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AWARD NUMBER: W81XWH-16-1-0042

TITLE: Dysregulated Signaling in Crohn's Disease

PRINCIPAL INVESTIGATOR: Dr. Kevin Haigis

RECIPIENT:
BETH ISRAEL DEACONESS MEDICAL CENTER, INC
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REPORT DATE: Sept 2019

TYPE OF REPORT: Final

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

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7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

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U.S. Army Medical Research and Materiel Command
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12. DISTRIBUTION / AVAILABILITY STATEMENT

Approved for public release; distribution is unlimited.

13. SUPPLEMENTARY NOTES

14. ABSTRACT

Crohn's disease (CD) is a chronic and debilitating inflammatory disorder of the small and large intestine. The lack of concrete molecular mechanistic insight into disease pathogenesis severely hampers the search for new and effective targeted therapies. In particular, the role that protein signal transduction cascades play in CD is not well understood. We are utilizing an approach that integrates mouse modeling, proteomics, and computational analysis to identify signaling pathways that drive the onset and progression of intestinal inflammation in mouse models. We previously identified MIP-1a and MIP-1b as potential therapeutic targets and we have begun the dose-finding studies for neutralizing antibodies that are required for the preclinical evaluation of efficacy in the T cell transfer model of colitis. We have also begun to establish experimental cohorts for the IL-2Ra and IL-10/Tlr4 mouse models of colitis so that we can perform signaling analysis. Significant progress has been made on the characterization of colitis in TNFdeltaARE animals. Here, we have completed mass spectrometry analysis of signaling, creating a novel dataset. We are performing bioinformatic analysis and we anticipate progressing to functional studies over the coming year.

15. SUBJECT TERMS
Crohn's disease, signal transduction, targeted therapies, systems biology, bioinformatics

16. SECURITY CLASSIFICATION OF:			**17. LIMITATION OF ABSTRACT**	**18. NUMBER OF PAGES**	**19a. NAME OF RESPONSIBLE PERSON**
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TABLE OF CONTENTS

1. **INTRODUCTION:** Narrative that briefly (one paragraph) describes the subject, purpose and scope of the research.

Crohn's disease (CD) is a chronic and debilitating inflammatory disorder of the small and large intestine. Few effective therapies are available to the 700,000 CD patients living in the United States. CD stems from complex combinatorial interactions between polymorphic genetic loci and environmental stimuli. Despite recent advances in our understanding of the genetics and immunology of CD, on a patient-by-patient basis there is often no clearly identifiable trigger for the disease. The lack of concrete molecular mechanistic insight into disease pathogenesis severely hampers the search for new and effective targeted therapies. In particular, the role that protein signal transduction cascades play in CD is not well understood. Our overarching goal is to understand CD-related protein signaling at a level that will allow us to identify new therapeutic strategies. To this end, we utilize an approach that integrates mouse modeling, proteomics, and computational analysis to identify signaling pathways that drive the onset and progression of intestinal inflammation in mouse models. Our working hypothesis is that genetically and environmentally diverse individuals can present with common symptoms of CD because they share a common dysregulated protein signaling state in their gastrointestinal tracts. By extension, we postulate that new therapeutic strategies will emerge from a better understanding of CD-related protein signaling. Therefore, the objectives of this study were to (1) identify the signaling pathways that are dysregulated in CD and (2) to determine which of these dysregulated pathways are pathogenic.

2. **KEYWORDS:** Provide a brief list of keywords (limit to 20 words).

Crohn's disease, inflammatory bowel disease, signal transduction, cytokines, mass spectrometry, targeted therapies

3. **ACCOMPLISHMENTS:** The PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency Grants Officer whenever there are significant changes in the project or its direction.

What were the major goals of the project?
List the major goals of the project as stated in the approved SOW. If the application listed milestones/target dates for important activities or phases of the project, identify these dates and show actual completion dates or the percentage of completion.

Aim 1. To elucidate the mechanisms linking MIP-1α and MIP-1β signaling to colitis.

Aim 2. To develop data-driven computational models of signaling in mouse models of CD.

Aim 3. To analyze the global proteome and phospho-proteome of affected tissues in mouse models of CD.

What was accomplished under these goals?

For this reporting period describe: 1) major activities; 2) specific objectives; 3) significant results or key outcomes, including major findings, developments, or conclusions (both positive and negative); and/or 4) other achievements. Include a discussion of stated goals not met. Description shall include pertinent data and graphs in sufficient detail to explain any significant results achieved. A succinct description of the methodology used shall be provided. As the project progresses to completion, the emphasis in reporting in this section should shift from reporting activities to reporting accomplishments.

