Heparan sulfate is a clearance receptor for aberrant extracellular proteins

Eisuke Itakura, Momoka Chiba, Takeshi Murata, and Akira Matsuura

Corresponding Author(s): Eisuke Itakura, Chiba University,

Review Timeline:

Submission Date:	2019-11-26
Editorial Decision:	2019-12-26
Revision Received:	2020-01-06

Monitoring Editor: Tamotsu Yoshimori

Scientific Editor: Andrea Marat

Transaction Report:

(Note: With the exception of the correction of typographical or spelling errors that could be a source of ambiguity, letters and reports are not edited. The original formatting of letters and referee reports may not be reflected in this compilation.)

DOI: https://doi.org/10.1083/jcb.201911126

December 26, 2019

RE: JCB Manuscript #201911126

Dr. Eisuke Itakura
Chiba University,
Department of Biology
1-33 Yayoi-cho
Inage-ku, Chiba-shi, Chiba 263-8522
Japan

Dear Dr. Itakura:

Thank you for submitting your revised manuscript entitled "Heparan sulfate is a clearance receptor for aberrant extracellular proteins". The manuscript has now been assessed by expert reviewers, whose reports are appended below. As you will see, both reviewers are very positive about your study. We would be happy to publish your paper in JCB pending text changes to address the reviewer concerns and final revisions necessary to meet our formatting guidelines (see details below). In addition, additional experiments to examine if the results are specific to Clusterin as suggested by reviewer #2, while not essential, are highly encouraged. If you do include additional data I expect to assess your revised manuscript without further external peer review. Please advise the editorial office if you intend to perform additional experiments, if so the standard timeframe for experimental revisions is 3-4 months.

To avoid unnecessary delays in the acceptance and publication of your paper, please read the following information carefully.

A. MANUSCRIPT ORGANIZATION AND FORMATTING:

Full guidelines are available on our Instructions for Authors page, http://jcb.rupress.org/submission-guidelines#revised. **Submission of a paper that does not conform to JCB guidelines will delay the acceptance of your manuscript.**

1) Text limits: Character count for Articles is < 40,000, not including spaces. Count includes title page, abstract, introduction, results, discussion, acknowledgments, and figure legends. Count does not include materials and methods, references, tables, or supplemental legends.

2) Figures limits: Articles may have up to 10 main text figures.

3) Figure formatting: Scale bars must be present on all microscopy images, * including inset magnifications (alternatively you may indicate the width of the inset). Molecular weight or nucleic acid size markers must be included on all gel electrophoresis.

4) Statistical analysis: Error bars on graphic representations of numerical data must be clearly described in the figure legend. The number of independent data points (n) represented in a graph must be indicated in the legend. Statistical methods should be explained in full in the materials and methods. For figures presenting pooled data the statistical measure should be defined in the figure

legends. Please also be sure to indicate the statistical tests used in each of your experiments (either in the figure legend itself or in a separate methods section) as well as the parameters of the test (for example, if you ran a t-test, please indicate if it was one- or two-sided, etc.). Also, if you used parametric tests, please indicate if the data distribution was tested for normality (and if so, how). If not, you must state something to the effect that "Data distribution was assumed to be normal but this was not formally tested."

5) Abstract and title: The abstract should be no longer than 160 words and should communicate the significance of the paper for a general audience. The title should be less than 100 characters including spaces. Make the title concise but accessible to a general readership.

6) Materials and methods: Should be comprehensive and not simply reference a previous publication for details on how an experiment was performed. Please provide full descriptions in the text for readers who may not have access to referenced manuscripts.

7) Please be sure to provide the sequences for all of your primers/oligos and RNAi constructs in the materials and methods. You must also indicate in the methods the source, species, and catalog numbers (where appropriate) for all of your antibodies. Please also indicate the acquisition and quantification methods for immunoblotting/western blots.

8) Microscope image acquisition: The following information must be provided about the acquisition and processing of images:
a. Make and model of microscope
b. Type, magnification, and numerical aperture of the objective lenses
c. Temperature
d. Imaging medium
e. Fluorochromes
f. Camera make and model
g. Acquisition software
h. Any software used for image processing subsequent to data acquisition. Please include details and types of operations involved (e.g., type of deconvolution, 3D reconstitutions, surface or volume rendering, gamma adjustments, etc.).

