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PRINCIPAL INVESTIGATOR: Jingsong Zhou

CONTRACTING ORGANIZATION: University of Texas at Arlington, Arlington, TX

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Jingsong Zhou

E-Mail: jingsong.zhou@uta.edu

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University of Texas at Arlington
701 S. Nedderman Drive, Box 19145
Arlington, TX 76019-0145

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14. ABSTRACT

ALS is a fatal neuromuscular disease. Failure of the respiratory muscle is a main cause of mortality in ALS patients. Defects in neuromuscular junctions (NMJs) and progressive NMJ loss occur at early stages, thus stabilizing and preserving NMJs represents a potential therapeutic strategy to slow ALS disease progression. This project is aim to explore a potential role of a muscle-derived protein MG53 in maintaining the NMJ and diaphragm muscle membrane integrity, and to establish the efficacy and safety for using recombinant human MG53 protein (rhMG53) to treat ALS in the mouse model. Since MG53 is already present in human blood circulation under normal physiologic conditions, therapeutic approach with modulation of MG53 function or systemic administration of rhMG53 can potentially be a safe biologic reagent for treatment of ALS.

15. SUBJECT TERMS

None listed.

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

ALS is a fatal neuromuscular disease. Failure of the respiratory muscle is a main cause of mortality in ALS patients. Defects in neuromuscular junctions (NMJs) and progressive NMJ loss occur at early stages, thus stabilizing and preserving NMJs represents a potential therapeutic strategy to slow ALS disease progression. While mitochondria-mediated reactive oxidative species (ROS) links to the etiology of ALS, the mechanisms that underlie NMJ degeneration in ALS are largely unknown. During respiration, the diaphragm constantly undergoes contraction-relaxation, a process that leads to injury to the muscle membrane. Inadequate repair of injury to the sarcolemma can disrupt NMJ integrity and contribute to diaphragm wasting in ALS. MG53 is an endogenous protein in human body that serves essential roles in nucleating assembly of repair patches at membrane injury sites. Genetic ablation of MG53 results in defective membrane repair and tissue regenerative capacity. A series of studies have shown that recombinant human MG53 (rhMG53) protein protects various cell types against membrane disruption when applied to the extracellular environment in animal models. We ***hypothesize that MG53-mediated membrane repair contributes to maintenance of NMJ integrity in ALS***. Since NMJ is an active site of neuron/muscle crosstalk, we postulate that membrane repair defects originate from NMJ. A vicious cycle of mitochondrial dysfunction/membrane repair defects leads to increased vulnerability of NMJ to stress-induced injury as part of ALS pathology. Thus, this project has two specific aims: (1) To elucidate the physiological role of MG53-mediated membrane repair in ALS. Specifically, the SOD1G93A mice will be cross-bred with MG53 knockout and ctPA-MG53 mice to evaluate MG53's physiological role in regulating the degeneration of NMJ associated with ALS progression. These studies will test whether elevated level of MG53 in circulation has protective role for NMJ integrity in ALS. (2) To conduct proof-of-concept study testing rhMG53 as a novel therapeutic means for improving NMJ integrity to treat ALS. We propose to establish the efficacy and safety for using rhMG53 protein to treat ALS in the mouse model. Since MG53 is already present in blood circulation under normal physiologic conditions, therapeutic approach with modulation of MG53 function or systemic administration of rhMG53 can potentially be a safe biologic reagent for treatment of ALS.

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

ALS (Amyotrophic Lateral Sclerosis), NMJ (neuromuscular junction), MG53, rhMG53 (recombinant human MG53), Mitochondria, Skeletal muscle

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.

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.

Major activities, specific objectives, significant results

Aim 1: Elucidate the physiological role of MG53-mediated membrane repair in ALS.

Subtask 1.1: Cross-breeding of *SOD1*^{G93A} mice with *mg53-/-* and ctPA-MG53 mice (1-18 months)
Subtask 1.2: Dissect the role of MG53 in preservation of muscle and NMJ integrity in ALS (6-18 months)
Subtask 1.3: Dissect the function of MG53 in maintenance of mitochondria function in ALS (12-24 months)

Milestones:
1) Physiologic role of MG53 in ALS established. (Completed)
2) ALS mice with knockout of MG53 show exacerbated diaphragm muscle injuries. (Ongoing and partially completed)
3) Elevated MG53 in circulation preserves NMJ integrity in ALS mice. (Completed)

Back-cross the *mg53-/-* and ctPA-MG53 mice (with the genetic background of C57) to the same gene background (B6SJL) of the ALS SOD1^{G93A} mice used in our lab: As stated in the previous Annual Technical Report, Dr. Zhou's lab was relocated from Kansas City University (KCU) to UTA in July 2018. The DOD grant was relinquished from KCU to UTA. The fund started in March 2019. Following the MTA (Material Transfer Agreement between the Ohio State University (OSU) and UTA) signed on Feb 11, 2019, the *mg53-/-* and ctPA-MG53 mice were transferred to UTA in March 2019. After the quarantine and foster breading, we initiated the process of back-crossing those mouse models to the genetic background (B6SJL), which is the genetic background of our G93A mouse model. The Covid-situation had a large impact on our progress since early 2020, however, we managed to complete cross-breeding of 5 generations to change the gene background. In our previous Annual Technical Report submitted on March 03, 2021, we reported that we had completed 3rd round of back-crossing. The below is the data summary of the cross-breeding *mg53-/-* and ctPA-MG53 mice with WT (B6SJL) and G93A mice up to date.

