organizes a weekly series of lectures by nationally and internationally acclaimed researchers from diverse areas of expertise.

8. **Pediatric Faculty Research Conference** – The Pediatric Department organizes weekly formal research presentations by UT Southwestern faculty. I will also have the opportunity to present my research in this forum annually, and to receive feedback from pediatric faculty, including, but not limited to, the Chairman and Vice Chairman of Pediatrics.

9. **Pediatric GI Core Curriculum** - The Division of Pediatric Gastroenterology organizes these weekly lectures and journal club sessions, which will reinforce my clinical skills and further expand my understanding of GI pathophysiology.

10. **Excellence in Immunology** – The UTSW Graduate School of Biomedical Sciences Department of Immunology organizes this weekly series of lectures by experts from national leading research institutions on the latest immunology research.

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<th>Yr</th>
<th>Activity Timeline</th>
<th>%Effort</th>
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| 1  | Research: Delineating mechanism of periostin expression in fibroblast and epithelial cells.  
Learning Objectives: Site-directed mutagenesis, ChIP Assay, Fibrosis (Keystone), Ethics in Clinical Science (DCS 5105).  
Clinical: 6-8 weeks inpatient wards or consultation service. | 80 5 15 |
| 2  | Research: Investigating pro-fibrotic effects of IL-13, IL-4, and periostin in fibroblasts. Prepare manuscript #1.  
Learning Objectives: Organotypic 3D culture protocol, Immunohistochemical staining, Responsible Conduct of Research (DCS 5107).  
Clinical: 6-8 weeks inpatient wards or consultation service | 80 5 15 |
| 3  | Research: Constructing 3D cultures and performing immunohistochemical staining on human histology specimens. Prepare manuscript #2 and review paper on EoE.  
Learning Objectives: Grant Writing/Funding Strategies (DCS 5106), Conceptual Biostatistics (DCS 5309)  
Clinical: 6-8 weeks inpatient wards or consultation service | 80 5 15 |
| 4  | Research: Preparing manuscript #3 and generating preliminary data for R01 grant  
Learning Objectives: Pathology of Mouse Models for Human Disease (Jackson Laboratory)  
Clinical: 6-8 weeks inpatient wards or consultation service | 80 5 15 |
Dear Review Committee Members,

We wish to express our great enthusiasm for serving as the primary mentors for Dr. Edaire Cheng who is submitting a proposal entitled "The Role of Periostin and Th2 Cytokines in Eosinophilic Esophagitis Fibrogenesis" for a Mentored Clinical Scientist Research Career Development Award. Edaire is a pediatric gastroenterologist who has been pursuing her research interest in eosinophilic esophagitis (EoE) under our guidance. At the Dallas VA Medical Center and UT Southwestern Medical Center (UTSW), we lead a robust, multi-disciplinary, translational esophageal research program that utilizes cell culture models, animal models, and patient studies to investigate diseases of the esophagus. Edaire has become an integral member of our research group, and we are delighted with her progress. She has quickly mastered laboratory techniques, and she has demonstrated an outstanding work ethic and keen intellect.

During her T32 and fellow-to-faculty awards, Edaire focused on epithelial cell biology. She successfully established novel, telomerase-immortalized esophageal squamous cell lines from patients with EoE. Her studies using these cell lines, recently published in *Gut*, have shown that proton pump inhibitors (PPIs) block Th2 cytokine-induced expression of esophageal eotaxin-3, a potent eosinophil chemoattractant. Prior to this seminal publication, it was assumed that inhibition of gastric acid secretion was the only important effect of PPIs and, therefore, only an acid-peptic disorder like gastroesophageal reflux disease (GERD) could respond to PPIs. Edaire’s work has challenged this assumption, and has provided a plausible molecular mechanism whereby PPIs might have beneficial effects in EoE, even in the absence of GERD.

During the tenure of this award, Edaire’s career development plan will be extended to the area of stromal/mesenchymal biology in order to define the mechanisms underlying the esophageal fibrosis that is the major cause of morbidity in EoE. Edaire already has written a review article on esophageal fibrogenesis in EoE that has been accepted for publication in the *American Journal of Physiology*. She has met with Dr. Fredrick Grinnell, an expert in fibrogenesis at UTSW who has agreed to be on her mentorship committee and to provide advice and support for her studies. To study how interactions between epithelial and stromal compartments contribute to fibrogenesis, Edaire will train in the laboratory of Dr. Anil Rustgi, Director of the Center for Molecular Studies in Digestive and Liver Diseases at the University of Pennsylvania, on the construction of 3D organotypic esophageal constructs that contain both epithelial and stromal cells. Edaire also will continue her bi-monthly teleconferences with Dr. Glenn Furuta, Professor of Pediatrics at the University of Colorado and leader of The International Gastrointestinal Eosinophil Researchers (TIGERS), to discuss the clinical relevance and implications of her studies.

As Edaire’s primary mentors, we will continue to meet with her informally on a daily basis, and formally on a weekly basis to discuss her progress. We will provide her training in presentation methods, manuscript writing, study design, data analysis, statistical techniques, grant writing, and the responsible conduct of research. We also will help Edaire to develop animal models for exploring the roles of specific molecular pathways in fibrogenesis, as outlined in the “Future Directions” section of her proposal. Edaire will attend and present at our weekly laboratory meetings at the Dallas VA. In addition, she will participate in journal clubs and research conferences at UTSW and Children's Medical Center, and she will present her research data at the weekly Pediatric Faculty Research Conference. These presentations will provide organized fora for Edaire to receive constructive criticism and advice from her peers in the Department of Pediatrics. She will also attend lectures at UTSW given by visiting faculty on topics relevant to her research (University Lecture Series and Excellence in Immunology), and she will attend the annual meetings of the American Gastroenterological Association and the North American Society for Pediatric Gastroenterology, Hepatology, and Nutrition. Edaire will have formal courses in grant writing and funding, biostatistics, and bioethics, which are available at UTSW through the Graduate School of Biomedical

UT Southwestern Medical School 5323 Harry Hines Blvd. / Room K5.140C / Dallas, Texas 75390-9151 / (214) 645-6344 / Fax (214) 645-6294
marlyn.mayo@utsouthwestern.edu
Sciences and the Integrative Biology Graduate Program. She will also take extramural courses in Fibrosis (Keystone Symposia) and Mouse Models (Jackson Laboratory). Our expectation is that Edaire will have 2-3 original research articles, 1-2 review papers, and a number of co-author publications by the completion of her career development award. We are working closely with Drs. Andrew Feranchak and Julio Perez-Fontan in the Division of Pediatric Gastroenterology and Department of Pediatrics to transition Edaire from her laboratory at the Dallas VA to her own translational research program based at Children’s Medical Center. We anticipate beginning the transition in the 3rd year of her 4-year award, with the understanding that we will be continually available for guidance and technical support. Edaire’s project is derived entirely from her clinical interest in the mechanisms of fibrosis in EoE, and she will take the entire project with her as the foundation for her independent-investigator career. As has been the case for her T32, fellowship-to-faculty, and K12 awards, Edaire’s salary and protected time will come from the Department of Pediatrics and Division of Pediatric Gastroenterology. We will provide the remaining support for the successful conduct of the proposed experiments in the form of guidance, training, technical and financial help as needed.

Dr. Spechler is the Chief of Gastroenterology at the Dallas VA, Professor of Medicine and holder of the Berta M. And Cecil O. Patterson Chair in Gastroenterology at UTSW. He will provide guidance in the clinically relevant components of study design and in the statistical analyses of data. Dr. Souza is Professor of Medicine, a member of the Simmons Cancer Center, and a faculty member in the Integrative Biology Graduate Program at UTSW. She also has served on the VA Merit Review Subcommittee in Oncology and on the Clinical, Integrative, and Molecular Biology (CIMG) study section for NIH. We have successfully mentored many GI fellows, postdocs, and junior faculty in the past, and we have received additional protected time specifically to mentor Edaire. The following table lists our current mentees in addition to Edaire:

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<th>Name</th>
<th>Dates of Training</th>
<th>Type of Mentoring</th>
<th>Current Position</th>
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<tbody>
<tr>
<td>Linda A. Feagins, MD</td>
<td>7/03 - present</td>
<td>T32 Fellow; ACG Research Award; Faculty Research</td>
<td>Assistant Professor of Medicine, Dept of GI, DVAMC and UTSW</td>
</tr>
<tr>
<td>Xiaofang Huo, Ph.D.</td>
<td>12/07 - present</td>
<td>Postdoctoral Research</td>
<td>Postdoctoral Student</td>
</tr>
<tr>
<td>David Wang, M.D., Ph. D.</td>
<td>9/09 - present</td>
<td>VA CDA</td>
<td>Assistant Professor of Medicine, Dept of Hematology/ Oncology, DVAMC and UTSW</td>
</tr>
<tr>
<td>Thai Pham, M.D.</td>
<td>9/10 - present</td>
<td>Faculty Research</td>
<td>Assistant Professor, Dept of Surgery, DVAMC and UTSW</td>
</tr>
<tr>
<td>Arianne Theiss, Ph.D.</td>
<td>8/10 - present</td>
<td>Faculty Research/K08</td>
<td>Associate Investigator, Baylor Research Institute</td>
</tr>
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We believe that our mentorship and multi-disciplinary translational esophageal research program will provide Edaire with continued growth in designing research questions and approaches that are truly “translational” in nature. Edaire has 80% protected time for her research and career development activities, and her highly supportive Department Chair and Division Chief have kept her clinical and administrative assignments at a minimum. Together with Edaire, we have designed a four-year career development plan that will result in her becoming an independent, physician-scientist investigator. Her environment will be enriched through our weekly one-on-one meetings, weekly lab meeting/journal clubs, and interactions with other members of our esophageal research group. We are excited and delighted to serve as mentors for such a promising young researcher as Edaire, and we are totally committed to fostering her research career.

Sincerely,

Rhonda F. Souza, M.D.
Professor of Medicine
UT Southwestern Medical Center

Stuart Jon Spechler, M.D.
Chief, Division of Gastroenterology, Dallas VA Medical Center
Professor of Medicine, UT Southwestern Medical Center
September 1, 2012

Center for Scientific Review
National Institute of Health
6701 Rockledge Drive, MSC 7710
Bethesda, MD 20817

Re: [Name], MD, NIH K08 Grant application

Dear Review Committee Members:

I strongly support [Name]'s application for the NIH Mentored Career Development Award entitled “The Role of Periostin and Th2 Cytokines in Eosinophilic Esophagitis Fibrogenesis.” I look forward to serving on her advisory committee providing guidance with her research project and career development.

[Name] became interested in eosinophilic esophagitis during her pediatric gastroenterology fellowship at UT Southwestern Medical Center. She joined Dr. Rhonda Souza’s laboratory and has worked primarily on Th2 cytokine-induced eotaxin-3 expression in esophageal epithelial cell lines established from patients with EoE and GERD. Her work led to a first-authored publication in Gut entitled “Omeprazole blocks eotaxin-3 expression by oesophageal squamous cells from patients with eosinophilic oesophagitis and GORD.” In the future, [Name] plans to study the roles of Th2 cytokines and periostin in fibrogenesis using newly established esophageal fibroblast cell lines. She will characterize the pro-fibrotic effects of IL-13, IL-4, and periostin on esophageal fibroblasts by evaluating myofibroblast activation, extracellular matrix protein production, and fibroblast collagen-matrix contraction. [Name] has chosen an excellent pair of mentors to guide this work, Dr. Rhonda Souza and Dr. Stuart Spechler. Their combined expertise in esophageal diseases makes them an ideal mentoring team for [Name] development as a physician scientist in the field of gastroenterology.

I am a professor in the Department of Cell Biology at the University of Texas Southwestern Medical Center. My research focuses on understanding features of tissue fibrosis and motile and mechanical interactions between fibroblasts and collagen matrices. Thus, I feel particularly well suited to provide intellectual and technical support to [Name] in her investigations involving fibroblast biology. Besides being available to her in an ongoing basis to answer questions, and I will meet formally twice a year to evaluate her career development and research progress. I understand the types of scientific and career development required to succeed as a young investigator today, and I look forward to sharing these insights with [Name] over the next 5 years.

