AWARD NUMBER: W81XWH-20-1-0088

TITLE:
Pursuing the Therapeutic Potential of the SUMOylation System by Characterizing the Mechanisms That Regulate SUMO Levels in the Cell

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REPORT DATE:
March 2021

TYPE OF REPORT:
Annual Report

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

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	**2. REPORT TYPE**	**3. DATES COVERED**
March 2021	Annual	01Feb2020-31Jan2021

4. TITLE AND SUBTITLE

Pursuing the Therapeutic Potential of the SUMOylation System by Characterizing the Mechanisms That Regulate SUMO Levels in the Cell

5a. CONTRACT NUMBER
W81XWH-20-1-0088

5b. GRANT NUMBER

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)

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5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

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

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8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)

U.S. Army Medical Research and Development Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT

Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

The SUMOylation system, a type of protein regulatory system present in all cells, is rapidly increased upon exposure to different types of stress, including influenza virus infection. For influenza, if the increase is large enough, the virus can't multiply and infection is blocked. The goal of this study is to determine whether a process known as alternative splicing, which produces small but meaningful changes in the templates used to make proteins, plays a central role in regulating the activity of the SUMOylation system. To explore this possibility, we proposed to measure the abundance of the different templates that code for the SUMO proteins (the central players in the SUMOylation system) under different types of stress conditions. We also proposed to study how changing the proportion of those templates affects the ability of the SUMOylation system to respond to stress and characterize the activity of new forms of the SUMO proteins that up to now had remained unknown.
Although the activities proposed for the first year of this grant were greatly affected by the SARS-CoV-2 pandemic, we made some progress on the development of some of the constructs proposed for achieving some of the experimental goals.

15. SUBJECT TERMS

SUMO, SUMOylation, alternative splicing, variants, isoforms, qRT-PCR.

16. SECURITY CLASSIFICATION OF:			**17. LIMITATION OF ABSTRACT**	**18. NUMBER OF PAGES**	**19a. NAME OF RESPONSIBLE PERSON**
a. REPORT	**b. ABSTRACT**	**c. THIS PAGE**			USAMRMC
Unclassified	Unclassified	Unclassified	Unclassified	5	**19b. TELEPHONE NUMBER** *(include area code)*

TABLE OF CONTENTS

1. Introduction

Post-translational modifications are changes affecting the chemical composition of proteins after being synthesized within the cell. Post-translational modifications play essential roles in the cell, altering protein activitiy, protein localization, and protein turn-over. SUMOylation, the covalent attachment of a SUMO protein to a target protein, is now well known to play an essential regulatory role within the cell. Importantly, cellular stress, as the one triggered by exposure to high-temperature, cold-shock, and viral infection, triggers massive increases in overall SUMO conjugation to cellular proteins. This event, referred to as a Global Increase in Cellular SUMOylation or GICS, is known to exert a protective effect. Our group previously demonstrated that large increases in SUMOylation block influenza virus replication, therefore stopping the viral life cycle.

In spite its relevance, the mechanisms that regulate the rapid increases in cellular SUMOylation triggered by stress remain mostly unknown. The ***main goal*** pursued by these studies is to achieve an initial characterization of the molecular mechanisms regulating the cellular levels and activity of the three main SUMO modifiers in the cell, SUMO1-3. Our ***hypothesis*** is that the levels of these modifiers and the degree to which they are conjugated to their cellular targets are deeply affected by the alternative splicing of their primary transcripts and post-translational feedback mediated by the SUMO proteins encoded by the alternatively spliced variants, hereafter referred to as the SUMOalpha isoforms. To achieve our main goal, we will pursue ***two specific aims***: **i)** ***Characterize the contribution of alternative splicing to the regulation of SUMO levels in the cell***; and, ***ii) Characterize the functional properties of the SUMOalpha isoforms***.

For our first year of research, we aimed to pursue the following specific aims:

A. Characterize the contribution of alternative splicing in the regulation of cellular SUMO levels

A.1. Quantify mRNA levels for all SUMO1-3 variants under normalcy and stress conditions.

A.2. Design and implement the use of siRNAs and morpholinos to decrease and increase the cellular levels of specific SUMO variants, respectively .