A. ctPA-MG53 back-crossing with B6SJL mice

1. 2nd backcrossing with WT (B6SJL)

Litters/Date of Birth	ctPA-MG53^{+}	ctPA-MG53^{-}
1. DOB 1/27/2020	Male: 1; Female: 4	Male: 1; Female: 1
2. DOB 2/18/2020	Male: 1; Female: 1	Male: 1; Female: 3
3. DOB 3/20/2020	Male: 3; Female: 2	Male: 5; Female: 0

2. 3rd backcrossing with WT (B6SJL)

Litters/Date of Birth	ctPA-MG53^{+}	ctPA-MG53^{-}
DOB 8/27/2020	Male: 3; Female: 0	Male: 1; Female: 2

3. 4th backcrossing with WT/G93A (B6SJL):

Litters/Date of Birth	ctPA-MG53^{+}	ctPA-MG53^{-}
1. DOB 11/17/2020	Male: 2; Female: 2	Male: 3; Female: 1
2. DOB 1/25/2021	Male: 2; Female: 1	Male: 3; Female: 1
3. DOB 1/27/2021	Male: 1; Female:2	Male: 5; Female: 2
4. DOB 2/26/2021	Male: 2; Female: 1	Male: 5; Female: 0
5. DOB 5/4/2021	Male: 1; Female: 0	Male: 2; Female: 0

4. 5th backcrossing with WT/G93A (B6SJL):

Litters/Date of Birth	ctPA-MG53^{+}	ctPA-MG53^{-}
1. DOB 6/19/2021	Male: 4; Female: 1	Male: 2; Female: 2
2. DOB 7/29/2021	Male: 4; Female: 2	Male: 3; Female: 3
3. DOB 7/30/2021	Male: 2; Female: 0	Male: 1; Female: 1
4. DOB 8/13/2021	Male: 4; Female: 2	Male: 3; Female: 1
5. DOB 8/17/2021	Male: 1; Female: 3	Male: 3; Female: 1
6. DOB 9/9/2021	To be genotyped	To be genotyped

B. Life span comparation between ctPA-MG53-G93A and G93A mice

From the 4th backcrossing, we also paired ctPA-MG53 mice with G93A mice, which also had the B6SJL background, to produce ctPA-MG53-G93A and the G93A littermates. Currently we have bred 8 liters and obtained 35 G93A and ctPA-MG53-G93A littermates, among which 13 mice have reached their end of life (EOL), and 21 mice (recently bred) are still alive.

Geno	Up to 9/25/21	Reached EOL	Mean life span(days)	SD
G93A_male	14	7	128.3	9.9
ctPA-MG53-G93A _male	8	3	124.3	2.5
G93A_female	3	0		
ctPA-MG53-G93A_female	10	3	138.0	7.0

C. The life span of untreated G93A (B6SJL) mice in our colony

The mice listed in Table B are the mice after 5th back-crossing, their genetic background should be close to our untreated G93A mice colony. To compare the back-crossed G93A and ctPA-MG53-

G93A mice with our untreated G93A mice (with pure B6SJL genetic background), we have also examined the life span of our untreated G93A mice.

Male	Survival days		Female	Survival days	Combined survival days
1	162		1	135	
2	132		2	124	
3	128		3	124	
4	130		4	129	
5	120		5	145	
6	134		6	151	
7	116		7	135	
8	132		8	150	
9	133		9	148	
10	113		10	139	
11	129		11	150	
12	130		12	146	
13	131		13	148	
14	135				
mean	**130**			**140**	**135 (male+female)**
SD	**11**			**10**	**11**

Discussion of the current results of the life span (**B/C**):