In summary, I strongly support [Name]'s application and recommend her without any reservation for the NIH Mentored Career Development Award. I believe she has exceptional potential to become a successful physician scientist.

Sincerely,

Frederick Grinnell
Re:  MD, NIH K08 Grant application

Dear Review Committee,

I am delighted to write this letter of support for Dr. application for the NIH Mentored Career Development Award. Briefly, I have known since she was a second-year pediatric gastroenterology fellow where she first expressed interest in eosinophilic esophagitis (EoE) and have always been impressed by her enthusiasm and engagement with this disease especially as it relates to esophageal remodeling. Let me begin by saying that I have had extensive experiences with post graduate training in my present role as the Chair of the Scientific Oversight Committee for the Pediatric Gastroenterology Fellowship Program at University of Colorado Denver School of Medicine, (2 fellows per year, 3 year fellowship-6 fellows over the 3 years) and previous roles as Associate Training Program Director for the Pediatric Gastroenterology Fellowship Program (5 fellows per year, 3 year fellowship-45 trainees over a decade) and Chief Resident at Baylor College of Medicine (136 residents). I can state without reservation that falls in the top 5% of this group; she is one of the few who has accrued the necessary training and skills and who possesses the inherent personality to “make it” as an independently funded investigator. As a physician scientist, she will bring not only novel ideas to this field but also translate these into practical solutions for patients. In this regard, she has thoughtfully aligned herself with successful funded investigators who have shaped her into a vibrant talent focusing on an area in desperate need of new research.

For the past 3 years, has worked primarily on Th2 cytokine-induced eotaxin-3 expression in esophageal epithelial cell lines established from patients with EoE and GERD. In this project, I served as a content mentor. During this time, Drs. Souza, Spechler and I have had bi-monthly teleconferences to discuss both clinical and research issues related to EoE and to review her progress. Her hard work culminated into a first-authored publication in the leading journal “Gut” (impact factor 10.11) entitled “Omeprazole blocks eotaxin-3 expression by oesophageal squamous cells from patients with eosinophilic oesophagitis and GORD”. In her current proposal, will expand her work by studying the role of Th2 cytokines and periostin in fibrogenesis using esophageal fibroblast cell lines that she recently developed. In my opinion, has devised a challenging but important research project which will contribute significantly to the field of EoE by determining an underlying mechanism defining subepithelial fibrosis; this type of project has great potential to identify novel therapeutic targets and to provide her with preliminary data necessary for her R01 grant application. Her proposed studies are novel, well planned, and completely feasible in her environment and in the time frame of a K award. During our conversations, it has been clear that she is committed to this proposal and possesses the intellectual curiosity to bring her to the next level of independence. As her advisor, I will meet her formally to discuss her progress twice yearly, in addition, we will continue to have bi-monthly teleconferences and continue to meet face to face at national conferences such as Digestive Disease Week and North American Society for Pediatric Gastroenterology, Hepatology, and Nutrition Annual Meeting.

In summary, is an extremely talented researcher with the full potential to be a productive, independent investigator in gastroenterology in a burgeoning field. I support this outstanding candidate and will give her all the support and guidance that I possibly can to help her succeed as a physician scientist.

Sincerely,
September 10, 2012

[Redacted]

UT Southwestern Medical Center
Department of Pediatrics
5323 Harry Hines Boulevard
Dallas TX 75390

Dear [Redacted],

It is a pleasure for me to collaborate with you on your project investigating the role of periostin and Th2 cytokines in EoE fibrogenesis.

As you are aware, I am a board certified pathologist with recognized expertise in gastrointestinal pathology. I am also associated with Miraca Life Sciences which receives endoscopic biopsies from over 1,500 gastroenterologists in 40 states.

In the past, I have successfully collaborated with your mentors on numerous projects. Most recently, I performed and interpreted immunohistochemical staining on rodent esophageal tissue for them that was published in *Gastroenterology*.

As part of our collaboration, I am happy to examine histology specimens from human tissues. I would be happy to provide you with any technical support that you may need.

Best wishes,

Robert M. Genta, M.D.
Chief of Academic Affairs, Miraca Life Sciences
Clinical Professor of Pathology and Medicine (Gastroenterology)
University of Texas Southwestern Medical Center at Dallas

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Tel: +1-214-596-7440
Fax: +1-214-596-2274
robert.genta@utsouthwestern.edu
September 1, 2012

[Instructor's Name], MD
Instructor
University of Texas Southwestern Medical Center
Department of Pediatrics
Division of Gastroenterology, Hepatology & Nutrition
5323 Harry Hines Boulevard
Dallas, TX 75390

Dear [Instructor's Name],

As Division Chief of Gastroenterology and Director of the Center for Molecular Studies in Digestive and Liver Diseases at the University of Pennsylvania, I would like to emphasize our future support of your K08 proposal entitled "The Role of Periostin and Th2 Cytokines in Eosinophilic Esophagitis Fibrogenesis". The proposed use of organotypic three-dimensional (3D) culture model will be a tool for you to determine the role of Th2 cytokine-mediated epithelial-mesenchymal signaling in fibrogenesis.

As you know, we have developed organotypic 3D culture, a form of human tissue engineering to reconstitute squamous epithelial in vitro, and have extensively used this model to investigate the molecular mechanisms of esophageal epithelial biology and tumor biology. Our protocol has been published in Nature Protocol, and we have successfully published data using this method in several high impact journals. Since you have established telomerase-immortalized esophageal squamous epithelial cell lines and esophageal fibroblasts lines, the organotypic 3D culture protocol will allow you to create a stratified epithelium with the epithelial cells overlying a mesenchymal-like layer comprised of the esophageal fibroblasts. Therefore, you can explore potential epithelial-mesenchymal signaling that may be involved in fibrogenesis.

We will be happy to provide you hands-on-training for the organotypic 3D culture at our institution and any additional technical support, ensuring your success to your proposed work. Best wishes and we look forward to collaborating with you in the near future.

Sincerely yours,

[Anil K. Rustgi, M.D.]

Anil K. Rustgi, M.D.

AKR:mkf
INSTITUTIONAL ENVIRONMENT

**University of Texas Southwestern Medical Center:** The University of Texas Southwestern (UTSW) Medical Center is a world renowned academic center. The mission of UTSW is to perform high-impact and internationally recognized research, to educate the next generation of leaders in patient care, biomedical science and disease prevention, and to deliver patient care that brings UTSW’s scientific advances to the bedside. UTSW offers three degree-granting schools including the UT Southwestern Medical School and the UT Southwestern Graduate School of Biomedical Sciences. UTSW has a track-record of fostering basic and clinical research emphasized by over 3,500 funded research projects and over $400 million annual extramural funding. The excellence of the institution is reflected by the high caliber faculty including 5 Nobel Prize laureates, 19 members of the National Academy of Sciences, and 19 members of the Institute of Medicine.

**Dr. Rhonda Souza,** a Professor of Medicine at UTSW and the Dallas VA Medical Center, is an international authority on molecular mechanisms underlying gastroesophageal reflux disease (GERD) and Barrett’s esophagus. **Dr. Stuart Spechler,** a Professor of Medicine and the Berta M. and Cecil O. Patterson Chair in Gastroenterology at UTSW and the Chief of Gastroenterology at the Dallas VA Medical Center, is an internationally-recognized expert on esophageal diseases including EoE, GERD, Barrett’s esophagus, and esophageal cancer. Both Drs. Souza and Spechler will be available to me on a daily basis to discuss my progress and research design. **Dr. Federick Grinnell** is a Professor of Cell Biology as UTSW, and is best known for his work and contribution in tissue fibrosis, including the discovery of fibronectin. Not only will Dr. Grinnell be a key member of my advisory committee, he has offered to provide any intellectual and technical support regarding fibroblast biology from his laboratory.

The **UT Southwestern Clinical and Translational Alliance for Research (UT-STAR)** is part of the national consortium of medical research institutions that share a common vision to improve human health by transforming the research and training environment to enhance the efficiency and quality of clinical and translational research. Its goals are to accelerate the translation of laboratory discoveries into treatments for patients, to engage communities in clinical research efforts, and to train a new generation of clinical and translational researchers. In conjunction with the Department of Clinical Sciences and Graduate School of Biomedical Sciences, UT-STAR offers courses in grant writing, biostatistics, and ethics, which I plan to attend during the award period. Furthermore, the Graduate School of Biomedical Sciences organizes the **University Lecture Series** which are weekly series of lectures by nationally and internationally acclaimed researchers from diverse areas of expertise enhancing the intellectual climate of the medical center and giving me the opportunity to learn about cutting-edge research from around the world. The Department of Immunology offers **Excellence in Immunology Lecture Series** where nationally recognized experts present the latest immunology research.

Although the studies for this proposal will be performed at the Dallas VA Medical Center, there is a wealth of resources at UTSW such as the **core facilities** including the Transgenic Mouse Core, DNA Microarray Core, and Protein Chemistry Technology Core that may be of utility for future aspects of my project.

**Dallas VA Medical Center:** The research facility at the Dallas VA Medical Center has all the **resources and equipment** (as outlined in **Facilities & Other Resources** and **Equipment**) readily available for me to execute the studies in my proposal. Furthermore, the overall scientific environment at Dallas VA Medical Center is an excellent one. The small number of investigators encourages multi-investigator collaborations and frequent interactions/discussions. Drs. Souza and Spechler and I, along with the other investigators, meet on a weekly basis for **journal club** and to discuss preliminary data which stimulates an intellectual dialogue. This nicely complements the large university environment offered by UT Southwestern.

**Children’s Medical Center:** The Department of Pediatrics organizes weekly **Pediatric Faculty Research Conferences** in which I will have the opportunity to present my research in this forum along with other pediatric investigators, and to receive constructive feedback from the department including the Chairman and Vice Chairman of Pediatrics. I will also attend **Pediatric GI Division Core Curriculum**, which is weekly presentations, including journal club, and will maintain my clinical acumen and expand my understanding of GI pathophysiology. In addition, the **Department of Pediatric Research Office and Grants** will provide me for any administrative support and oversight on conducting my research.
September 24, 2012

Center for Scientific Review
National Institutes of Health
6701 Rockledge Drive
Bethesda, MD 20817

Re: [Name], MD - NIH K08 PA-11-193 Application

Dear Members of the Study Section:

I am writing on behalf of the University of Texas Southwestern Medical Center as Dallas to confirm the institutional commitment of [Name] faculty appointment and to fully support her application of a K08 mentored career development award. It is clear that [Name] has performed superbly during her pediatric gastroenterology fellowship and as a scholar in the Child Health Research Career Development Award program. She has already generated a substantial body of novel scientific data. Furthermore, she has formulated a sound innovative research plan centered on understanding the biological underpinnings of eosinophilic esophagitis. She possesses the crucial tools of a solid commitment to basic and translational research, high-quality original ideas, a strong work ethic, and the intellectual curiosity to become a top-notch scientist.

Our commitment: [Name] is currently an Instructor on the Clinical Scholars track in the Department of Pediatrics. Her appointment and salary support are not contingent upon the receipt of the K08 award. Her time will be 80% protected for her research activities. Her Division Director Dr. Drew Feranchak and I are personally committed to ensure that she has every opportunity to develop as an independent investigator, and we plan to provide her with all the resources needed at every stage of what I am sure will be an extremely prolific career. She will have limited commitments to Departmental functions and clinical responsibilities. UT Southwestern will provide her with all the administrative and financial support required for this proposal. In support of her research on eosinophilic esophagitis, [Name] has chosen Drs. Rhonda Souza and Stuart Spechler to be her mentors. These senior faculty members of the Department of Medicine are internationally renowned experts on esophageal diseases and have an established record in training junior faculty members. [Name] has been provided laboratory space adjacent to Dr. Souza's laboratory in the Dallas VA Medical Center to facilitate daily interactions with Drs. Souza and Spechler.

The institutional environment: UT Southwestern is a leading research institution with over $400 million per year in extramural research support, 5 Nobel Laureates, 19 faculty in the National Academy of Sciences, and 19 members of the Institute of Medicine. There is a strong campus-wide commitment to the support of talented and promising scientists and there are numerous mechanisms on campus designed to assist young faculty to foster their research careers. There is easy access to core laboratories of the Department of Pediatrics and the Medical Center to provide education and assist with complex projects. There is also a strong collegial relationship between the clinical and basic science departments, which has been a critical factor in the success of many young investigators like [Name].

