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TITLE: Alternative RNA splicing of CSF3R in Promoting Myelodysplastic Syndromes

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RECIPIENT: Virginia Commonwealth University – Massey Cancer Center

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Dr. Seth Corey

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13. SUPPLEMENTARY NOTES

14. ABSTRACT
More effective therapies for myelomdysplastic syndromes (MDS) can be developed if we know more about how the disease develops. One of the most exciting advances has been the identification of mutations in genes encoding splicing factors. These occur in 50 - 70% of all adult patients with MDS. These proteins acts as a machine to process instructions (messenger RNA) that lead to the production of a specific protein. We have identified that the receptor for the most important growth factor for the production of granulocytes (the white blood cells most affected in MDS) is subject to splicing. These splicing changes result in a defective receptor, which fails to instruct blood cells to mature. We have developed a test to identify which specific splicing factor is involved in processing the messenger RNA for this receptor. We are identifying that specific splicing factor and whether there is any required post-translational modification of the splicing factor. This knowledge will inform us on how MDS begins and how to interrupt its development and progression to leukemia. Also, we have found that this defective receptor results in too much growth and too little differentiation. We have identified that splicing factors such as U2AF1 and post-translational modification involving phosphorylation contribute to processing of the message for the granulocyte colony stimulating factor receptor. SRSF2 may also play a role in regulating CSF3R. We are developing a mouse model that will allow us to describe in greater, more accurate detail the molecular changes and cell behaviors due to that defective receptor. Our work could allow us to screen for drugs that correct the MDS condition by correcting the faulty splicing and may advance the use of the receptor as a clinical laboratory tool.

15. SUBJECT TERMS
Splicing factor, myelodysplastic syndromes, CSF3R

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.

   A major distinguishing feature of myelodysplastic syndromes (MDS), the most common form of acquired bone marrow failure, is the presence of recurrent mutations in one of the genes encoding a component of the splicing machinery. These mutations are found in 50-85% of individuals with MDS. However, little is known of their impact on normal and abnormal hematopoiesis. Our lab studies the signal transduction of Granulocyte Colony Stimulating Factor Receptor (GCSFR, the gene is *CSF3R*). The alternative splicing of *CSF3R*, which is associated with MDS, provides a robust model to reveal the mechanisms by which aberrant splicing promotes myelodysplasia and determine cell fate.

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

   Splicing factor, myelodysplastic syndromes, CSF3R

3. **ACCOMPLISHMENTS:** The PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official 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.

   Specific Aim 1. Determine the splicing mechanism involved in processing the *CSF3R* gene into transcripts encoding a full-length GCSFR and a truncation, differentiation-impaired GCSFR. We will construct a minigene reporter cassette and test the predicted mechanisms. We will determine which signaling pathways promote intron retention and permit expression of full-length GCSFR so to target this step pharmacologically.

   Specific Aim 2. Fully characterize the aberrant proximal phosphoprotein and distal gene regulatory networks and correlate with an in vivo model of a truncated GCSFR. We will compare the signaling and gene expression profiles in murine and human CD34+ hematopoietic stem cells and correlate phenotypically with a retroviral transduction/transplantation model by expressing alternative splice form. in the context of *Csf3r-/-* mice.

   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.

1. Major activities: We have interrogated all the splicing factors known to be recurrently mutated in myelodysplastic syndromes by studying the wild-type and mutated forms co-expressed with the CSF3R minigene. We have also studied these relationships in the context of post-translational modifications.

2. Specific objectives: Determine which splicing factor and which post-translational modification, if any, affects the alternative splicing of *CSF3R*, which results in a pro-proliferative, differentiated-impaired granulocyte colony stimulating factor receptor.

3. Significant results: First we studied aberrant splicing of *CSF3R* in non-hematopoietic cell lines. We transfected 293FT cells with *CSF3R* minigene and either wildtype *SF3B1* or mutant K700E and wildtype *U2AF1* or mutant S34F have shown that *SF3B1* mutant K700E enhances *CSF3R* splicing that results in increased Class IV transcript levels while *U2AF1* mutant S34F inhibits splicing that results in decreased Class IV transcript levels compared to wildtype.

   Next, we studied aberrant splicing of *CSF3R* in hematopoietic tissues. Exogenous expression of *CSF3R* minigene and *U2AF1* S34F in K562 cells showed significantly decreased Class IV:I compared to wildtype *U2AF1*. In contrast, K562 cells over-expressing *SRSF2* P95H showed significantly increased Class IV:I compared to wildtype *SRSF2*. Exogenous expression of *SF3B1* K700E in K562 cells produced in an additional PCR band that likely resulted from an alternate 3' splice site.