1) The life span of the ctPA-MG53-G93A male mice (n=3) has no significant difference comparing with the male G93A mice (n=7).
2) While the 3 female ctPA-MG53-G93A mice show a mean life span of 138 days, the 3 G93A female mice from the cross-breeding have not reached the EOL yet. However, it is known that female G93A mice have a longer life span than the male G93A mice. Based on our own data, the female G93A mice in our colony have a mean life span of 140 days. Thus, we could predict that the ctPA-MG53-G93A female mice will have a similar life span as cross-bred G93A female mice.
3) Based on current data, we conclude that enhanced endogenous MG53 expression in skeletal muscle and circulation seems not to improve the life span of G93A mice. This is against our original thoughts, in which the increased endogenous MG53 level in skeletal muscle and circulation should help to maintain the sarcolemma and NMJ integrity and slow down the ALS progression. However, this unexpected outcome could be a new discovery, indicating that endogenous MG53 lost membrane-repair function in ALS, even in the condition of overexpression. Our study in Aim 2 has shown that MG53 formed aggregates in skeletal muscle in both ALS mice and ALS patients. It is likely that overexpressed MG53 in ctPA-MG53-G93A mice also forms aggregates as endogenous MG53 due to mitochondria-mediated oxidative stress, thus, lost the membrane-repair function. This outcome redirected our original thinking to test a potential virus-mediated gene therapy by enhancing MG53 expression level in G93A mice, which could run to a wrong direction.
4) Note that at the current stage, we have limited animal numbers of cross-bred G93A and ctPA-MG53-G93A (total 6 ctPA-MG53-G93A and 7 G93A in both genders). We have more

back-crossed mice (waiting for genotyping and life span evaluation) that can be used to further confirm this result before we can submit a manuscript for publication.

D. mg53-/- back-crossing with B6SJL mice

1. 2nd backcrossing with WT (B6SJL)

Litters/Date of Birth	$MG53^{+/-}$(hemizygous)	$MG53^{+/+}$(wild type)
DOB 1/27/2020	Male: 4; Female: 9	Male: 3; Female: 3

2. 3rd backcrossing with WT (B6SJL)

Litters/Date of Birth	$MG53^{+/-}$(hemizygous)	$MG53^{+/+}$(wild type)
DOB 8/27/2020	Male: 2; Female: 0	Male: 1; Female: 3

3. 4th backcrossing with WT (B6SJL)

Litters/Date of Birth	$MG53^{+/-}$(hemizygous)	$MG53^{+/+}$(wild type)
1. DOB 11/18/2020	Male: 2; Female: 3	Male: 1; Female: 1
2. DOB 2/12/2021	Male: 2; Female: 0	Male: 1; Female: 0
3. DOB 3/8/2021	Male: 0; Female: 3	Male: 0; Female: 3

4. 5th backcrossing with G93A (B6SJL)

Litters/Date of Birth	$MG53^{+/-}$(hemizygous)	$MG53^{+/+}$(wild type)
1. DOB 5/11/2021	Male: 1; Female: 1	Male: 1; Female: 1
2. DOB 8/1/2021	Male: 1; Female: 1	Male: 1; Female: 0
3. DOB 9/10/2021	To be genotyped	To be genotyped

E. Breeding G93A-mg53-/- mice

We next self-crossed $MG53^{+/-}$ to produce mg53-/-, the MG53 knockout mice in B6SJL background. Up to 9/25, we have bred 5 liters and obtained 6 male and 3 female mg53-/- mice.

Note it is very difficult to breed and maintain G93A-mg53-/-. First, while crossing G93A-mg53-/- with mg53-/- can produce G93A-mg53-/-, there will be no littermate G93A that can be obtained. Secondly, female G93A mice have very low fertility, while male G93A mice can only breed for one month at the age of 2-3 months old. As the female parent can't be a G93A, we couldn't cross a male G93A-$MG53^{+/-}$ with a female G93-$MG53^{+/-}$. Our strategy to produce G93A-mg53-/- is described as following:

(1) Cross G93A mice with $MG53^{+/-}$ or mg53-/- to obtain G93A-$MG53^{+/-}$

(2) In one cage, we pair one male G93A-$MG53^{+/-}$ with one mg53-/- female and one WT female. Theoretically, the pups have the genotypes listed in the following table

B6SJL x G93A-$MG53^{+/-}$	Mg53-/- x G93A-$MG53^{+/-}$
25% G93A (used as littermate control) 25% G93A-$MG53^{+/-}$ 25% $MG53^{+/-}$ 25% wild type (B6SJL)	25% G93A-mg53-/- 25% G93A-$MG53^{+/-}$ 25% mg53-/- 25% $MG53^{+/-}$

Although the G93A-mg53-/- and G93A are from different mothers, they have the same father and are bred at the same time in the same cage, so are expected to give more consistent and similar gene background. The mice of other genotypes, wild type, MGKO and G93A-MG53$^{+/-}$, can also be used in further breeding to produce more G93A-MGKO and G93A.

Up to 9/25/21, we have obtained 11 G93A-MG53$^{+/-}$ (4 males, 11 females) from 4 liters. Two liters of pups from mg53-/- x G93A-MG53$^{+/-}$ were also obtained. For one liter, only two pups survived, but none were G93A-mg53-/-. Another liter is still waiting for genotyping.