Overall, I believe that [Name] has been provided with a uniquely supportive environment here at UT Southwestern in which to begin her career. The Department of Pediatrics offers its most enthusiastic support of [Name]'s application for the K08 mentored career development award. We are firmly committed to [Name]'s development as an independent physician-scientist and to her long-term career success. Receipt of this award would be a fundamental step towards this success.

Sincerely,

Julio Pérez Fontán, M.D.
Interim Chairman
SPECIFIC AIMS

Eosinophilic esophagitis (EoE) is a recently recognized, immune-mediated disease characterized clinically by symptoms of esophageal dysfunction and histologically by eosinophil-predominant inflammation. The chronic esophageal eosinophilia of EoE is associated with tissue remodeling characterized by prominent fibrosis in the lamina propria, a pattern that is seldom, if ever, observed in fibrotic esophageal disorders other than EoE. This unique remodeling causes the esophageal rings and strictures that frequently complicate EoE, and underlies the mucosal fragility that predisposes to painful mucosal tears in the EoE esophagus. The pathogenesis of fibrosis in EoE is not understood, and presently no treatment has been shown to prevent tissue remodeling in this disorder. Understanding the mechanisms underlying remodeling will identify therapeutic targets to prevent EoE complications.

While emerging studies suggest that secretory products of eosinophils and mast cells, as well as cytokines produced by epithelial and mesenchymal cells in the esophagus contribute to the remodeling process, the complex interactions among these cell types and the mechanisms by which molecular signals drive EoE fibrosis are unknown. Studies from our laboratory, as well as others, have demonstrated a prominent role for Th2 cytokines, including IL-13 and IL-4, in EoE. In our preliminary experiments using novel, non-neoplastic esophageal fibroblast cell lines that we developed, we have found that IL-13 and IL-4 induce a number of fibrogenic genes including the periostin gene that encodes an extracellular matrix (ECM) protein. Interestingly, we discovered that the Th2 cytokines are potent inducers of periostin in our esophageal squamous epithelial cell lines. Periostin, which is highly upregulated in the EoE esophagus, is known to contribute to fibrosis in a number of extra-esophageal organs by forming crosslinks with other ECM proteins and by interacting with integrins in cell membranes to initiate or intensify other fibrogenic effects. Currently, the impact of periostin in esophageal fibrosis has not been adequately studied. Furthermore, the transcriptional regulation of periostin is unclear, and the precise mechanisms underlying how IL-13, IL-4, and periostin promote fibrosis in EoE are not defined.

We hypothesize that, in the esophagus of EoE patients, IL-13 and/or IL-4 drive fibrogenesis by increasing esophageal mucosal expression of periostin. To test this hypothesis, we will use our novel, telomerase-immortalized esophageal fibroblast and esophageal squamous epithelial cell lines, as these cell types represent essential components of the esophageal mucosa. We will also use these cell lines to create organotypic 3D cell cultures to study signaling between the two mucosal compartments (epithelium and mesenchyme) in a system uncontaminated by inflammatory cells. Based on our preliminary data, we propose three specific aims:

**Aim 1. To delineate the mechanism whereby IL-13 and IL-4 regulate periostin expression by esophageal fibroblasts and squamous epithelial cells.**

a. We will establish that IL-13/IL-4 signaling and STAT3 machinery are functional in our fibroblast and epithelial cell lines.
b. We will explore IL-13 and IL-4 effects on transcriptional activation and post-transcriptional regulation of periostin.
c. We will target the Jak-STAT3 pathway for intervention using Jak inhibitors.

**Aim 2. To characterize the “direct” and “indirect” pro-fibrotic effects of IL-13, IL-4, and periostin on esophageal fibroblasts.**

a. We will determine the direct pro-fibrotic effects of IL-13 and IL-4 on esophageal fibroblasts by measuring myofibroblast transdifferentiation, ECM protein production, and fibroblast contraction.
b. We will determine the direct IL-13/IL-4-induced, periostin-dependent, pro-fibrotic effects on esophageal fibroblasts.
c. We will determine the indirect pro-fibrotic effects of IL-13 and IL-4 by treating esophageal fibroblasts with conditioned media from IL-13- and IL-4-exposed esophageal squamous cells.

**Aim 3. To confirm that IL-13 and/or IL-4 can cause STAT3-mediated periostin expression and fibrotic effects in organotypic models, and to correlate STAT3 pathway activation, periostin expression, and fibrosis in esophageal biopsy specimens from EoE patients.**

a. We will perform functional evaluations of IL-13/IL-4 signaling and periostin expression between the mucosal compartments (i.e. epithelial and mesenchyme), and assess effects on sub-epithelial fibrosis in organotypic 3D cell cultures.
b. We will correlate STAT3 and periostin expression with markers of fibrosis in patient specimens. Overall, our goal is to understand the pathophysiological roles of IL-13, IL-4, periostin, epithelial cells, and fibroblasts in the subepithelial fibrosis that complicates EoE, and to identify potential molecular targets to prevent tissue remodeling in EoE.
**RESEARCH STRATEGY**

**Significance**

Eosinophilic esophagitis (EoE) recently has been defined as a chronic, immune/antigen-mediated disease characterized clinically by symptoms related to esophageal dysfunction and histologically by eosinophil-predominant inflammation.\(^1\) EoE, which has increased in incidence dramatically over the past decade, affects both children and adults, and can severely impair their quality of life. In EoE, food and aeroallergens activate a Th2 immune response, resulting in the production of Th2 cytokines such as interleukin (IL)-13 and IL-4.\(^2\)\(^3\) These cytokines stimulate the esophagus to express eotaxin-3, a potent eosinophil chemoattractant thought to play a key role in drawing eosinophils to the esophagus in EoE.\(^4\)\(^5\) Eosophageal epithelial injury results from the release of toxic eosinophil degranulation products. Prolonged eosinophil-predominant inflammation also can result in tissue remodeling characterized by subepithelial fibrosis, hyperplasia of the epithelium, angiogenesis, and hypertrophy of esophageal smooth muscle.\(^6\)\(^7\)\(^8\)\(^9\)\(^10\)\(^11\)\(^12\) The pattern of dense, subepithelial fibrosis in the lamina propria (Figure 1) appears to be unique to EoE. This pattern is found rarely, if ever, in other fibrotic esophageal disorders such as peptic and radiation-induced esophageal strictures, in which fibrosis typically involves the submucosa predominantly. Remodeling in EoE can cause serious complications including esophageal ring and stricture formation, which are responsible for the dysphagia and episodes of food impaction typical of EoE.\(^16\)\(^17\)\(^18\) Tissue remodeling also can result in esophageal dysmotility, which might contribute to the dysphagia and chest pain of EoE, and in fragility of the esophageal mucosa, which predisposes to painful mucosal tears that can occur spontaneously or with instrumentation of the EoE esophagus.

The study of tissue remodeling in EoE is especially challenging because the process largely involves the deep layers of the esophageal mucosa, as well as the submucosa and muscularis propria, and techniques for sampling and imaging these layers with microscopic precision are limited. Thus, the pathogenesis of tissue remodeling in EoE is not completely understood, but studies suggest that secretory products of eosinophils and mast cells, as well as cytokines produced by inflammatory cells, epithelial cells and stromal cells in the esophagus all contribute to the process. The goals of treatment are the relief of symptoms and the prevention of EoE complications. A number of studies have established the efficacy of various treatments in relieving EoE symptoms and decreasing esophageal eosinophilia, but no study has shown that any treatment can prevent the tissue remodeling that causes EoE complications.\(^19\)\(^20\)\(^21\)\(^22\) A better understanding of the mechanisms underlying tissue remodeling might identify novel therapeutic targets that could be used to prevent the serious consequences of EoE.

1. **Role of Fibroblasts in Fibrogenesis**

Fibrogenesis is part of the normal repair process triggered by epithelial injury. Injured epithelial cells and infiltrating immune cells release mediators that can initiate and regulate fibrogenesis. Pro-fibrotic cytokines like IL-13 and pro-fibrotic growth factors such as TGFβ can activate quiescent fibroblasts to transdifferentiate into myofibroblasts. These myofibroblasts play key roles in the synthesis, deposition, organization, and degradation of extracellular matrix (ECM) proteins including collagen, fibronectin, tenascin-C, and periostin. Myofibroblasts also express large amounts of α smooth muscle actin (αSMA) and, by virtue of their contractile phenotype, myofibroblasts can cause wound contraction. Although limited fibrogenesis is a normal response to tissue injury, severe injury or chronic inflammation can cause the deposition of large quantities of ECM protein and the excess matrix contraction that characterize fibrosis. Extensive subepithelial fibrosis often is observed in the esophagus of adults and children with EoE.\(^8\)\(^12\)\(^14\)\(^15\)\(^29\)

1. **Th2 cytokines (IL-13 and IL-4) have pro-fibrotic effects**

IL-4 and IL-13, Th2 cytokines that are over-produced in allergic disorders, have direct pro-fibrotic and remodeling effects in a number of diseases including asthma, atopic dermatitis, schistosomiasis, and chronic colitis.\(^30\)\(^31\)\(^32\)\(^33\) IL-4 and IL-13 can induce the expression of activated fibroblast markers [αSMA, fibronectin, connective tissue growth factor (CTGF)] and regulate the expression of matrix proteins [collagen, matrix metalloproteinases (MMP), periostin].\(^38\)\(^39\)\(^40\)\(^41\) In addition to these pro-fibrotic effects, IL-13 and IL-4 also can induce fibroblasts to produce pro-inflammatory substances, including the eosinophil chemoattractant eotaxin.\(^42\)\(^43\)\(^44\)\(^45\)
IL-13 and IL-4 share many of the same biological effects, probably because both cytokines bind the Type II IL-4 receptor, which is comprised of IL-4Rα and IL-13Rα1 subunits (see Figure 4 below). Binding of the Type II IL-4 receptor by either ligand activates the Signal Transducer and Activator of Transcription (STAT)6 signaling pathway, which mediates the transcriptional regulation of IL-13 and IL-4 target genes. Both IL-13 and IL-4 also can activate the STAT3 and Insulin Receptor Substrate (IRS) signaling cascades, and only IL-4 can bind the Type I IL-4 receptor through its gamma chain (γC) subunit. IL-13 has a complex relationship with TGFβ. There appear to be both TGFβ-dependent and TGFβ-independent pathways by which IL-13 exerts its pro-fibrotic effects. IL-13 can induce the production of latent TGFβ (i.e. TGFβ bound to latency-associated protein) in macrophages, and IL-13 also can upregulate MMP- and plasmin protease-mediated activation of latent TGFβ, thereby enabling TGFβ to exert its pro-fibrotic effects.

In EoE, IL-13 appears to induce esophageal remodeling through effects that are independent of eosinophils. One animal model of EoE involves rtTA-CC10-IL-13 mice that over express IL-13 in the lung and esophagus when induced with doxycycline. This IL-13 overexpression causes esophageal remodeling with fibrosis and stricture formation, even in rtTA-CC10-IL-13 mice that are deficient in eosinophils. Thus, these findings challenge the notion that therapies directed only at eosinophils will be sufficient to prevent fibrosis and remodeling in EoE.

2. Periostin is highly expressed in EoE

The ECM protein periostin, a 90 kDa disulfide-linked protein, has the ability to crosslink with other matrix proteins including tenascin-C, fibronectin, and collagen. Periostin also can bind integrins in the cell membrane, an interaction that has the potential to initiate a variety of biological effects including cell proliferation, migration, and differentiation. Binding of periostin to the integrins αvβ3, αvβ5, and αvβ6 has been shown to trigger crosstalk with epidermal growth factor receptor (EGFR), which initiates the Akt/protein kinase B (PKB) and focal adhesion kinase (FAK)-mediated signaling pathways. Periostin is expressed in many disease states that involve remodeling such as myocardial infarction, asthma, and cancer. In addition, periostin has been implicated in upregulating TGFβ activation in epithelial cells, an effect that might perpetuate a fibrogenic signaling cycle. Although TGFβ1, IL-13, and IL-4 all appear to have a role in regulating periostin, the transcriptional regulation of this matrix protein is incompletely understood.