Aim 1. To elucidate the mechanisms linking MIP-1α and MIP-1β signaling to colitis. Our initial goal in this project was to establish the minimum dose and dosing schedule that provides complete and persistent inhibition of MIP-1α and/or MIP-1β in the mouse colon. We treated animals with a range of doses of MIP-1α and MIP-1β neutralizing antibodies, but we were not able to reliably detect inhibition of MIP-1α/β signaling in the colon. Because our modeling analysis (see Aims 2 and 3) successfully identified kinases that are activated during colitis, we shifted the efforts of Aim 1 to studying the mechanisms of action of dysregulated kinases and and evaluating efficacy of those kinases in preclinical trials.

Aim 2. To develop data-driven computational models of signaling in mouse models of CD. The goal of this aim was to identify new therapeutic targets through computational modeling of protein signaling data (either from Luminex analysis or from mass spectrometry). In the first example of this approach, we used mass spectrometry (MS) to measure the proteome and phospho-proteome of colons from the TCT model of colitis. At the same time, we measured transcriptional changes that occur during inflammation, allowing us to determine similarities and differences among different data streams. We learned that phospho-proteomic analysis provides a dramatically different picture of the molecular pathogenesis of colitis relative to proteomics and transcriptomics and analysis of the phospho-proteome identified PAK1, as serine/threonine kinase, as a potential therapeutic target in IBD. Using a small molecule inhibitor, we found that PAK1 activity is required to maintain active colitis. This work was published in *Science Signaling* in 2018 (REF 1 below), but we chose not to continue the study of PAK1 in this context because of concerns about translational relevance. Although PAK1 inhibition is effective in a pre-clinical setting, inhibitors are associated with significant cardiotoxicity in human patients, limiting their potential usefulness.

In a second study, we identified mTOR activation in the colons of mice with colitis by applying computational modeling to Luminex-based targeted proteomic measurements. As with our PAK1 studies, we showed that inhibition of mTOR signaling with rapamycin could attenuate inflammation. We then went on to study the mechanism linking mTOR activation to colitis. We found that activated mTOR inhibits epithelial differentiation in the colon. Undifferentiated cells secrete pro-inflammatory cytokines to promote colitis. Thus, inhibition of mTOR promotes differentiation, alters the expression of inflammatory cytokines, and suppresses inflammation. This work was published *PLoS Biology* in 2018 (REF 2 below).

Extended studies. In the course of the multi-omics analysis that identified PAK1 as a therapeutic target, we noted that, although our studies of signaling in mouse models require validation in humans, it is difficult to compare omics data from humans and experimental models. As a result, we developed an informatic framework for comparing mouse and human gene expression data (REF 3 below). Although this paper is not directly related to the study of IBD, it was borne out of the computational analyses we were developing to study the mechanisms driving colitis in mice.

Aim 3. To analyze the global proteome and phospho-proteome of affected tissues in mouse models of CD. We performed global MS on colons from the TCT model of colitis and small intestines from the TNFdeltaARE model of ileitis. In the TCT model, we were able to quantify 8,696 proteins and 14,388 phosphorylation sites. In the process of analyzing our MS data, we developed a new computational approach (SKAI – Substrate-based kinase activity inference) that allows us to infer the activation of a kinase based on the level of phosphorylation of its substrates in a phospho-proteomic data set. One of the major insights revealed by this analysis is that the kinases activating during colitis in the TCT model are largely distinct from those activated during ileitis in the TNFdeltaARE model. This observation indicates that different mouse models of IBD are distinct in their molecular pathogenesis, just as human patients are likely distinct from one another.

Another insight from the MS data was the activation of MK2, a serine/threonine kinase that functions downstream of p38 MAPK. Based on this observation, we treated animals with colitis with ATI-450, an orally available small molecule inhibitor of MK2 and found that the drug dramatically reduced inflammation. Our paper reporting the development of SKAI and the identification of MK2 as a therapeutic target in IBD is now in press in *Integrative Biology* (REF 4 below). We are very optimistic about MK2 as a therapeutic target for IBD and we are continuing to study its mechanism of action. We are also evaluating whether combinatorial inhibition of MK2 and mTOR can improve outcomes in our preclinical model.