F. Life span of G93A-MG53+/- and G93A mice:

During the above breeding, we have obtained 16 mice of G93A-MG53$^{+/-}$ and G93A, ten of which have reached their EOL.

Geno	Up to 9/25/21	Reached EOL	Mean life span(days)	SD
G93A_male	4	2	133.0	2.8
G93A-MG53$^{+/-}$_male	4	2	136.5	7.8
G93A_female	1	1	126.0	
G93A-MG53$^{+/-}$_female	7	5	150.2	6.9

Discussion on the preliminary results of the life span (**F**):

1) From our preliminary results, we obtained an interesting phenomenon. Those 5 female G93A-MG53+/- mice seems have a longer life span (also compared with untreated G93A mice with a mean life span of 140 days, listed in **Table C**). It is possible that loss of one copy of MG53 gene (MG53+/-) in G93A mice may lead to less expression of MG53 in skeletal muscle of G93A mice, leading to less aggregation of MG53 in the later stage G93A mice. If this is the case, it would indicate that the endogenous MG53 aggregation in later stage of G93A mice has a gain-of-function to promote muscle wasting due to MG53-mediated unfolded protein response (UPR). This idea also supports that overexpression of MG53 in ctPA-MG53-G93A mice does not show a beneficial effect. However, at the current stage, this is only a speculation/hypothesis. We need to perform more research to answer: *a)* If this phenomenon can be further approved by increasing experimental animal numbers; *b)* Whether the G93A-MG53+/- mice show reduced MG53 expression level and protein aggregation; *c)* If this effect is gender-dependent and why.
2) We are still in the process to obtain G93A-mg53-/- mice to determine whether ALS G93A mice with knockout of MG53 show exacerbated diaphragm muscle injuries.

G. Dissect the function of MG53 in maintenance of mitochondrial function in ALS:

Mitochondrial defect is a pathological hallmark of ALS muscle. Our previous study (Xiao 2018) shows that muscle cells (myofibers) of ALS G93A mice exhibit oxidative stress, which may impact muscle membrane integrity. Our previous studies (Zhou 2010, Yi 2011) also showed that depolarized mitochondrial appears first near the neuromuscular junction (NMJ) in G93A muscle. With the support of DOD, we found that NMJ was more susceptible to injury in G93A muscle, and demonstrated that mitochondrial dysfunction-mediated excessive oxidative stress is associated with the disruption of cell membrane integrity at NMJ and sarcolemma of the ALS muscle.

While the endogenous MG53 protein (including the overexpression of MG53 protein in ctPA-MG53-G93A mice) show dysfunction in the muscle membrane-repair, the intravenous application of exogenous recombinant MG53 (rhMG53) formed membrane repair patches on the diaphragm muscle and reduced the leakage of muscle membrane in G93A mice. In the extra vivo experiments, we examined the effect of rhMG53 in maintaining mitochondrial function by incubating the isolated live muscle myofibers with rhMG53 in a culture medium to mimic the effect of rhMG53 in circulation. We have demonstrated that rhMG53 reduced the muscle membrane injury during a high energy laser-induced muscle membrane damage, and drastically reduced the oxidative stress level in G93A muscle myofibers. The completed set of these data were included in one abstract for the 2021 65th Biophysical Society Annual Meeting (Abstract list #1), and one publication in Antioxidants (Publication list #1).

Aim 2: Conduct proof-of-concept study testing efficacy and safety of rhMG53 to treat ALS in mice.

Subtask 2.1: Production and quality controls of rhMG53 and PEG-rhMG53. (1-12 months)
Subtask 2.2: Pharmacokinetic (PK) assessment of PEG-rhMG53 in ALS mice. (6-12 months)
Subtask 2.3: In vivo efficacy and safety assays with rhMG53 and PEG-rhMG53 in ALS mice. (6-24 months)

Milestones:

1) Produce 2 grams of rhMG53, sufficient for pre-clinical studies (Completed-last report)
2) In vitro QC assays to ensure purity and function of rhMG53. Endotoxin level of rhMG53 < 10 EU/mg (Completed-last report)
3) PEGylation improves PK of rhMG53 in ALS mice (Completed-last report)
4) Intravenous or subcutaneous administration of rhMG53 or PEG-rhMG53 improves the integrity of diaphragm and NMJ in ALS mice. (completed-last report)
5) Repetitive intravenous or subcutaneous administration of rhMG53 does not produce adverse effects in ALS mice. (completed-last report).

As reported in the last progress report, the milestones Aim 2 have been achieved and the whole set of data now were published on "Antioxidants". (Publication list #1)

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.

Nothing to Report

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.

Nothing to Report

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:** D*escribe 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).