In the esophagus of EoE patients, periostin has been shown to be one of the most highly upregulated (46-fold) genes, second in upregulation magnitude only to the eotaxin-3 gene. Blanchard et al. have shown that TGFβ1 and IL-13 can induce periostin expression in primary esophageal fibroblasts, and IL-13 can induce periostin expression in primary epithelial cells. They also have demonstrated that eosinophil recruitment is significantly decreased in allergen-challenged, periostin-null mice, and that periostin increases eosinophil adhesion to fibronectin, suggesting a role for periostin in eosinophil recruitment and trafficking. These observations support the notion that periostin plays an important role in EoE remodeling.

Innovation

Innovative aspects of the proposal include:

1. Elucidating the mechanisms of cytokine-induced and periostin-mediated fibrosis in the esophagus. Although Th2 cytokines are known to induce fibrosis in other organs, there are few data on how these cytokines may initiate esophageal fibrosis in EoE. We will delineate the mechanism of how periostin is regulated by IL-13 and IL-4 in epithelial cells and fibroblasts derived from the human esophagus. We will also define the pro-fibrotic effects IL-13, IL-4, and periostin on the esophageal mucosa, and the complex relationship between signaling events in the epithelial and mesenchymal compartments of the esophagus that result in the characteristic subepithelial fibrosis of EoE.

2. Novel reagents. We will use novel, telomerase-immortalized esophageal fibroblast and squamous epithelial cell lines that we have developed, and that recapitulate signaling events observed in primary biopsy tissues for in-depth mechanistic studies. We will also use a compelling integrative model by constructing organotypic 3D cell cultures with our cell lines to explore the complex relationship between the two mucosal compartments.

3. Translational studies. Aim 3 was designed to correlate our in vitro findings in cell lines and in 3D tissue constructs regarding Th2 cytokine-mediated periostin expression and fibrogenesis with markers of fibrosis in esophageal mucosal biopsy specimens from patients with EoE.
**Approach**

For the studies below in Aims 1-3, we will use the telomerase-immortalized fibroblast cell lines FEE4-T, BEFhT, and NeoFib2T and the telomerase-immortalized squamous epithelial cell lines EoE1-T and EoE2-T, unless otherwise noted, because prior studies have shown that both fibroblasts and epithelial cells can express periostin in response to Th2 cytokines.58

**Aim 1:** To delineate the mechanism whereby IL-13 and IL-4 regulate periostin expression by esophageal fibroblasts and squamous epithelial cells.

**Rationale:** Our preliminary data show that IL-13 and IL-4 upregulate periostin in both esophageal fibroblasts and epithelial cells, but the mechanism underlying these effects is not clear. TGFβ is known to upregulate periostin, and IL-13 can upregulate activated TGFβ in extraesophageal cells. In our esophageal cell lines, however, our preliminary data show that IL-13 and IL-4 at doses and time points optimized by our prior studies59 have little effect on TGFβ1 mRNA expression, but a robust response in periostin mRNA expression. Thus, we will focus on the TGFβ-independent pathway by which IL-13 and IL-4 regulate periostin expression, and we will investigate effects of targeting this pathway.

**Preliminary Data:** We have created novel, non-neoplastic, telomerase-immortalized esophageal fibroblast cell lines from esophageal mucosal biopsies taken from a patient with EoE (FEE4-T), a patient with GERD (BEFhT), and a patient with Barrett’s esophagus post-ablation (NeoFib2T). Using techniques previously reported in our laboratory, primary cultures of esophageal fibroblasts were retrovirally infected with human telomerase.60 These fibroblast cell lines express fibroblast markers such as αSMA, vimentin, and fibroblast surface protein (Figure 2A-B), and they do not express epithelial markers such as cytokeratins (Figure 2B). They also express receptors for IL-13 and IL-4 (Figure 2C). Stimulation of these fibroblast lines with IL-13 or IL-4 did not increase TGFβ1 mRNA expression by more than 3-fold as detected by QRT-PCR (Figure 2D). Since a cycle threshold change < 3 by QRT-PCR generally is not considered meaningful, these data suggest that Th2 cytokines have little effect on TGFβ1 production in esophageal fibroblasts. In our EoE fibroblast cell line FEE4-T, we found that Th2 cytokine stimulation for 48 hours induced periostin mRNA expression, as did stimulation with TGFβ1, which we used as a positive control (Figure 3A). In addition, we found that our telomerase-immortalized esophageal squamous epithelial cell lines derived from EoE patients (EoE1-T and EoE2-T)59 also expressed periostin after Th2 cytokine stimulation (Figure 3B).

**Subaim 1a.** Establishing that IL-13/IL-4 signaling and STAT3 machinery are intact and functional
The Type II IL-4 receptor activates STAT3 signaling (Figure 4). The cytoplasmic tails of the receptor subunits IL-13Rα1 and IL-4Rα are associated with tyrosine kinases of the Janus family (Jak 1-2 and Tyk2). When activated by either IL-13 or IL-4 ligands, the receptor subunits heterodimerize and enhance Jak activity. IL-13Rα1 activates Jak2 and/or Tyk2. IL-4Rα activates Jak1. Subsequently, these can initiate STAT3 activation and phosphorylation. Once phosphorylated, STAT3 dimerizes and translocates to the nucleus to initiate transcription of target genes. We reviewed the periostin promoter sequence (-2000bp) and found 4 potential STAT3 binding sites [based on the putative binding sequence TTC(N)AA]. Thus, we suspect that IL-13 and IL-4 regulate periostin via the STAT3 pathway. Since Th2 cytokines have been shown to induce periostin in fibroblasts, we will first establish that the STAT3 mechanism is functional and intact in our novel fibroblast cell lines. We have already demonstrated that this machinery is intact and functional in our epithelial cell lines. After IL-13 or IL-4 stimulation, Jak1, Jak2, and Tyk2 phosphorylation will be determined by Western blot (Abcam). Subsequently, pSTAT3 in the cytoplasm and nucleus will be determined to confirm STAT3 phosphorylation (Abcam) and nuclear translocation using the NE-PER Nuclear and Cytoplasmic Extraction kit (Thermo Fisher Scientific, Rockford, IL). Lastly, we will confirm expression of well-known STAT3 target gene such as c-myc, p21/WAF1, cyclin-D, and BCL2 by real-time PCR.

Subaim 1b. Determining IL-13 and IL-4 transcriptional activation and post-transcriptional regulation on periostin

**Periostin Transcriptional Activity:** Recently, we obtained a periostin-luciferase promoter construct (−2000bp) from Franco et al. We will transiently transfet our fibroblasts and epithelial cells with the luciferase promoter and renilla reporter as an internal control. We will then stimulate transfected cells with IL-13 or IL-4, and determine periostin promoter activation by dual-luciferase detection (Promega, Madison, WI). I have successfully performed chemical-based transfections and luciferase assays in previous studies. We also plan to use various deletion constructs to identify the relevant promoter region. Based on these findings, we will assess specificity to STAT3 by performing site-directed mutagenesis using the QuikChange II site-Directed Mutagenesis Kit (Stratagene, La Jolla, CA) to introduce single or double mutations within the STAT3 binding sites on the periostin promoter. This technique has been successfully performed in the Souza lab and will be one of my learning objectives. We will also knock down STAT3 using siRNA (Thermo Scientific Dharmacon) in our fibroblasts and epithelial cells. We will confirm that STAT3 binds to the relevant site on the periostin promoter with ChIP assay. We will immunoprecipitate the DNA with STAT3 antibody and perform PCR with primers designed to amplify the region of the periostin promoter flanking the binding site. The Souza lab has performed ChIP assay successfully in numerous other studies, and I will be performing this technique under the guidance of Dr. Souza as a learning objective. Finally, to confirm active transcription, we will perform ChiP assay for RNA Polymerase II binding the promoter.

**Periostin mRNA Stability:** To see if there is a component of delayed mRNA degradation, we will assess mRNA stability as we have previously described. After stimulation with a Th2 cytokine, we will inhibit further mRNA synthesis with 5, 6-dichlorobenzimidazole riboside (DRB) (Sigma-Aldrich, St. Louis, MO). We will then measure periostin mRNA levels over time in the presence or absence of the Th2 cytokine, to see if IL-13 or IL-4 regulates post-transcription by enhancing mRNA stability.

Subaim 1c. Targeting Jak-STAT3 pathway for intervention.

Recently, small molecule Jak inhibitors have been developed to treat autoimmune diseases (e.g. rheumatoid arthritis and psoriasis) and hematological malignancies. We will identify the pertinent Janus kinase involved in periostin regulation to use as a potential therapeutic target. We will first use a pan-Jak inhibitor (Jak Inhibitor I, EMD Millipore Chemicals, Billerica, MA) that inhibits Jak1-3 and Tyk2 to implicate this pathway in the signaling. Next, we will use agents known to target the Janus kinases selectively. AZD1480 (Selleck Chemicals, Houston, TX) is a selective Jak2 inhibitor. Tofacitinib (CP-690550) is a selective inhibitor...
of Jak3 commercially available through Selleck Chemicals and currently in clinical trials.65 There is no selective Jak1 inhibitor. However, Ruxolitinib (INCB018424) is an inhibitor of both Jak1 and Jak 2. Ruxolitinib is currently in clinical trials, and available through Selleck Chemicals.66 Periostin mRNA and protein expression will be determined in the presence and absence of the Jak inhibitors. To verify specificity, we will also use selective Jak siRNAs (Thermo Scientific Dharmacon).

**Anticipated results, potential pitfalls and alternative approaches:** We anticipate that periostin will be regulated via IL-13-IL-4-STAT3 activation in both squamous epithelial cells and fibroblasts based on our preliminary data. If STAT3 does not increase or only partially increases periostin expression, then we will study STAT6 as an alternative transcription factor regulating periostin. As shown in Figure 4, IL13/IL-4 signaling can activate STAT6. Because of the relative homology between the binding sequences of STAT3 and STAT6, STAT6 could potentially bind the periostin promoter. Lastly, if STAT3 and STAT6 are not involved in periostin regulation, then we will consider the IRS1/2 pathway which can also be initiated by IL-13/IL-4 signaling (Figure 4). IRS1/2 can activate the PI3K/Akt and Ras/MAPK pathways. Our promoter deletion construct analysis will narrow down the relevant transcription factor binding site for periostin. We then would use the rVista 2.0 program to identify conserved transcription factor binding sites and cross reference the results with known PI3K/Akt and/or Ras/MAPK transcription factors. Other alternative approaches include widening our search using a protein/DNA array from Panomics (Fremont, CA) that will allow us to profile 56 to 345 transcription factors at once, followed by cross referencing with the conserved transcription factor sites identified by rVista and with the PI3K and Ras pathways for candidate selection. Based on these findings, we would then interrogate for the relevant transcription factor for inducing periostin promoter activity using the techniques discussed. If none of the IL-13/IL-4 associated transcription factors are involved in periostin regulation, then the possibility remains that the Th2 cytokines engage the TGFβ pathway by facilitating activation of latent TGFβ. To determine if activation of latent TGFβ is playing a role, we can silence TGFβ with siRNA, and determine if there is still periostin expression with Th2 cytokine stimulation.

**Aim 2. To characterize the “direct” and “indirect” pro-fibrotic effects of IL-13, IL-4, and periostin on esophageal fibroblasts.**

**Rationale:** Studies in extra-esophageal diseases suggest that IL-13 and IL-4 have pro-fibrotic effects. In EoE, however, it is not clear what types of pro-fibrotic effects these Th2 cytokines have on esophageal fibroblasts. IL-13 and IL-4 may have direct effects on fibroblasts by inducing (1) myofibroblast transdifferentiation, (2) ECM protein production, and (3) contraction. We believe these effects are mediated by Th2-cytokine-induced periostin expression by the fibroblasts themselves. Since epithelial cells can also respond to Th2 cytokines by expressing periostin, IL-13 and IL-4 may also have indirect effects on the fibroblasts by inducing epithelial cells to express periostin, which then signals in a paracrine fashion to the fibroblasts in the lamina propria (i.e. mesenchyme). This aim will explore both direct and indirect effects of IL-13, IL-4, and periostin on fibrosis.