   Using shRNA, we performed knockdown of *LUC7L2* in K562 and U937 and found no differences in Class IV:I compared to the controls; however, we are identifying differential gene expression patterns involved in proliferation and hematopoiesis in U937, HL60, and NB4 cells. To further validate our findings, we created *LUC7L2*-deficient cells in HL60 and K562 using CRISPR/Cas9 gene editing. We have confirmed protein loss of LUC7L2 in HL60 and K562 cells, and are now proceeding with analysis of *CSF3R* and RNA-Seq. We are also developing the CSF3R Class IV mouse.

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.

A doctoral student Ann Wang is performing this study under the PI's supervision. Ms Wang has participated in seminars, scientific integrity courses, RNA biology coursework, and presents in

cancer biology department seminars for trainees and journal clubs. She participates in three weekly lab meeting for Drs. Corey, Maciewjewski, and Padgett.

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.

Publications:
1. Wang B, Mehta HM. Cytokine receptor splice variants in hematologic diseases. **Cytokine**. 2020 Mar;127:154919. doi: 10.1016/j.cyto.2019.154919. Epub 2019 Dec 6. PMID: 31816579
2. Garg B, Mehta HM, Wang B, Kamel R, Horwitz M, Corey SJ. Inducible expression of a disease-associated ELANE mutation impairs granulocytic differentiation without eliciting an unfolded protein response. **J Biol Chem**. 2020 295(21):7492-7500. PMID:32299910

Presentations:
1. MDS-Associated Splicing Factor Mutations Promote Alternative Splicing of *CSF3R*" at the 2019 Splicing Factor Mutations and RNA Biology in Cancer Workshop at Yale University (New Haven, CT).
2 Low LUC7L2 Levels Decrease Expression of Genes Implicated in Myeloid Neoplasia Associated with -7/del(7q). for the American Society of Hematology Annual Meeting in December 2019 in Orlando, FL.
3. MDS-Associated Splicing Factor Mutations Promote Alternative Splicing of CSF3R. Cleveland Clinic Lerner Research Institute Research Day. November 2019

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.

N/A.

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).

We have tentatively identified U2AF1 and tyrosine phosphorylation as a splicing factor and post-translational modification that regulate the processing of the CSF3R transcript. We have also identified possible role for SRSF2. These data may identify pathways for therapeutic targeting in MDS.

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.

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 PD/PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official 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:

   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.

   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.

   None

   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.

   None

   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

   None

   Significant changes in use or care of vertebrate animals

   None

Significant changes in use of biohazards and/or select agents
None

6. 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. Austin F, Oyarbide U, Massey G, Grimes M, Corey SJ. Synonymous mutation in TP53 results in a cryptic splice site affecting its DNA-binding site in an adolescent with two primary sarcomas. **Pediatr Blood Cancer** 2017; 64(11). doi: 10.1002/pbc.26584.
  2. Wang B, Mehta HM. Cytokine receptor splice variants in hematologic diseases. **Cytokine**. 2020 Mar;127:154919. doi: 10.1016/j.cyto.2019.154919. Epub 2019 Dec 6. PMID: 31816579
  3. Garg B, Mehta HM, Wang B, Kamel R, Horwitz M, Corey SJ. Inducible expression of a disease-associated ELANE mutation impairs granulocytic differentiation without eliciting an unfolded protein response. **J Biol Chem** 2020 295 (21), 7492-7500. PMID:32299910

  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).*

  N/A

  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.*

  N/A

- **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.

N/A

- **Technologies or techniques**
  Identify technologies or techniques that resulted from the research activities. Describe the technologies or techniques were shared.

  N/A

  Inventions, patent applications, and/or licenses
  Identify inventions, patent applications with date, and/or licenses that have resulted from the research. 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.

  None

  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;*
  - *physical 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.*

N/A

7. 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 com

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

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.

Organization Name: Cleveland Clinic
Location of Organization: (if foreign location list country), Cleveland OH
Partner's contribution to the project (identify one or more)

- *Financial support;* start-up support for Dr Corey
- *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);* collaboration with Drs. Richard Padgett and Jaroslaw Maciejewski.
- *Personnel exchanges (e.g., project staff and/or partner's staff use each other's facilities, work at each other's site); and*
- *Other:* access to bioinformatic data on RNA-Seq.

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS: For collaborative awards, independent reports are required from BOTH the Initiating Principal Investigator (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.

9. 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.

1. Wang B, Mehta HM. Cytokine receptor splice variants in hematologic diseases. **Cytokine**. 2020 Mar;127:154919. doi: 10.1016/j.cyto.2019.154919. Epub 2019 Dec 6. PMID: 31816579
2. Garg B, Mehta HM, Wang B, Kamel R, Horwitz M, Corey SJ. Inducible expression of a disease-associated ELANE mutation impairs granulocytic differentiation without eliciting an unfolded protein response. **J Biol Chem**, 2020 295 (21), 7492-7500. PMID:32299910