ALS patients experience rapid deterioration of skeletal muscle, especially diaphragm wasting that significantly impacts their life span and life quality. Although as a motor neuron disease, ALS appears to be a combination of "dying-forward" and/or "dying-back" pathophysiological processes, starting in cortical motor neurons and glia or at the muscle and neuromuscular junction (NMJ). It is possible that the balance of these processes differs in subsets of ALS patients, and multiple mechanisms have been proposed for how they may interrelate. Since 2006, Zhou laboratory has started to examine the contribution of skeletal muscle dysfunction in ALS pathogenesis. We have established that dysregulated Ca^{2+} signaling and mitochondrial dysfunction play a major role in skeletal muscle wasting in ALS mouse model.

With the DOD support, in this project we have fundamental discoveries in understanding the role of skeletal muscle in ALS and identified a compound that could be further developed as a potential therapeutic means to treat ALS.

(1) We provided solid experimental evidence to demonstrate that muscle membrane damage is a key factor underlying the severe muscle wasting in ALS, a pathological mechanism

that has not been explored before. The enhanced injury in diaphragm muscle membrane occurs early in ALS mice before the onset of ALS clinical symptoms. A modest exercise drastically enhanced the diaphragm muscle membrane injury in ALS mice. Our data may explain the controversial outcomes of exercise training on ALS animal models and patients and provide an underlying mechanistic explanation on why diaphragm pacing was found to be associated with reduced survival in ALS patients with respiratory insufficiency. Based on our findings, one should be cautious in designing exercise-related protocols or diaphragm pacing as alternative interventions to mitigate ALS.

(2) MG53 protein has been shown as an essential component of the cell membrane repair machinery. With this DOD support, we for the first time demonstrated that neuromuscular junction is an active site of injury repair by MG53, and the endogenous MG53 membrane repair-function is lost in ALS, even in the condition of long-term overexpression of MG53 in skeletal muscle of ALS mice. We further discovered that mitochondrial dysfunction-mediated oxidative stress likely caused MG53 protein aggregation and limit its membrane repair function. The pathological feature of MG53 protein aggregation was also demonstrated in skeletal muscle of both sporadic and familial ALS patients, indicating that MG53-associated injury of muscle membrane and neuromuscular junction could be a key pathological mechanism underlying progressive muscle wasting and diaphragm dysfunction in ALS. The discovered new mechanism of ALS muscle wasting sheds new light on developing novel therapies to treat ALS via improving mitochondrial function, and muscle membrane and neuromuscular integrity.

(3) A series of studies have shown that recombinant human MG53 (rhMG53) protein protects various cell types against membrane disruption when applied to the extracellular environment and ameliorates pathology associated with muscular dystrophy, acute lung and kidney injury, myocardial infarction, and ischemic brain damage in animal models. With this DOD support, we first demonstrated that exogenously administered rhMG53 forms repair patches and reduces membrane leakage of the ALS diaphragm muscle. Reducing cell membrane leakage can improve the intracellular milieu and mitigate mitochondrial ROS production, thus improving the intrinsic membrane repair function of MG53 to preserve the integrity of NMJ and muscle membrane. Indeed, the systemic administration of rhMG53 to the ALS mice after disease onset preserved innervation of the diaphragm and prolonged the lifespan.

(4) In rodents and humans, MG53 is present at low levels in blood circulation under normal physiologic conditions. Given that we observed higher circulating levels in the G93A mice than wild-type littermates, and a correlation with serum CK measurements in the early stage of ALS, it is possible that ALS patients will also show different levels of circulating MG53. We intend to examine this further in future studies, including the possibilities that ***1)*** a one-time measurement of MG53 early in disease course could be useful as a “prognostic biomarker” of the rate of disease progression, ***2)*** a “predictive biomarker” to identify/cohort select patients who might best respond to therapeutic strategies designed to stabilize/repair damage to myofibers, or ***3)*** a pharmacodynamic biomarker to demonstrate the therapeutic effect of treatments to preserve myofiber membrane integrity, including exogenously administered rhMG53.

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 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:*

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.

Due to the Zhou lab translocation, the quarantine and back-crossing of mg53$^{-/-}$ and ctPA-MG53 mouse models to B6SJL genetic background caused some delay. Additionally, the Covid-19 pandemic has tremendous impact on our research. The Texas Governor put the state on lockdown for several weeks. UTA was also closed for several weeks prior to implementing tele/remote working. Research laboratories were closed for a longer period of times before the university executive staff executed a plan for continued operations. Even after the lab reopening, the research activity has been impacted due to taking turns to working in the lab to keep social distance, working from home, limiting the animal numbers in the facility center, delayed purchase deliveries, etc. All our lab members did not complete the vaccine until this May after the vaccine became available to the UTA community. We have made efforts and completed the major goals of this proposed project, with one milestone partially completed and ongoing to examine whether ALS mice with knockout of MG53 show exacerbated diaphragm muscle injuries. The outcome of the MG53 knockout study will provide additional understanding of the physiological role of MG53-mediated membrane repair in ALS. However, it will not change the conclusion of ***(a)*** the ALS muscle exhibit enhanced muscle membrane injury, ***(b)*** the endogenous MG53 lost membrane repair function in ALS muscle, and ***(c)*** rhMG53 as a novel therapeutic means for improving NMJ and muscle membrane integrity to treat ALS.