B. Characterize the functional properties of the SUMOalpha isoforms.

B.1. Develop all major tools needed for this aim, including plasmid clones needed for expression of the normal SUMOs and SUMOalpha isoforms in mammalian and bacterial vectors, and purification of the proteins expressed in bacterial systems.

2. Keywords

SUMO, SUMOylation, alternative splicing, variants, isoforms, qRT-PCR.

3. Accomplishments

The SARS-CoV-2 pandemic had a dramatic effect in the El Paso region and, as a consequence, access to our research laboratories by our students and research personnel was substantially restricted starting on March 2020. This has drastically limited our ability to perform the experiments that were proposed for this first year of the grant. Nevertheless, some accomplishments were achieved as listed and described below. To facilitate their assessment, they are listed in relation to the specific aims initially proposed for year 1, as previously presented above.

A. Characterize the contribution of alternative splicing in the regulation of cellular SUMO levels

A.1. Quantify mRNA levels for all SUMO1-3 variants under normalcy and stress conditions.

The experiments for quantifying the SUMO3 variants under normalcy, heat shock, and influenza infection have already been performed. The data analysis is currently in progress as the constructs required to generate the regression curve required to calculate the copy number of the different variants are still being developed.

A.2. Design and implement the use of siRNAs and morpholinos to decrease and increase the cellular levels of specific SUMO variants, respectively.

All morpholinos required have been designed. However, only some of them have been tested in tissue culture. Nevertheless, the data obtained so far indicates that our approach allows the specific and efficient alteration in splicing events that were expected.

B. Characterize the functional properties of the SUMOalpha isoforms.

B.1. Develop all major tools needed for this aim, including plasmid clones needed for expression of the normal SUMOs and SUMOalpha isoforms in mammalian and bacterial vectors, and purification of the proteins expressed in bacterial systems.

All the primers required for the development of the missing plasmids have been designed and some of them have been ordered and implemented in the development of the recombinant plasmids needed. However, there are still a few plasmids needed, most of which are currently being developed. Nevertheless, the PCR conditions needed for the specific amplification of the desired sequences have already been standardized.

4. Impact
This project challenges the current dogma of how global cellular SUMOylation levels are regulated and introduces a new paradigm in which alternative splicing and the protein isoforms produced by this process, the SUMOalpha isoforms, play key roles in the regulation of the cellular levels and activity of the SUMO proteins. Furthermore, the experiments currently under way will provide essential data on an untapped area of SUMO research, assess the potential use of siRNAs and morpholinos as tools to regulate global cellular SUMOylation levels, and could lead to the development of innovative therapeutic and prophylactic approaches to combat influenza viral infections and improve patient outcomes following ischemic events.

5. Changes/Problems
The main challenged we faced during our first year of grant support was the limited access to our research areas imposed by the SARS-CoV-2 pandemic. This greatly affected our ability to perform most of the tasks initially planned for the first year of grant support. Therefore, we will be requesting a no-cost extension to have the time required to perform all the experiments initially proposed and accomplish our research goals.

6. Products
There are no products to report.

7. Participants & Other Collaborating Organizations

Name:	Myriah Acuña
Project Role:	Graduate Student/Research Associate
Nearest person month worked:	4
Contribution to project:	Ms. Acuña has performed most of the qRT-PCR analyses related to this grant.
Funding Support:	Teaching Assistantship and current grant.

Name:	Alejandra Flores
Project Role:	Undergraduate Student
Nearest person month worked:	4
Contribution to project:	Ms. Flores has been involved in the development of some of the plasmids needed for expression of the SUMO isoforms in mammalian and bacterial cells.
Funding Support:	MARC program at UTEP

Name:	Andrea Garcia
Project Role:	Undergraduate Student
Nearest person month worked:	4
Contribution to project:	Ms. Garcia has played a key role in maintaining our mammalian cell lines, standardizing the stress conditions, performing the stress experiments, and collecting the stressed cells and purifying the RNA to be used in qRT-PCR analyses. Ms. Garcia has also been involved in developing the constructs required to generate the standard curves used for quantitative analyses of the qRT-PCR data generated.
Funding Support:	SURPASS program at UTEP.

There are no other organizations to report.

8. Special Reporting Requirements
None**.**

9. Appendices
None**.**