**Preliminary Data:** We explored the expression of markers of myofibroblast transdifferentiation (αSMA), ECM protein production (fibronectin and periostin), and a pro-inflammatory mediator (eotaxin-3) in our fibroblasts after stimulation with TGFβ1, IL-13, or IL-4 (Figure 5). Th2 cytokine stimulation caused a minimal increase in αSMA and a marked increase in periostin and eotaxin-3 mRNA expression. In contrast, TGFβ1 stimulation moderately increased both αSMA and fibronectin mRNA expression, but had no

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**Figure 5.** FEE4-T and BEFhT were stimulated by TGFβ1 (10 ng/ml), IL-13 (100 ng/ml), or IL-4 (100 ng/ml) for 48 hrs. Relative fold-change in mRNA expression compared to control (unstimulated) cells was determined for αSMA, fibronectin, periostin, and eotaxin-3 by real-time PCR.
effect on periostin and eotaxin-3. These findings suggest that the effects of IL-13 and IL-4 are specific in the induction of periostin and eotaxin-3, and that the induction of these pro-fibrotic and pro-inflammatory mediators is not a generalized response to all fibrosis-inducing agents.

**Subaim 2a. Determining the direct pro-fibrotic effects of IL-13 and IL-4 on esophageal fibroblasts.**

We will treat fibroblasts in vitro with varying doses (1-100 ng/ml) of IL-13 or IL-4 for 48 hours, and explore the effects on fibroblast activation by examining myofibroblast transdifferentiation, ECM protein production, and fibroblast contraction as detailed below. TGFβ1 (10 ng/ml), a well know inducer of fibrosis, will be used as a positive control.

**Myofibroblast transdifferentiation:** Myofibroblasts adopt a contractile phenotype and are considered intermediates between fibroblasts and smooth muscle cells. Therefore, while still expressing vimentin (intermediate filaments expressed by fibroblasts), myofibroblasts also express more αSMA, which is the actin isoform specifically expressed in smooth muscles cells and the major constituent of the contractile apparatus. In myofibroblasts, large amounts of αSMA are organized into myofilaments along a line of tension, whereas fibroblasts express low amounts of unorganized αSMA in the cytoplasm. We will perform immunofluorescence staining for αSMA (Sigma-Aldrich) and vimentin (Santa Cruz Biotechnologies, Santa Cruz, CA). Total actin (Sigma-Aldrich) will be our loading control since it should remain unaltered as fibroblast transdifferentiate into myofibroblasts. In vitro studies involving TGFβ-activated myofibroblast transdifferentiation involve 3-10 days of treatment, although 5 days seem to induce consistent increases in αSMA expression. Therefore, for this study we will perform time course studies over 3-5 days.

**ECM protein production:** Although activated fibroblasts can express a number of proteins that make up the extracellular matrix, based on EoE and fibrosis literature, we have selected to examine ECM proteins that can bind to periostin including fibronectin, collagen I, collagen III, collagen V, tenascin C, and periostin itself. mRNA expression levels will be determined by real-time PCR and protein levels for fibronectin, collagens, and tenascin C will be determined by Western blot (Santa Cruz and Abcam).

**Contraction:** To evaluate the direct effects of IL-13 and IL-4 on esophageal fibroblast function (i.e. contraction), we will use collagen gel matrix contraction assays (Cell Biolabs, San Diego, CA). These assays will allow us to assess fibroblast contractility by measuring collagen gel size in a floating matrix model or a stressed matrix model. For both of these collagen contraction models, fibroblasts will be seeded into collagen gel matrices. After the gel polymerizes, the matrices will be released from the wells of the culture wells, which initiates contraction. The floating matrix model evaluates the ability of the fibroblasts to contract in a tension-free environment. We will treat the cells with IL-13, IL-4, and TGFβ1 by adding these agents into the media in which the matrices are floating. Lysophosphatidic acid (10 μM) (Sigma-Aldrich), a strong agonist for contraction, will be used as a positive control. We have successfully performed contraction assays using TGFβ1, which caused fibroblast contraction within 3 hours, with a maximal effect observed by 48 hours (Figure 6). At each time point, the gel size (i.e. area) was determined by ImageJ. The percent contraction was calculated by the following equation: (1 - gel size / pre-contraction gel size) x 100%. We will also transdifferentiate the fibroblasts into myofibroblasts by treating with IL-13, IL-4, or TGFβ1 for 5 days prior to seeding into the collagen matrices. Then we will evaluate the contractility of transdifferentiated fibroblasts in the floating matrix model compared to non-transdifferentiated fibroblasts. The stressed matrix model evaluates the ability of the fibroblasts to transdifferentiate into myofibroblasts and contract under “stressed” or tension-loaded conditions. We will culture the fibroblasts in the collagen matrices for 5 days in the presence or absence of IL-13, IL-4, or TGFβ1. During this time, the matrices will not be released from the wall and will remain under tension. Myofibroblasts transdifferentiation is known to correlate with development of stress in the matrix. Thus, we expect myofibroblasts to orient along lines of tension and develop prominent stress fibers and fibronexus junctions (intercellular junctions between myofibroblasts and matrix protein). After 5 days, the matrices will be released to initiate contraction, which will be measured over 48 hours.
Subaim 2b. Determining the direct IL-13/IL-4-induced, periostin–dependent, pro-fibrotic effects on esophageal fibroblasts.

Our preliminary data demonstrate that Th2 cytokine stimulation induces periostin expression, which we anticipate will exert pro-fibrotic effects. Thus, to determine the periostin-dependent effects of Th2 cytokines, we will generate stable knockdown fibroblast cell lines by silencing the periostin gene with SMARTvector 2.0 Human Lentiviral shRNA particles (Thermo Scientific Dharmacon). We will study both the cell population and selected clones after confirming periostin knockdown by Western blots. We will determine pro-fibrotic effects of IL-13 and IL-4 that are dependent on periostin expression using the methods outlined in subaim 2a. In addition, we will also overexpress periostin using a periostin cDNA clone (Origene, Rockville, MD) as a second method to confirm our findings.

Subaim 2c. Determining the indirect effects of IL-13 and IL-4 on esophageal fibroblasts

We will explore whether the Th2 cytokine-induced periostin expressed by epithelial cells might contribute to pro-fibrotic effects on fibroblasts in the lamina propria. We will treat epithelial cells in vitro with IL-13 or IL-4 for 48 hours, and then collect the epithelial cell conditioned media, which will be used to treat the fibroblasts. Because recombinant IL-13 or IL-4 will be in the conditioned media, we will need to knock down the receptors for IL-13 or IL-4 in the fibroblasts to ensure that they do not respond to the recombinant IL-13 or IL-4. Therefore, we will stably knock down IL-13Rα1 or IL-4Ra in the fibroblasts with SMARTvector 2.0 Human Lentiviral shRNA particles (Thermo Scientific Dharmacon), and confirm the results by RT-PCR. We will use RT-PCR to confirm knockdown because, in preliminary studies, we found that the antibodies for these receptors were inadequate for detection by Western blotting. We will also confirm that the receptors are functionally inactive by treating with IL-13 or IL-4, and demonstrating the absence of downstream signaling molecule such as STAT3 and the target genes outlined in subaim 1a. We will then treat the fibroblasts with the conditioned media and determine fibroblast activation by myofibroblast transdifferentiation, ECM protein production, and contraction as described in subaim 2a. We will use a commercially available ELISA assay (SA Biosciences) to measure periostin in the conditioned media. We will then treat the fibroblasts with human recombinant periostin (R&D Systems) in doses found in our conditioned media for 48 hours and examine its effects on fibroblast activation using the same read-outs as outlined in subaim 2a.

Anticipated results, potential pitfalls and alternative approaches: In subaim 2a, we anticipate that fibroblast activation will require 48 hours of treatment. However, myofibroblast transdifferentiation may require up to 5 days of treatment. Thus, in order to achieve myofibroblast transdifferentiation, we may need to extend the treatment time. Collagen synthesis in the fibroblast is likely dependent on the presence of ascorbic acid. Therefore, we will evaluate mRNA expression of collagen genes. If protein expression does not correlate with mRNA levels, then we will need to add ascorbic acid into the media to facilitate lysine and proline hydroxylation of the preprocollagen peptides, and then repeat Western blotting. In subaim 2b, we anticipate that periostin protein will be highly expressed in fibroblasts as indicated by our preliminary data, and will exert significant pro-fibrotic effects. If periostin has minimal pro-fibrotic effects, then we will explore other IL-13/IL-4-inducible proteins such as CTGF, TNFα, eotaxin-1, and eotaxin-3 using the techniques described above. Based on our review of the EoE and fibrosis literature, we have selected these mediators as alternative because they have a high likelihood of being involved in EoE remodeling. In subaim 2c, if we are unable to knock down the IL-13 or IL-4 receptors, then an alternative would be to add neutralizing antibodies to IL-13 or IL-4 (Abcam) into the epithelial conditioned media before treating the fibroblasts. In subaim 2c, we will also use recombinant periostin to examine its direct effects on fibroblasts. However, there are several isoforms of periostin created by alternative splicing in its C-terminal domain, and the recombinant periostin we will be using is the full-length variant. Although the significance and function of the various isoforms are unknown, it is conceivable that the full-length variant will not be effective in our cells. Thus, an alternative approach would be to determine the dominant variant form expressed by the epithelial cells and the fibroblasts by performing 3’ rapid amplification of cDNA ends (RACE) technique to determine the sequence of the 3’ region of the transcript variant mRNA. We can use the 3’ RACE kit by Life Technologies (Life Technologies, Grand Island, NY). Once we determine the sequence, we will purchase the isoform commercially available or, if the isoform is not available, we will use the mammalian recombinant protein expression system (Freestyle Protein Expression System, Invitrogen) to create the protein. As noted above, it is possible that periostin will have minimal pro-fibrotic effects. Subaim 2c will determine if the conditioned media itself has pro-fibrotic effects and if so, we will determine other epithelial cell–secreted mediators that may cause fibroblast activation. However, there are no ELISA kits available to identify an array of pro-fibrotic proteins to address this issue. Therefore, we will use the methods outlined in subaim 2c.
STAT3 activation. we anticipate that there will be no periostin expression, confirming that periostin expression is dependent on comprised of these knockout cells. Since we are using both epithelial and fibroblast STAT3 knockdown cells, SMARTvector 2.0 Human Lentiviral shRNA technology (Thermo Scientific Dharmacon), and create 3D cultures Subaim 3a. Functional evaluations of IL-13/IL-4 signaling and periostin expression between the mucosal compartments (i.e. epithelial and mesenchyme) and assess effects on sub-epithelial fibrosis in organotypic 3D cultures

Rationale: In our first two aims, we will (1) delineate the mechanisms whereby IL-13 and IL-4 upregulate periostin and (2) characterize the effects of IL-13, IL-4, and periostin on fibroblasts directly and indirectly. In this aim, we propose to confirm our findings in cell lines grown in monolayer using an organotypic 3D culture model. Ideally, an in vitro model for EoE should recapitulate the three dimensional structure of the esophagus using a single epithelial cell type grown along with stromal fibroblasts. Those are the features of organotypic cultures, which allow for analyses of the molecular contributions of epithelial cells and fibroblasts to the development of disease in a tissue-like context. In addition, these organotypic cultures will enable us to explore in detail the role of IL-13, IL-4, and periostin in signaling from the epithelial compartment to mesenchymal compartment without the confounding influence of immune cells (i.e. eosinophils). We have selected this approach to confirm epithelial-to-mesenchymal signaling because most endoscopic biopsies of the esophageal mucosa contain only epithelium and, therefore, are not of sufficient depth to provide information on fibroblasts in the lamina propria. For example, a recent study evaluated the depth of 1,692 endoscopic esophageal biopsy tissue pieces and found that fewer than 11% contained subepithelial lamina propria. However, if this information is to be used to design new therapeutic strategies to prevent EoE complications, then it will be necessary to confirm that the events identified in our cell lines also occur in epithelial and stromal cells in patients in vivo. Therefore, we will use biopsy specimens from patients with EoE to confirm that the mechanisms elaborated in our in vitro studies are relevant in patients.