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

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. Jianxun Yi, Ang Li, Xuejun Li, Ki Ho Park, Xinyu Zhou, Frank Yi, Yajuan Xiao, Dosuk Yoon, Tao Tan, Lyle W. Ostrow, Jianjie Ma and Jingsong Zhou. (2021). MG53 Preserves Neuromuscular Junction Integrity and Alleviates ALS Disease Progression. ***Antioxidants***, 2021, 10, 1522. (acknowledgement of federal support: yes)
     https://doi.org/10.3390/antiox10101522

  2. Ang Li, Xuejun Li, Jianxun Yi, Jianjie Ma, Jingsong Zhou. (2021). Butyrate Feeding Reverses CypD-Related Mitoflash Phenotypes in Mouse Myofibers. ***Int J Mol Sci***. 2021 Jul; 22(14): 7412. (acknowledgement of federal support: yes)
     https://doi.org/10.3390/ijms22147412

  3. Jingsong Zhou. (2021). Ca^{2+}-mediated coupling between neuromuscular junction and mitochondria in skeletal muscle. ***Neuroscience Letters***. Volume 754 (29), May 2021, 135899. (acknowledgement of federal support: yes)
     https://doi.org/10.1016/j.neulet.2021.135899

  4. Kerrie Downing, Rhonda Prisby, Venu Varanasi, Jingsong Zhou, Zui Pan, Marco Brotto. (2021). Old and new biomarkers for volumetric muscle loss. ***Current Opinion in Pharmacology***. Volume 59, August 2021, Pages 61-69. (acknowledgement of federal support: yes)
     https://doi.org/10.1016/j.coph.2021.05.001

5. Xinyu Zhou, Ang Li, Pei-hui Lin, Jingsong Zhou, Jianjie Ma. (2021). TRIC-A regulates intracellular Ca2+ homeostasis in cardiomyocytes. ***Pflugers Arch***. 2021; 473(3): 547–556. (acknowledgement of federal support: No)
https://doi.org/10.1007/s00424-021-02513-6

6. Ang Li, Jianxun Yi, Xuejun Li, Jingsong Zhou. (2020). Physiological Ca^{2+} Transients Versus Pathological Steady-State Ca2+ Elevation, Who Flips the ROS Coin in Skeletal Muscle Mitochondria. ***Front Physiol***. 2020; 11: 595800. (acknowledgement of federal support: yes)
https://doi.org/10.3389/fphys.2020.595800

7. Ang Li, Jingsong Zhou, Randall B. Widelitz, Robert H. Chow, Cheng-Ming Chuong. (2020). Integrating Bioelectrical Currents and Ca^{2+} Signaling with Biochemical Signaling in Development and Pathogenesis. ***Bioelectricity***. September 2020; 2(3): 210–220. (acknowledgement of federal support: No)
https://doi.org/10.1089/bioe.2020.0001

8. Xinyu Zhou, Ki Ho Park, Daiju Yamazaki, Pei-hui Lin, Miyuki Nishi, Zhiwei Ma, Liming Qiu, Takashi Murayama, Xiaoqin Zou, Hiroshi Takeshima, Jingsong Zhou and Jianjie Ma. (2020). ***Circulation Research***. 2020 Feb 14; 126(4): 417–435. (acknowledgement of federal support: No)
https://doi.org/10.1161/CIRCRESAHA.119.316241

9. Zehua Bian, Qiang Wang, Xinyu Zhou, Tao Tan, Ki Ho Park, H. Fritz Kramer, Alan McDougal, Nicholas J. Laping, Sanjay Kumar, T. M. Ayodele Adesanya, Matthew Sermersheim, Frank Yi, Xinxin Wang, Junwei Wu, Kristyn Gumpper, Qiwei Jiang, Duofen He, Pei-Hui Lin, Haichang Li, Fangxia Guan, Jingsong Zhou, Mark J. Kohr, Chunyu Zeng, Hua Zhu, Jianjie Ma. (2019). Sustained elevation of MG53 in the bloodstream increases tissue regenerative capacity without compromising metabolic function. ***Nat Commun***. 2019; 10: 4659. (acknowledgement of federal support: No)
https://doi.org/10.1038/s41467-019-12483-0

10. Jingsong Zhou, Ang Li, Xuejun Li, Jianxun Yi. (2019). Dysregulated mitochondrial Ca^{2+} and ROS signaling in skeletal muscle of ALS mouse model. ***Arch Biochem Biophys***. 2019 Mar 15; 663: 249–258. (acknowledgement of federal support: No)
https://doi.org/10.1016/j.abb.2019.01.024