What opportunities for training and professional development has the project provided?
If the project was not intended to provide training and professional development opportunities or there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe opportunities for training and professional development provided to anyone who worked on the project or anyone who was involved in the activities supported by the project. "Training" activities are those in which individuals with advanced professional skills and experience assist others in attaining greater proficiency. Training activities may include, for example, courses or one-on-one work with a mentor. "Professional development" activities result in increased knowledge or skill in one's area of expertise and may include workshops, conferences, seminars, study groups, and individual study. Include participation in conferences, workshops, and seminars not listed under major activities.

The majority of work on this proposal was done by three members of the Haigis lab. Based on his two first author papers from this work, Dr. Lyons moved on to a senior position at a biotechnology start-up. Samantha Dale Strasser was a graduate student who developed the computational SKAI approach. She recently defended her Ph.D. thesis and is co-first author on the paper describing the approach. Phaedra Ghazi was a technician who did all of the in vivo validation for the SKAI paper. She was also a co-first author on that paper and is now in graduate school at the University of Utah.

How were the results disseminated to communities of interest?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how the results were disseminated to communities of interest. Include any outreach activities that were undertaken to reach members of communities who are not usually aware of these project activities, for the purpose of enhancing public understanding and increasing interest in learning and careers in science, technology, and the humanities.

REFERENCES

The work has resulted in several papers that are published or in the process of being published:

1. Lyons, D.K. Brubaker, P.C. Ghazi, K.R. Baldwin, A. Edwards, M. Boukhali, S.D. Strasser, L. Suarez-Lopez, Y-J Lin, V. Yajnik, J.L. Kissil, W. Haas, D.A. Lauffenburger, K.M. Haigis. 2018. Integrated *in vivo* multi-omics analysis identifies p21-activated kinase signaling as a driver of colitis. *Sci. Signal.* 11: eaan3580. PMID: 29487189.

2. J. Lyons, P.C. Ghazi, A. Starchenko, A. Tovaglieri, K.R. Baldwin, E.J. Poulin, J.J. Gierut, C. Genetti, V. Yajnik, D.T. Breault, D.A. Lauffenburger, K.M. Haigis. 2018. The colonic epithelium plays an active role in promoting colitis by shaping the tissue cytokine profile. *PLoS Biol.* 16: e2002417. PMID: 29596476.

3. D.K. Brubaker, E.A. Proctor, K.M. Haigis, D.A. Lauffenburger. 2019. Computational translation of genomic responses from experimental model systems to humans. *PLoS Comp. Biol.* 15: e1006286. PMID: 30629591.

4. S.D. Strasser, A. Starchenko, J. Lyons, P.C. Ghazi, M. Boukhali, A. Edwards, D.K. Brubaker, B.A. Joughin, W. Haas, D.A. Lauffenburger, K.M. Haigis. 2019. Substrate-based kinase activity inference: interpreting phosphoproteomic data using computational enrichment analysis. *Int. Biol.* In press.

What do you plan to do during the next reporting period to accomplish the goals?
If this is the final report, state "Nothing to Report."

Describe briefly what you plan to do during the next reporting period to accomplish the goals and objectives.

Nothing to Report.

4. **IMPACT:** Describe distinctive contributions, major accomplishments, innovations, successes, or any change in practice or behavior that has come about as a result of the project relative to:

What was the impact on the development of the principal discipline(s) of the project?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how findings, results, techniques that were developed or extended, or other products from the project made an impact or are likely to make an impact on the base of knowledge, theory, and research in the principal disciplinary field(s) of the project. Summarize using language that an intelligent lay audience can understand (Scientific American style).

Our identification of three different therapeutic targets in mouse models of colitis – mTOR, Pak1, and Mk2 – opens the door for exploring how broadly they are also activated in the human disease. With this knowledge in-hand, we will be able to design clinical trials, as clinically relevant drugs are available for all three kinases. In the case of mTOR, we also identified a novel cellular mechanism – inhibition of differentiation – that plays a key role in the maintenance of chronic inflammation.

Beyond the therapeutic implications of our work, we have also developed a computational algorithm that allows us to infer kinase activation from global phospho-proteomic data. Although we used this approach to analyze our data from mouse models of colitis, it is adaptable to mass spectrometry data from any experimental system. We believe that it will be extremely useful to the broad scientific community.

What was the impact on other disciplines?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how the findings, results, or techniques that were developed or improved, or other products from the project made an impact or are likely to make an impact on other disciplines.

Nothing to Report.