Subaim 3a. Functional evaluations of IL-13/IL-4 signaling and periostin expression between the mucosal compartments (i.e. epithelial and mesenchyme) and assess effects on sub-epithelial fibrosis in organotypic 3D cultures

The organotypic 3D culture protocol will allow us to create a stratified epithelium with esophageal squamous epithelial cells overlying a mesenchymal-like compartment comprised of esophageal fibroblasts once the culture is brought to liquid-air interface. These cultures can then be paraffin embedded and processed for histological evaluation. Dr. Anil Rustgi, Professor of Medicine at the University of Pennsylvania School of Medicine, has developed a successful protocol for 3D esophageal cultures, and he has agreed to provide me training on this technique at his institution as one of my learning objectives (please refer to letter of collaboration). We plan to use our epithelial cell lines and our fibroblast cell lines to create the 3D model on porous membrane cell culture inserts. Treatments can be applied to the media in the bottom well underlying the fibroblast layer and both epithelial and fibroblast cells can “communicate” with the media constituents.

To confirm our findings in the monolayer cell culture from aim 1, we will assess periostin expression by immunohistochemistry (IHC) staining in 3D cultures. This aim will be performed in conjunction with Dr. Robert Genta, an expert gastrointestinal pathologist who practices both at the Dallas VA Medical Center and at Miraca Life Sciences, Inc. in Irving, Texas. The Souza lab, in collaboration with Dr. Genta, has successfully performed IHC on human and rat esophageal tissue specimens for phospho-ERK1/2 and IL-8, and performing IHC will be a learning objective for me. We will then prepare STAT3 knockout epithelial cells and fibroblasts using SMARTvector 2.0 Human Lentiviral shRNA technology (Thermo Scientific Dharmacon), and create 3D cultures comprised of these knockout cells. Since we are using both epithelial and fibroblast STAT3 knockout cells, we anticipate that there will be no periostin expression, confirming that periostin expression is dependent on STAT3 activation.

To confirm our findings in aim 2, we will perform IHC staining for markers of fibrosis and fibroblast activation such as αSMA, collagen, fibronectin, tenascin C, and periostin (all antibodies are available from Abcam). To evaluate the role of IL-13 in epithelial-to-mesenchymal compartment signaling, we will use the IL-13 receptor knockout fibroblasts developed in subaim 2c, and will similarly develop IL-13 receptor knockout epithelial cells. We will then create 3D cultures with three different combinations of cells: (1) IL-13Ra1 knockout epithelial cells plus fibroblasts, (2) epithelial cells plus IL-13Ra1 knockout fibroblasts, (3) IL-13Ra1

SABiosciences Human Fibrosis RT2 PCR array (Qiagen, Valencia, CA) to provide an unbiased approach to evaluate for expression of 84 key genes involved in dysregulated tissue remodeling using our IL-13/IL-4 treated epithelial cell mRNAs. Based on these findings, we will then select the likely candidates for ELISA analysis and quantification in the conditioned media. Depending on the mediators identified, we will use recombinant forms of the mediators to treat the fibroblasts and determine their role in fibroblast activation.

Aim 3. To confirm that IL-13 and/or IL-4 can cause STAT3-mediated periostin expression and fibrotic effects in organotypic models, and to correlate STAT3 pathway activation, periostin expression, and fibrosis in esophageal biopsy specimens from EoE patients.

Rationale: In our first two aims, we will (1) delineate the mechanisms whereby IL-13 and IL-4 upregulate periostin and (2) characterize the effects of IL-13, IL-4, and periostin on fibroblasts directly and indirectly. In this aim, we propose to confirm our findings in cell lines grown in monolayer using an organotypic 3D culture model. Ideally, an in vitro model for EoE should recapitulate the three dimensional structure of the esophagus using a single epithelial cell type grown along with stromal fibroblasts. Those are the features of organotypic cultures, which allow for analyses of the molecular contributions of epithelial cells and fibroblasts to the development of disease in a tissue-like context. In addition, these organotypic cultures will enable us to explore in detail the role of IL-13, IL-4, and periostin in signaling from the epithelial compartment to mesenchymal compartment without the confounding influence of immune cells (i.e. eosinophils). We have selected this approach to confirm epithelial-to-mesenchymal signaling because most endoscopic biopsies of the esophageal mucosa contain only epithelium and, therefore, are not of sufficient depth to provide information on fibroblasts in the lamina propria. For example, a recent study evaluated the depth of 1,692 endoscopic esophageal biopsy tissue pieces and found that fewer than 11% contained subepithelial lamina propria. However, if this information is to be used to design new therapeutic strategies to prevent EoE complications, then it will be necessary to confirm that the events identified in our cell lines also occur in epithelial and stromal cells in patients in vivo. Therefore, we will use biopsy specimens from patients with EoE to confirm that the mechanisms elaborated in our in vitro studies are relevant in patients.

Subaim 3a. Functional evaluations of IL-13/IL-4 signaling and periostin expression between the mucosal compartments (i.e. epithelial and mesenchyme) and assess effects on sub-epithelial fibrosis in organotypic 3D cultures

The organotypic 3D culture protocol will allow us to create a stratified epithelium with esophageal squamous epithelial cells overlying a mesenchymal-like compartment comprised of esophageal fibroblasts once the culture is brought to liquid-air interface. These cultures can then be paraffin embedded and processed for histological evaluation. Dr. Anil Rustgi, Professor of Medicine at the University of Pennsylvania School of Medicine, has developed a successful protocol for 3D esophageal cultures, and he has agreed to provide me training on this technique at his institution as one of my learning objectives (please refer to letter of collaboration). We plan to use our epithelial cell lines and our fibroblast cell lines to create the 3D model on porous membrane cell culture inserts. Treatments can be applied to the media in the bottom well underlying the fibroblast layer and both epithelial and fibroblast cells can “communicate” with the media constituents.

To confirm our findings in the monolayer cell culture from aim 1, we will assess periostin expression by immunohistochemistry (IHC) staining in 3D cultures. This aim will be performed in conjunction with Dr. Robert Genta, an expert gastrointestinal pathologist who practices both at the Dallas VA Medical Center and at Miraca Life Sciences, Inc. in Irving, Texas. The Souza lab, in collaboration with Dr. Genta, has successfully performed IHC on human and rat esophageal tissue specimens for phospho-ERK1/2 and IL-8, and performing IHC will be a learning objective for me. We will then prepare STAT3 knockout epithelial cells and fibroblasts using SMARTvector 2.0 Human Lentiviral shRNA technology (Thermo Scientific Dharmacon), and create 3D cultures comprised of these knockout cells. Since we are using both epithelial and fibroblast STAT3 knockout cells, we anticipate that there will be no periostin expression, confirming that periostin expression is dependent on STAT3 activation.

To confirm our findings in aim 2, we will perform IHC staining for markers of fibrosis and fibroblast activation such as αSMA, collagen, fibronectin, tenascin C, and periostin (all antibodies are available from Abcam). To evaluate the role of IL-13 in epithelial-to-mesenchymal compartment signaling, we will use the IL-13 receptor knockout fibroblasts developed in subaim 2c, and will similarly develop IL-13 receptor knockout epithelial cells. We will then create 3D cultures with three different combinations of cells: (1) IL-13Ra1 knockout epithelial cells plus fibroblasts, (2) epithelial cells plus IL-13Ra1 knockout fibroblasts, (3) IL-13Ra1

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knockout epithelial cells plus IL-13Rα1 knockout fibroblasts. Cultures will be treated with IL-13, and IHC staining for the same fibrosis markers will be performed. By knocking out IL-13Rα1 receptor in the epithelial cells, we will impair the epithelial cells from responding to IL-13 stimulation and, subsequently, any epithelial-to-mesenchymal signaling induced by IL-13. Conversely, by knocking out IL-13Rα1 receptor in fibroblasts, we will impair the fibroblasts from responding to IL-13 stimulation and, thus, any fibrotic effects in the fibroblast layer will be a result of epithelial-to-mesenchymal signaling. In the same fashion, we will evaluate the role of IL-4 in epithelial-to-mesenchymal signaling using IL-4Ra shRNA in our epithelial cells and fibroblasts.

To evaluate the role of periostin in epithelial-to-mesenchymal signaling, we will use the periostin knockout fibroblasts in subaim 2b, and we will similarly develop periostin knockout epithelial cells. We will then create 3D cultures with three different combinations of cells: (1) periostin knockout epithelial cells plus fibroblasts, (2) epithelial cells plus periostin knockout fibroblasts, (3) periostin knockout epithelial cells plus periostin knockout fibroblasts. Cultures will be treated with IL-13 or IL-4, and IHC staining for fibrosis markers will be performed.

Subaim 3b. Evaluation of STAT3 and periostin expression and markers of fibrosis in patient biopsy specimens

This aim will be performed in conjunction with Drs. Stuart Spechler and Robert Genta. We have chosen to perform these analyses on archived biopsy specimens from EoE patients available through Dr. Genta at Miraca Life Sciences. Miraca (formerly Caris) Life Sciences is a specialized gastrointestinal pathology group that receives endoscopic biopsy specimens for diagnostic purposes from over 1,500 gastroenterologists in 40 states. During the past 3 years, Miraca processed esophageal biopsy specimens from approximately 7,000 patients with EoE.

To correlate STAT3 pathway activation with periostin expression, we will perform IHC staining using antibodies against pSTAT3 and periostin on serial sections of esophageal biopsy tissues. We will also correlate STAT3 activation with the degree of fibrosis identified by Masson trichrome technique, which stains for collagen. Finally we will correlate periostin expression with IHC staining for αSMA, fibronectin, and tenascin C.

We will score the tissue specimens based on the intensity of the staining and on the percentage of cells that stain for a particular protein. The intensity will be scored on a scale of 0 to 3 (0 = no staining; 1 = minimal, but specific staining; 2 = moderate staining; 3 = intense staining). We will determine each score individually and we will generate a combined score which will be the % positive cells X intensity of the positivity. We recognize that staining within a section is not likely to be uniform. Therefore, we will generate a combined staining score from 5 different high power fields per section, and we will average these to derive a final score for that tissue specimen. We will also score the tissue specimens on a 0 to 3 point scale for fibrosis based on amount of collagen bundles, thickness of bundles, and degree of cell loss in fibrotic areas in a manner similar to that of Lucendo et al. and Aceves et al.8, 9

Statistics: Sample size determinations and selection of appropriate statistical analyses were determined for this aim in consultation with Daisha J. Cipher, Ph.D., Associate Professor, University of Texas at Arlington School of Nursing and Biostatistical Consultant, Dallas VA Research Corporation.

Sample Size Determination: Power analysis using SAS 9.2 for Linux indicated that a total of 133 subjects provided at least 80% power to address aim 3 using Cramer’s phi correlations between periostin, collagen, αSMA, fibronectin, and tenascin C. This sample size estimate was based on a moderate anticipated effect size ($W = .30$), alpha of .05, two-tailed (see Table 1).

<table>
<thead>
<tr>
<th>Strength of Association ($W$)</th>
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Table 1: Sample Size and Power Considerations

Statistical Analysis: All study variables will be descriptively analyzed for measures of central tendencies. Tissue samples will be tested for the presence of phospho-STAT3 and periostin. Staining scores will be created for periostin, collagen, αSMA, fibronectin, and tenascin C using a grading scale of 0 to 3. Those samples that stain positively for periostin will be submitted to correlational analyses of the staining scores between periostin, collagen, tenascin C, fibronectin, and αSMA using Cramer’s phi coefficients.

Anticipated results, potential pitfalls and alternative approaches: In subaim 3a, if IHC staining does not provide adequate intensity, then as an alternative technique we can perform IF on the sections. Another alternative is processing the cultures for RNA and protein isolation. The epithelial layer can potentially be
peeled off with forceps from the matrix compartment; subsequently, these separate layers (epithelial and mesenchyme) can be homogenized in lysis buffer for RNA and protein isolation. Although in subaim 3a we will evaluate IL-13- and/or IL-4-induced fibroblast activation, there are no histological methods for evaluating fibroblast contractility. Thus, fibroblast contractility can only be determined in the in vitro studies outlined in aim 2. In subaim 3b, we estimate that 11% of 7,000 biopsies from the Caris database will provide us 770 potential biopsies with adequate lamina propria to evaluate. However, an additional resource for biopsies would be Dr. Rajeev Jain from the Dallas Endoscopy Center who is another of our collaborators. Dr. Jain collects over 700 EoE biopsies per year from the center. Masson trichrome staining is the method of choice for collagen staining but, if we encounter problems with the trichrome stain, Sirus red staining is an alternative approach for collagen detection.