11. Yajuan Xiao, Chehade Karam, Jianxun Yi, Lin Zhang, Xuejun Li, Dosuk Yoon, Huan Wang, Kamal Dhakal, Paul Ramlow, Tian Yu, Zhaohui Mo, Jianjie Ma and Jingsong Zhou. (2018). ROS-related Mitochondrial Dysfunction in Skeletal Muscle of an ALS Mouse Model during the Disease Progression. ***Pharmacol Res***. 2018 December; 138: 25–36. (acknowledgement of federal support: No)
https://doi.org/10.1016/j.phrs.2018.09.008.

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.*

1. Jianxun Yi, Ang Li, Xuejun Li and Jingsong Zhou. (2021). Mitochondrial defect exacerbates muscle membrane fragility at NMJ in ALS skeletal muscle. ***65th annual meeting of the Biophysical Society***. Feb. 2021.*

2. Xuejun Li, Ang Li, Jianxun Yi, Yanan Vickery and Jingsong Zhou. (2021). Butyrate improves mitochondrial biogenesis of an ALS cellular model. ***65th annual meeting of the Biophysical Society***. Feb. 2021.

3. Ang Li, Xuejun Li, Jianxun Yi, Jingsong Zhou. (2021). Butyrate reverses CypD-mediated mPTP opening phenotypes in mouse myofibers. ***65th annual meeting of the Biophysical Society***. Feb. 2021. *

4. Xuejun Li, Jianxun Yi, Ang Li, Marco Brotto, Jingsong Zhou. (2020). Butyrate Modulates Mitochondrial Bioenergetics of Cultured Motor Neuron Cells with Overexpression of an ALS mutation SOD1G93A. ***64th annual meeting of the Biophysical Society***. Feb. 2020. San Diego, CA, USA.

5. Ang Li, Xuejun Li, Jianxun Yi, Xinyu Zhou, Ki Ho ParK, Miyuki Nishi, Hiroshi Takeshim, Jianjie Ma, and Jingsong Zhou (2020). TRIC-A Channel Modulates Ca2+ Homeostasis in Mitochondria. ***64th annual meeting of the Biophysical Society***. Feb. 2020. San Diego, CA, USA.

- **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. Describe the technologies or techniques were shared.

  We have advanced following techniques, which are shared in the publication of item #1:

  (1) We have advanced the techniques to evaluate the diaphragm muscle membrane integrity using live cell confocal imaging.

  (2) We have advanced the techniques to quantify the innervation of neuromuscular junction of adult diaphragm muscle using confocal microscopy Z-scanning for 3D-reconstitution.

  (3) We have advanced the techniques of PEGylation of rhMG53.

  (4) We have advanced the techniques of pharmacokinetic evaluation of PEG-rhMG53 in rodents.

- **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.

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;*
  - *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.*

In this current project, we have generated new mouse models of ctPA-MG53-G93A, and G93A-mg53-/-. Due to the expression of ALS mutation of SOD1G93A, those mouse models will not be suitable for maintaining a mouse colony. However, after we complete the publication, we will share the method of how to generate those two mouse models.

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 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.)*

Name: Jianjie Ma

Project Role: Co-investigator

Researcher Identifier: N/A

Nearest person month worked: 1.5 months

Contribution to Project: Dr. Ma's laboratory discovered the MG53 gene as a membrane repair molecule. Dr. Ma and Dr. Zhou have long productive collaboration history in Ca^{2+} signaling, mitochondrial function, cell membrane repair, muscle physiology and diseases. Particularly for this project, in addition to provide his expertise in the biology and physiology of MG53 in cell membrane repair, Dr. Ma worked closely with Dr. Zhou in developing methodologies for this proposed study, data analysis and interpretation, and manuscript writing. He also made available all reagents related to MG53, including antibody, cDNA, mg53-/- and ctPA-MG53 mouse models, to this project.

A research scientist in Dr. Ma's laboratory with a total 12-month effort dedicated the effort to the development studies with rhMG53 protein purification, PEGylation and formulation of PEG-

rhMG53 protein, and the protein quality control of the rhMG53 and PEG-rhMG53 for in vivo efficacy studies.

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

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://ebrap.org/eBRAP/public/index.htm*** *for each unique award.*

QUAD CHARTS: *If applicable, the Quad Chart (available on https://www.usamraa.army.mil/Pages/Resources.aspx) 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.