What was the impact on technology transfer?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe ways in which the project made an impact, or is likely to make an impact, on commercial technology or public use, including:

- *transfer of results to entities in government or industry;*
- *instances where the research has led to the initiation of a start-up company; or*
- *adoption of new practices.*

Nothing to Report.

What was the impact on society beyond science and technology?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how results from the project made an impact, or are likely to make an impact, beyond the bounds of science, engineering, and the academic world on areas such as:

- *improving public knowledge, attitudes, skills, and abilities;*
- *changing behavior, practices, decision making, policies (including regulatory policies), or social actions; or*
- *improving social, economic, civic, or environmental conditions.*

Nothing to Report.

5. **CHANGES/PROBLEMS:** The Project Director/Principal Investigator (PD/PI) is reminded that the recipient organization is required to obtain prior written approval from the awarding agency Grants Officer whenever there are significant changes in the project or its direction. If not previously reported in writing, provide the following additional information or state, "Nothing to Report," if applicable:
6.

Nothing to Report.

Changes in approach and reasons for change
Describe any changes in approach during the reporting period and reasons for these changes. Remember that significant changes in objectives and scope require prior approval of the agency.

Nothing to Report.

Actual or anticipated problems or delays and actions or plans to resolve them
Describe problems or delays encountered during the reporting period and actions or plans to resolve them.

Nothing to Report.

Changes that had a significant impact on expenditures
Describe changes during the reporting period that may have had a significant impact on expenditures, for example, delays in hiring staff or favorable developments that enable meeting objectives at less cost than anticipated.

Nothing to Report.

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents
Describe significant deviations, unexpected outcomes, or changes in approved protocols for the use or care of human subjects, vertebrate animals, biohazards, and/or select agents during the reporting period. If required, were these changes approved by the applicable institution committee (or equivalent) and reported to the agency? Also specify the applicable Institutional Review Board/Institutional Animal Care and Use Committee approval dates.

Significant changes in use or care of human subjects

Nothing to Report.

Significant changes in use or care of vertebrate animals.

Nothing to Report.

Significant changes in use of biohazards and/or select agents

Nothing to Report.

7. PRODUCTS: List any products resulting from the project during the reporting period. If there is nothing to report under a particular item, state "Nothing to Report."

- **Publications, conference papers, and presentations**
  Report only the major publication(s) resulting from the work under this award.

  Journal publications. *List peer-reviewed articles or papers appearing in scientific, technical, or professional journals. Identify for each publication: Author(s); title; journal; volume: year; page numbers; status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

1. Lyons, D.K. Brubaker, P.C. Ghazi, K.R. Baldwin, A. Edwards, M. Boukhali, S.D. Strasser, L. Suarez-Lopez, Y-J Lin, V. Yajnik, J.L. Kissil, W. Haas, D.A. Lauffenburger, K.M. Haigis. 2018. Integrated *in vivo* multi-omics analysis identifies p21-activated kinase signaling as a driver of colitis. *Sci. Signal.* 11: eaan3580. PMID: 29487189.

2. J. Lyons, P.C. Ghazi, A. Starchenko, A. Tovaglieri, K.R. Baldwin, E.J. Poulin, J.J. Gierut, C. Genetti, V. Yajnik, D.T. Breault, D.A. Lauffenburger, K.M. Haigis. 2018. The colonic epithelium plays an active role in promoting colitis by shaping the tissue cytokine profile. *PLoS Biol.* 16: e2002417. PMID: 29596476.

3. D.K. Brubaker, E.A. Proctor, K.M. Haigis, D.A. Lauffenburger. 2019. Computational translation of genomic responses from experimental model systems to humans. *PLoS Comp. Biol.* 15: e1006286. PMID: 30629591.

4. S.D. Strasser, A. Starchenko, J. Lyons, P.C. Ghazi, M. Boukhali, A. Edwards, D.K. Brubaker, B.A. Joughin, W. Haas, D.A. Lauffenburger, K.M. Haigis. 2019. Substrate-based kinase activity inference: interpreting phosphoproteomic data using computational enrichment analysis. *Int. Biol.* In press.

Books or other non-periodical, one-time publications. *Report any book, monograph, dissertation, abstract, or the like published as or in a separate publication, rather than a periodical or series. Include any significant publication in the proceedings of a one-time conference or in the report of a one-time study, commission, or the like. Identify for each one-time publication: Author(s); title; editor; title of collection, if applicable; bibliographic information; year; type of publication (e.g., book, thesis or dissertation); status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

Nothing to Report.