**Future Directions:** Once we have established the mechanisms of periostin expression and fibrogenesis in vitro and confirmed an association between periostin expression and subepithelial fibrosis in patients in vivo, we will investigate the intracellular signaling pathways and effector molecules (i.e. integrins) by which periostin causes fibroblast activation and focus on demonstrating the benefits of targeting periostin-mediated fibrogenesis in mouse models. In collaboration with Dr. David Wang, an Assistant Professor of Medicine with expertise in transgenic mouse models and a member of our research group at the Dallas VA, we plan to develop a murine model involving transgenic expression of periostin in the esophagus of eosinophil-deficient mice (∆dbl-GATA). This will enable us to examine the extent of fibrosis and interrogate periostin-induced fibrogenesis that is eosinophil-independent. Conversely, we can examine the therapeutic benefits of inhibiting periostin by crossing rtTA-CC10-IL-13 transgenic mice (which overexpress IL-13 in the esophagus and, therefore, develop esophageal fibrosis and eosinophilia) with periostin null mice Postn−/− (The Jackson Laboratory, Bar Harbor, ME). We plan to use these models to determine whether the pathways and/or specific proteins indentified in our proposal demonstrate promise as targets for therapeutic agents in EoE patients to prevent fibrosis and tissue remodeling.

**Timeline:** Aim 1, years 1-2.5; Aim 2, years 1.5-3; Aim 3, years 2.5-4.
INCLUSION OF WOMEN AND MINORITIES

N/A
PROTECTION OF HUMAN SUBJECTS
This Human Subjects Research will only use unidentifiable diagnostic specimens. The human cell lines utilized were established previously and no identifiable patient markers are associated with these cell lines. Human specimens for use in Specific Aim #3 will be obtained from archived tissue de-identified by a third party (Miraca Life Sciences) before being analyzed. Miraca Life Sciences is a specialized gastrointestinal pathology group that receives endoscopic biopsy specimens for diagnostic purposes from primarily adult gastroenterologists (over 1,500 gastroenterologists in 40 states). Since the archived biopsy specimens are derived from the demographic population that develops eosinophilic esophagitis, we expect the tissue to come mostly from young adult white males but may include females, minorities, and children.
### Study Title:
The Role of Periostin and Th2 Cytokines in Eosinophilic Esophagitis Fibrogenesis

**Total Planned Enrollment:** N/A

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* The “Ethnic Category: Total of All Subjects” must be equal to the “Racial Categories: Total of All Subjects.”
INCLUSION OF CHILDREN

N/A
SUMMARY STATEMENT

Application Number: 1 K08 DK099383-01

Principal Investigator

MD

Applicant Organization: UNIV OF TEXAS SW MED CTR

Review Group: DDK-C
Digestive Diseases and Nutrition C Subcommittee

Meeting Date: 03/13/2013
Council: MAY 2013
Requested Start: 07/01/2013

RFA/PA: PA11-193
PCC: NJP DDTR

Project Title: The Role of Periostin and Th2 Cytokines in Eosinophilic Esophagitis Fibrogenesis

SRG Action: Impact Score: 20

Human Subjects: 30-Human subjects involved - Certified, no SRG concerns
Animal Subjects: 10-No live vertebrate animals involved for competing appl.
Gender: 4A-Gender representation unknown, scientifically acceptable
Minority: 4A-Minority representation unknown, scientifically acceptable
Children: 4A-Child representation unknown, scientifically acceptable

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ADMINISTRATIVE BUDGET NOTE: The budget shown is the requested budget and has not been adjusted to reflect any recommendations made by reviewers. If an award is planned, the costs will be calculated by Institute grants management staff based on the recommendations outlined below in the COMMITTEE BUDGET RECOMMENDATIONS section.

ADMINISTRATIVE NOTE, BIOHAZARD
BIOHAZARD

SCIENTIFIC REVIEW ADMINISTRATOR’S NOTES

RESUME AND SUMMARY OF DISCUSSION: An application for a Mentored Clinical Scientist Research Career Development Award (Parent K08) was submitted by the University of Texas Southwestern Medical Center Dallas on behalf of Dr. [redacted]. The goal is to understand the pathophysiological roles of IL-13, IL-4, periostin, epithelial cells, and fibroblasts in the subepithelial fibrosis that complicates eosinophilic esophagitis (EoE), and to identify potential molecular targets to prevent tissue remodeling in EoE. Strengths of the application include a productive candidate with strong letters of support and a firm commitment to research; a clinically important research topic; an excellent team of mentors and collaborators; an outstanding research and training environment; and very strong institutional commitment. In addition to these strengths, concerns were also noted, including some weaknesses in the research plan, including the need to further characterize the immortalized fibroblasts; an overall lack of alternative interpretations of the data; and over-confidence in some of the model systems to answer the scientific questions. The overall merit of this application is considered to be outstanding, and four years of support are recommended.

DESCRIPTION (provided by applicant): This NIH Mentored Career Development Award proposal describes a 4 year training program for the candidate, a pediatric gastroenterologist with the long-term goal of becoming an independent and funded physician-scientist with a research focus on fibrogenesis and tissue remodeling in eosinophilic esophagitis (EoE). To accomplish these goals, the candidate and her mentors have developed an integrated plan including innovative scientific ideas, advanced training in the field of basic science and translational research, and a detailed career development plan. This proposal builds on the basic research developed during the candidate’s fellowship and K12 training, specifically on her preliminary findings of esophageal fibroblasts and esophageal epithelial cells expressing periostin, an extracellular matrix protein highly upregulated in the EoE esophagus. The chronic esophageal eosinophilia of EoE is associated with tissue remodeling characterized by a unique pattern of subepithelial fibrosis leading to painful mucosal tears, odynophagia, dysphagia, and strictures. The pathogenesis of fibrosis in EoE is not understood, and no treatment has been shown to prevent tissue remodeling. Previously, the candidate has demonstrated a prominent role for Th2 cytokines, IL-13 and IL-4, in EoE. She hypothesizes that, in the esophagus of EoE patients, IL-13 and/or IL-4 drive fibrogenesis by increasing esophageal mucosal expression of periostin. To test this hypothesis, she will use novel, telomerase- immortalized esophageal fibroblast and epithelial cell lines. She will also create organotypic 3D cell cultures to study signaling between the two mucosal compartments (epithelium and mesenchyme). She proposes three specific aims: (1) To delineate the mechanism whereby IL-13 and IL-4 regulate periostin expression by esophageal fibroblasts and squamous epithelial cells; (2) To characterize the “direct” and “indirect” pro-fibrotic effects of IL-13, IL-4, and periostin on esophageal fibroblasts; (3) To confirm that IL-13 and/or IL-4 can cause STAT3-mediated periostin expression and fibrotic effects in organotypic models, and to correlate STAT3 pathway activation, periostin expression, and fibrosis in esophageal biopsy specimens from EoE patients. This proposal seeks to identify potential molecular targets to prevent tissue remodeling in EoE. The candidate will perform these studies under the guidance of Drs. Rhonda Souza and Stuart Spechler, senior translational investigators with a record of research excellence in the molecular study of esophageal diseases. In addition, the advisory committee includes Dr. Glenn Furuta, an international authority on EoE, and Dr. Frederick Grinnell, a fibrosis expert. To augment her research and career development program, she has outlined advance training in specific methodologies and formal intramural and extramural coursework in fibrosis, grant writing, biostatistics, bioethics, and animal models of disease. The University of Texas Southwestern Medical Center, Dallas VA Medical Center,
and Children's Medical Center will provide the necessary resources and intellectual environment to foster this candidate's transition to an independent investigator.

**PUBLIC HEALTH RELEVANCE:** Eosinophilic esophagitis (EoE) is a recently recognized esophageal disorder associated with eosinophil- predominant inflammation and significant tissue remodeling characterized by prominent subepithelial fibrosis leading to painful mucosal tears, odynophagia, dysphagia, and ultimately esophageal strictures in children and adults. The pathogenesis of fibrosis in EoE is not understood and no treatment has been shown to prevent tissue remodeling in EoE. This proposal seeks to understand the pathophysiological roles of IL-13, IL-4, periostin, epithelial cells, and fibroblasts in the subepithelial fibrosis that complicates EoE, and to identify potential molecular targets to prevent tissue remodeling in EoE.

**CRITIQUE 1:**

**Candidate:** 2  
**Career Development Plan/Career Goals /Plan to Provide Mentoring:** 1  
**Research Plan:** 2  
**Mentor(s), Co-Mentor(s), Consultant(s), Collaborator(s):** 1  
**Environment & Commitment to the Candidate:** 1

**Overall Impact:**  
This is a K08 from an Instructor of Pediatrics specializing in gastroenterology at UT Southwestern who has held that position since 2011. The proposal is focused on eosinophilic esophagitis (EoE), a major health concern with a limited number of basic science models available for study. The candidate has generated immortalized (non-transformed) esophageal fibroblasts and squamous epithelial cells and has shown that Th2 cytokines drive fibrogenic genes in both fibroblasts and squamous epithelial cells— including one called periostin, which will be the focus of a major part of the research proposed here. The proposal will test the hypothesis that Th2 cytokines drive fibrosis associated with EoE by inducing periostin. To address this they will assess how Th2 cytokines induce periostin expression in fibroblasts and squamous IECs

1. **Candidate:**  
   **Strengths**
   - has an AGA fellow to faculty transition award
   - recent first authored publication in Gut related to this work
   - three first authored reviews/chapters including some directly related to the current proposal
   - excellent letters of support
   **Weaknesses**
   - Modest record of publication in the primary literature, likely owing to a relatively short duration in mentors lab thus far

2. **Career Development Plan/Career Goals & Objectives (K24 ONLY Plan to Provide Mentoring):**
   **Strengths**
   - Clearly delineated plan with the goal of establishing a niche in EoE research for the candidate
   **Weaknesses**
3. Research Plan:

Strengths
- Has an outside pathologist committed to assess biopsies
- Letter of support from 3-D organotypic culture expert, Anil Rustgi

Weaknesses
- None noted

4. Mentor(s), Co-Mentor(s), Consultant(s), Collaborator(s):

Strengths
- The co-mentors, Drs Souza and Spechler have a longstanding collaboration
- All mentors and advisors are Full Professors, three of them at UT Southwestern

Weaknesses
- One advisor, a former mentor, is in Colorado

5. Environment and Institutional Commitment to the Candidate:

Strengths
- The environment is outstanding, with a significant group of individuals dedicated to esophageal disorders
- Longstanding collaborative environment for research in esophageal disorders
- Candidate already has a faculty position with protected time
- Institutional commitment is very strong and clearly stated in the letter from Dr Fontan

Weaknesses
- Dr. Fontan is the interim chair of pediatrics

CRITIQUE 2:

Candidate: 2
Career Development Plan/Career Goals /Plan to Provide Mentoring: 3
Research Plan: 4
Mentor(s), Co-Mentor(s), Consultant(s), Collaborator(s): 2
Environment & Commitment to the Candidate: 4

Overall Impact:
This is an excellent application from an outstanding young physician-scientist. The candidate, Dr. is an Instructor in the Department of Pediatrics at Univ Texas Southwestern. Her career goals are centered on defining the mechanisms underlying eosinophilic esophagitis and has established herself in an exceptional environment to continue those studies. The mentorship team is comprised of Dr. Souza who will oversee the candidate's research and Dr. Spechler who will assist in translating
those findings to the clinic. This co-mentorship is strengthened by the long-track record of Drs Souza and Spechler and is demonstrated by joint multi-PI grants. The environment is outstanding and supported by a superb advisory committee at UTSW, Univ Colorado and Penn. The research plan seeks to define the signaling mechanisms whereby periostin, a gene significantly upregulated in human eosinophilic esophagitis, regulates fibrosis. Novel reagents support the three proposed specific aims. Preliminary data acquired during the candidates K12 award further support the application. The proposal is weakened somewhat by an overall lack of alternative interpretations of the data and over-confidence in some of the model systems to answer the scientific questions. However, these limitations are mitigated in many respects by the advisory panel who are leaders in the field and have experience mentoring physician-scientists to successful independent positions.