The original copies of journal articles are available online:

1. Jianxun Yi, Ang Li, Xuejun Li, Ki Ho Park, Xinyu Zhou, Frank Yi, Yajuan Xiao, Dosuk Yoon, Tao Tan, Lyle W. Ostrow, Jianjie Ma and Jingsong Zhou. (2021). MG53 Preserves Neuromuscular Junction Integrity and Alleviates ALS Disease Progression. Antioxidants, 2021, 10, 1522. (acknowledgement of federal support: yes)
https://doi.org/10.3390/antiox10101522

2. Ang Li, Xuejun Li, Jianxun Yi, Jianjie Ma, Jingsong Zhou. (2021). Butyrate Feeding Reverses CypD-Related Mitoflash Phenotypes in Mouse Myofibers. Int J Mol Sci. 2021 Jul; 22(14): 7412. (acknowledgement of federal support: yes)
https://doi.org/10.3390/ijms22147412

3. Jingsong Zhou. (2021). Ca2+-mediated coupling between neuromuscular junction and mitochondria in skeletal muscle. Neuroscience Letters. Volume 754 (29), May 2021, 135899. (acknowledgement of federal support: yes)
https://doi.org/10.1016/j.neulet.2021.135899

4. Kerrie Downing, Rhonda Prisby, Venu Varanasi, Jingsong Zhou, Zui Pan, Marco Brotto. (2021). Old and new biomarkers for volumetric muscle loss. Current Opinion in Pharmacology. Volume 59, August 2021, Pages 61-69. (acknowledgement of federal support: yes)
https://doi.org/10.1016/j.coph.2021.05.001

5. Xinyu Zhou, Ang Li, Pei-hui Lin, Jingsong Zhou, Jianjie Ma. (2021). TRIC-A regulates intracellular Ca2+ homeostasis in cardiomyocytes. Pflugers Arch. 2021; 473(3): 547–556. (acknowledgement of federal support: No)
https://doi.org/10.1007/s00424-021-02513-6

6. Ang Li, Jianxun Yi, Xuejun Li, Jingsong Zhou. (2020). Physiological Ca2+ Transients Versus Pathological Steady-State Ca2+ Elevation, Who Flips the ROS Coin in Skeletal Muscle Mitochondria. Front Physiol. 2020; 11: 595800. (acknowledgement of federal support: yes)
https://doi.org/10.3389/fphys.2020.595800

7. Ang Li, Jingsong Zhou, Randall B. Widelitz, Robert H. Chow, Cheng-Ming Chuong. (2020). Integrating Bioelectrical Currents and Ca2+ Signaling with Biochemical Signaling in Development and Pathogenesis. Bioelectricity. September 2020; 2(3): 210–220. (acknowledgement of federal support: No)
https://doi.org/10.1089/bioe.2020.0001

8. Xinyu Zhou, Ki Ho Park, Daiju Yamazaki, Pei-hui Lin, Miyuki Nishi, Zhiwei Ma, Liming Qiu, Takashi Murayama, Xiaoqin Zou, Hiroshi Takeshima, Jingsong Zhou and Jianjie Ma. (2020). Circulation Research. 2020 Feb 14; 126(4): 417–435. (acknowledgement of federal support: No)
https://doi.org/10.1161/CIRCRESAHA.119.316241

9. Zehua Bian, Qiang Wang, Xinyu Zhou, Tao Tan, Ki Ho Park, H. Fritz Kramer, Alan McDougal, Nicholas J. Laping, Sanjay Kumar, T. M. Ayodele Adesanya, Matthew Sermersheim, Frank Yi, Xinxin Wang, Junwei Wu, Kristyn Gumpper, Qiwei Jiang, Duofen He, Pei-Hui Lin, Haichang Li, Fangxia Guan, Jingsong Zhou, Mark J. Kohr, Chunyu Zeng, Hua Zhu, Jianjie Ma. (2019). Sustained elevation of MG53 in the bloodstream increases tissue regenerative capacity without compromising metabolic function. Nat Commun. 2019; 10: 4659. (acknowledgement of federal support: No)
https://doi.org/10.1038/s41467-019-12483-0

10. Jingsong Zhou, Ang Li, Xuejun Li, Jianxun Yi. (2019). Dysregulated mitochondrial Ca2+ and ROS signaling in skeletal muscle of ALS mouse model. Arch Biochem Biophys. 2019 Mar 15; 663: 249–258. (acknowledgement of federal support: No)
https://doi.org/10.1016/j.abb.2019.01.024

11. Yajuan Xiao, Chehade Karam, Jianxun Yi, Lin Zhang, Xuejun Li, Dosuk Yoon, Huan Wang, Kamal Dhakal, Paul Ramlow, Tian Yu, Zhaohui Mo, Jianjie Ma and Jingsong Zhou. (2018). ROS-related Mitochondrial Dysfunction in Skeletal Muscle of an ALS Mouse Model during the Disease Progression. Pharmacol Res. 2018 December; 138: 25–36. (acknowledgement of federal support: No)
https://doi.org/10.1016/j.phrs.2018.09.008.