Other publications, conference papers, and presentations. *Identify any other publications, conference papers and/or presentations not reported above. Specify the status of the publication as noted above. List presentations made during the last year (international, national, local societies, military meetings, etc.). Use an asterisk (*) if presentation produced a manuscript.*

Nothing to Report.

- **Website(s) or other Internet site(s)**
  List the URL for any Internet site(s) that disseminates the results of the research activities. A short description of each site should be provided. It is not necessary to include the publications already specified above in this section.

Nothing to Report.

- **Technologies or techniques**
  Identify technologies or techniques that resulted from the research activities. In addition to a description of the technologies or techniques, describe how they will be shared.

Nothing to Report.

- **Inventions, patent applications, and/or licenses**
  Identify inventions, patent applications with date, and/or licenses that have resulted from the research. State whether an application is provisional or non-provisional and indicate the application number. Submission of this information as part of an interim research performance progress report is not a substitute for any other invention reporting required under the terms and conditions of an award.

Nothing to Report.

- **Other Products**
  Identify any other reportable outcomes that were developed under this project. Reportable outcomes are defined as a research result that is or relates to a product, scientific advance, or research tool that makes a meaningful contribution toward the understanding, prevention, diagnosis, prognosis, treatment, and/or rehabilitation of a disease, injury or condition, or to improve the quality of life. Examples include:
  - *data or databases;*
  - *biospecimen collections;*
  - *audio or video products;*
  - *software;*
  - *models;*
  - *educational aids or curricula;*
  - *instruments or equipment;*
  - *research material (e.g., Germplasm; cell lines, DNA probes, animal models);*
  - *clinical interventions;*
  - *new business creation; and*
  - *other.*

Nothing to Report.

8. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?
Provide the following information for: (1) PDs/PIs; and (2) each person who has worked at least one person month per year on the project during the reporting period, regardless of the source of compensation (a person month equals approximately 160 hours of effort). If information is unchanged from a previous submission, provide the name only and indicate "no change."

Example:

Name:	*Mary Smith*
Project Role:	*Graduate Student*
Researcher Identifier (e.g. ORCID ID):	*1234567*
Nearest person month worked:	*5*
Contribution to Project:	*Ms. Smith has performed work in the area of combined error-control and constrained coding.*
Funding Support:	*The Ford Foundation (Complete only if the funding support is provided from other than this award).*

No change.

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

If the active support has changed for the PD/PI(s) or senior/key personnel, then describe what the change has been. Changes may occur, for example, if a previously active grant has closed and/or if a previously pending grant is now active. Annotate this information so it is clear what has changed from the previous submission. Submission of other support information is not necessary for pending changes or for changes in the level of effort for active support reported previously. The awarding agency may require prior written approval if a change in active other support significantly impacts the effort on the project that is the subject of the project report.

Nothing to report.

What other organizations were involved as partners?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe partner organizations – academic institutions, other nonprofits, industrial or commercial firms, state or local governments, schools or school systems, or other organizations (foreign or domestic) – that were involved with the project. Partner organizations may have provided financial or in-kind support, supplied facilities or equipment, collaborated in the research, exchanged personnel, or otherwise contributed.
Provide the following information for each partnership:
Organization Name:
Location of Organization: (if foreign location list country)

Partner's contribution to the project (identify one or more)

- *Financial support;*
- *In-kind support (e.g., partner makes software, computers, equipment, etc., available to project staff);*
- *Facilities (e.g., project staff use the partner's facilities for project activities);*
- *Collaboration (e.g., partner's staff work with project staff on the project);*
- *Personnel exchanges (e.g., project staff and/or partner's staff use each other's facilities, work at each other's site); and*
- *Other.*

Nothing to report.

9. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS: For collaborative awards, independent reports are required from BOTH the Initiating PI and the Collaborating/Partnering PI. A duplicative report is acceptable; however, tasks shall be clearly marked with the responsible PI and research site. A report shall be submitted to **https://ers.amedd.army.mil** for each unique award.

QUAD CHARTS: If applicable, the Quad Chart (available on https://www.usamraa.army.mil) should be updated and submitted with attachments.

10. APPENDICES: Attach all appendices that contain information that supplements, clarifies or supports the text. Examples include original copies of journal articles, reprints of manuscripts and abstracts, a curriculum vitae, patent applications, study questionnaires, and surveys, etc.