1. Candidate:

Strengths

- The candidate has a very good record of scientific productivity. She lists 5 papers published in excellent journals, with reviews and a book chapter published or accepted for publication.
- Significant preliminary data strongly attest to the scientific merit of the candidate and support a well written application.
- The applicant is working in an exceptional mentoring environment, with Dr. Spechler and Dr. Souza forming a very strong support structure for Dr. to matriculate into an independent investigator.
- The candidate has established a strong Advisory Committee to assist in the completion of her proposed research. Dr. has also recruited some superb physician-scientists in Drs. Rustgi and Dr. Genta to augment her hands-on training.
- Letters of reference are uniformly strong and attest to the candidate’s strong commitment to research and succeeding as a physician scientist.

Weaknesses

- There is a letter of support from the Interim Department Chair in Pediatrics, Dr. Fontan, who attests to both Departmental and Division support for Dr.. Given the interim tag it would have been nice to see an additional letter of support from the Division Chief to lend further evidence of long-term support for Dr. protected time.

2. Career Development Plan/Career Goals & Objectives

Strengths

- The 4-year training plan is well thought out, with clearly defined milestones for completing the proposed research. Two separate Ethics and Responsible Conduct courses is a strength of the proposal, as is the inclusion of a biostatistics course. Inclusion of detail syllabi for those courses would have lent significant strength.
- The current career development plan builds and integrates nicely with the completed research and career development of the K12.
- Daily and weekly meetings with the mentor and co-mentor are appropriate. The division of labor amongst the mentor will provide outstanding training. Meetings amongst the advisory committee of Dr. Furuta and Dr. Grinnell are bi-monthly and appropriate to the proposed research and the candidates career development.

Weaknesses
• While there are clear milestones for independence, additional evidence for the transition would strengthen the application. As an example, besides transitioning Dr. laboratory to UTSW from the Dallas VA, milestones or clearly specified and attainable plans for promotion to Assistant Professor should be detailed.

• The twice yearly meetings with Dr. Furuta and Dr. Grinnell were mentioned, but lacked details as to when and for how long these meetings would occur.

• It was not clear from the proposal or budget if Dr. was going to travel to The Jax Labs for the Pathology of Mouse Models course or if the training could be completed online or at UTSW. Does the UTSW animal facility offer a similar course? What is the rationale for a mouse pathology coursework for a research plan lacking mouse models?

3. Research Plan:

Strengths

• The research questions posed are hypothesis driven and build nicely from data the candidate has published in Gut and which was supported by the K12 award. The studies build iteratively from cell culture biochemical signaling and promoter analyses (Aim 1) to functional assays (Aim 2) through epithelial-fibroblast organoids and archived human specimens (Aim 3).

• The proposed studies, especially the molecular and biochemical analyses in Aim 1, will provide excellent development for the candidate. These studies should establish a solid foundation in molecular biology.

• Engineered immortalized fibroblast and esophageal squamous epithelial cells is a strength.

• Future plans move into possible transgenic mouse models are logical, but could be technically difficult as often mouse inflammation does not fully recapitulate human disease.

Weaknesses

• The studies should consider the potential for cytokine-induced changes in periostin on active TGFbeta. RT-PCR studies were proposed but may not impact activity. It is not clear if periostin regulates expression or activity? If it is the latter then TGFbeta activity should be assayed to validate studying TGFbeta-independent roles for IL13/IL4 in fibrogenesis.

• Studies in Aim 1 are rather straightforward but would be strengthened by inclusion of shRNA technology to test specificity for the STAT signaling. Given that squamous epithelium also respond to IL13/IL4 it would be nice to specify which cell is being tested with the pharmacological Jak inhibitors in Aim 1. These were proposed in Aim 3 organoid mixed culture system but should be defined in each of the cellular components of that assay in Aim 1.

• Alternative approaches lack consideration of alternative interpretation of the data or foresight regarding possible technical difficulties was noted in the functional studies and organoid 3D culture model system.

• The preliminary data has little statistical analyses.

4. Mentor(s), Co-Mentor(s), Consultant(s), Collaborator(s):

Strengths

• Both the mentor Dr. Souza and the co-mentor Dr. Spechler are exceptionally strong scientists with a track-record of mentorship of physician-scientists. The research strengths of the scientists are in the area of esophageal pathophysiology and will clearly benefit the research career of the candidate.
Both Dr. Souza and Dr. Spechler are well funded from the NIH and VA merit. They co-I several of the awards, lending further support that that mentorship team is tightly integrated.

The Advisory Committee of Dr. Furuta (Univ Colorado) and Dr. Grinnell (UTSW) are superb. The distance of Dr. Furuta has been overcome, mitigating that potential weakness. The inclusion of both scientists greatly strengthens the functional fibroblast studies proposed in aims 1 and 2.

Strong letters from the collaborators Dr. Rustgi and Dr. Genta.

Weaknesses
- The travel to train in the Rustgi laboratory is a strength, but is undermined by a lack of detail regarding the duration and specific modality of the training.

5. Environment and Institutional Commitment to the Candidate:

Strengths
- The environment and institutional commitment at UTSW is exceptional as evidenced by the letter from the mentors and Dr. Fortan, Interim Chairman of the Department of Pediatrics.
- The department has reserved 80% protected research time and has provided 300 square feet of wet-lab space for the candidate. The identified space is more than sufficient to complete the proposed studies and is strengthened by the close proximity and sharing environment of the mentor Dr. Souza.
- Dr. appointment as Instructor and salary support are not contingent on receipt of the K-award.

Weaknesses
- A letter from the Division chief re-iterating the support and long-term career development of the candidate would strengthen the application.
- There was no mention of defined goals or milestones for promotion to tenure-track faculty. The long-term institutional commitment to the candidate is not clearly defined.

Protections for Human Subjects:
Acceptable Risks and Adequate Protections
- de-identified archived specimens from a commercial source (Caris)

Data and Safety Monitoring Plan (Applicable for Clinical Trials Only):

Vertebrate Animals:
Acceptable

Biohazards:
Unacceptable
- no consideration of the lentiviral vectors which require an IBC protocol.

Training in the Responsible Conduct of Research:
Acceptable
Comments on Format (Required):
- The candidate states she has completed a biomedical ethics course with Dr. O'Day but no grade for the course was listed.

Comments on Subject Matter (Required):
- the subjects covered in the course appear to meet NIH guidelines but the inclusion of a detailed syllabus would have been useful

Comments on Faculty Participation (Required; not applicable for mid- and senior-career awards):
- each discussion section was proctored by PI, none of whom were listed on the application.

Comments on Duration (Required):
- 1.5 hours per session for 10 sessions.

Comments on Frequency (Required):
- the candidate took the class twice, but it was unclear how often the class met per semester.

Resource Sharing Plans:
Acceptable

Budget and Period of Support:
Recommended budget modifications or possible overlap identified:
- the justification does not include a breakout of the specific materials. there is also no breakdown as to the use of K01 budget for tuition of the scientific computing coursework.

CRITIQUE 3:

Candidate: 2
Career Development Plan/Career Goals /Plan to Provide Mentoring: 2
Research Plan: 1
Mentor(s), Co-Mentor(s), Consultant(s), Collaborator(s): 1
Environment & Commitment to the Candidate: 2

Overall Impact:
The strengths of this application include a very strong and productive applicant, an important area of investigation that would allow the applicant to further progress in her research career, a carefully structured career plan, outstanding mentors and advisory panel, and an excellent environment. Mild concerns revolve around the heavy reliance on transformed cells and the possibility for the proposal to be too ambitious.

1. Candidate:
Strengths
- 5 publications since 2010, of which two are first author publications, and one is in Gut; 2 book chapters.
- Strong commitment to research: previous support by a T32 training grant; current support through a K12 and a concomitant AGA Fellowship to Faculty transition award.
• Very strong letters of recommendation.

Weaknesses
• None noted

2. Career Development Plan/Career Goals & Objectives/Plan to Provide Mentoring

Strengths
• Carefully structured and detailed plan
• This proposal will allow the applicant to further develop her research area and extend it from epithelial cell biology to stromal/mesenchymal cell biology.

Weaknesses
• None noted

3. Research Plan:

Strengths
• Tissue remodeling and fibrosis is an important area of research and the specific aims are plausible.
• The proposed experiments are carefully structured and well documented, they are also supported by preliminary data.
• The Specific Aims show logical progression

Weaknesses
• The transformed cell lines, which are extensively utilized in the proposed experiments, should be further discussed or characterized. How well-differentiated and representative of normal esophageal fibroblasts are they? Can they be expected to spontaneously de-differentiate and/or trans-differentiate? How will the different fibroblasts, from normal controls, Barrett’s patients, and eosinophilic esophagitis patients, be used; will some of these fibroblasts constitute controls or will they all be expected to behave similarly?
• The proposed experiments may be too ambitious in their number.

4. Mentor(s), Co-Mentor(s), Consultant(s), Collaborator(s):

Strengths
• The two co-mentors, Dr. Stuart Spechler & Rhonda Souza, both established in their field, have collaborated extensively and have complementary expertise, clinical esophageal diseases for Dr. Spechler, and translational aspects for Dr. Souza.
• The advisory panel is also very strong. Drs. Grinnell, Furuta, and Genta are outstanding investigators capable of providing important contributions to the different aspects of this project (tissue fibrosis, Dr. Grinnell; eosinophilic esophagitis, Dr. Furuta; histopathology and biopsy specimen, Dr. Genta)
• Dr. Anil Rutgsi will provide expertise in organotypic culture.

Weaknesses
• The research emphasis and funding of Drs. Spechler and Souza have been mainly in the area of Barrett's esophagus.
5. Environment and Institutional Commitment to the Candidate:

Strengths
- Superb environment at UT Southwestern.
- Strong general commitment to applicant (appointment as Instructor, salary support, and 80% protected time).

Weaknesses
- None noted

Protections for Human Subjects:
Acceptable Risks and Adequate Protections

Inclusion of Women, Minorities and Children:
G1A - Both Genders, Acceptable
M1A - Minority and Non-minority, Acceptable
C1A - Children and Adults, Acceptable

Biohazards:
Acceptable

Training in the Responsible Conduct of Research:
Acceptable
Comments on Format (Required):
- Acceptable
Comments on Subject Matter (Required):
- Acceptable
Comments on Faculty Participation (Required; not applicable for mid- and senior-career awards):
- Acceptable
Comments on Duration (Required):
- Acceptable
Comments on Frequency (Required):
- Acceptable

Select Agents:
Not Applicable (No Select Agents)

Resource Sharing Plans:
Unacceptable
• Not addressed

Budget and Period of Support:
Recommend as Requested

THE FOLLOWING RESUME SECTIONS WERE PREPARED BY THE SCIENTIFIC REVIEW OFFICER TO SUMMARIZE THE OUTCOME OF DISCUSSIONS OF THE REVIEW COMMITTEE ON THE FOLLOWING ISSUES:

PROTECTION OF HUMAN SUBJECTS (Resume): ACCEPTABLE

INCLUSION OF WOMEN PLAN (Resume): ACCEPTABLE, G4A

INCLUSION OF MINORITIES PLAN (Resume): ACCEPTABLE, M4A

INCLUSION OF CHILDREN PLAN (Resume): ACCEPTABLE, C4A

SCIENTIFIC REVIEW ADMINISTRATOR’S NOTES: A Resource Sharing Plan was not proposed (critique 3). The consideration of biohazards was unacceptable (critique 2). The Plan for the Training in the Responsible Conduct of Research was acceptable.

COMMITTEE BUDGET RECOMMENDATIONS: The budget was recommended as requested.

Recommended direct cost levels are estimated and are subject to further adjustment based on the Institute’s standard budget calculation practices.


The impact/priority score is calculated after discussion of an application by averaging the overall scores (1-9) given by all voting reviewers on the committee and multiplying by 10. The criterion scores are submitted prior to the meeting by the individual reviewers assigned to an application, and are not discussed specifically at the review meeting or calculated into the overall impact score. Some applications also receive a percentile ranking. For details on the review process, see http://grants.nih.gov/grants/peer_review_process.htm#scoring.
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* Temporary Member. For grant applications, temporary members may participate in the entire meeting or may review only selected applications as needed.

Consultants are required to absent themselves from the room during the review of any application if their presence would constitute or appear to constitute a conflict of interest.