9) References: There is no limit to the number of references cited in a manuscript. References should be cited parenthetically in the text by author and year of publication. Abbreviate the names of journals according to PubMed.

10) Supplemental materials: There are strict limits on the allowable amount of supplemental data. Articles may have up to 5 supplemental display items (figures and tables). At the moment, you currently have 6 such items, therefore please combine two supplemental figures into one. Please be sure to correct the callouts in the text to reflect this change. Please also note that tables, like figures, should be provided as individual, editable files. A summary of all supplemental material should appear at the end of the Materials and methods section.

11) eTOC summary: A ~40-50-word summary that describes the context and significance of the findings for a general readership should be included on the title page. The statement should be written in the present tense and refer to the work in the third person.

12) Conflict of interest statement: JCB requires inclusion of a statement in the acknowledgements regarding competing financial interests. If no competing financial interests exist, please include the

following statement: "The authors declare no competing financial interests." If competing interests are declared, please follow your statement of these competing interests with the following statement: "The authors declare no further competing financial interests."

13) ORCID IDs: ORCID IDs are unique identifiers allowing researchers to create a record of their various scholarly contributions in a single place. At resubmission of your final files, please consider providing an ORCID ID for as many contributing authors as possible.

14) A separate author contribution section following the Acknowledgments. All authors should be mentioned and designated by their full names. We encourage use of the CRediT nomenclature.

B. FINAL FILES:

Please upload the following materials to our online submission system. These items are required prior to acceptance. If you have any questions, contact JCB's Managing Editor, Lindsey Hollander (lhollander@rockefeller.edu).

-- An editable version of the final text (.DOC or .DOCX) is needed for copyediting (no PDFs).

-- High-resolution figure and video files: See our detailed guidelines for preparing your production-ready images, http://jcb.rupress.org/fig-vid-guidelines.

-- Cover images: If you have any striking images related to this story, we would be happy to consider them for inclusion on the journal cover. Submitted images may also be chosen for highlighting on the journal table of contents or JCB homepage carousel. Images should be uploaded as TIFF or EPS files and must be at least 300 dpi resolution.

It is JCB policy that if requested, original data images must be made available to the editors. Failure to provide original images upon request will result in unavoidable delays in publication. Please ensure that you have access to all original data images prior to final submission.

The license to publish form must be signed before your manuscript can be sent to production. A link to the electronic license to publish form will be sent to the corresponding author only. Please take a moment to check your funder requirements before choosing the appropriate license.

Thank you for your attention to these final processing requirements.

Please contact the journal office with any questions, cellbio@rockefeller.edu or call (212) 327-8588.

Thank you for this interesting contribution, we look forward to publishing your paper in Journal of Cell Biology.

Sincerely,

Tamotsu Yoshimori, PhD
Monitoring Editor

Andrea L. Marat, PhD
Scientific Editor

Journal of Cell Biology

Reviewer #1 (Comments to the Authors (Required)):

In this manuscript, Itakura and colleagues convincingly show a novel pathway of degradation of extracellular misfolded proteins by autophagy. The authors developed a novel sensor system to assess the process and detailed thoroughly the molecular mechanism. As the topic is of interest and poorly understood, this Referee recommends its publication with just a few stylistic revisions.

-As the authors mention, how stressed proteins are internalized and degraded is poorly understood, and they nicely show that the CRED pathway is conserved among several cell lines derived from different human tissues. However, the authors didn't check the CRED pathway in primary tissues, all tissues or other model organisms. Thus, the claim that the process is "universal" seems a bit far-fetched. Please remove or rephrase all sentences related to this claim.
-Page 9, line 29: "the CRED pathway is a ubiquitous process in virtually all tissues". Similar to the previous comment, the CRED pathway is conserved in all human tissues tested.
-Page 10, lines 11-12: "Our results demonstrated that the CRED pathway results in Aβ degradation (Fig. 6)." The authors only show that AB is internalized with Clusterin and that Clusterin is degraded, but didn't prove Aβ degradation following this pathway. Please rephrase.

Reviewer #2 (Comments to the Authors (Required)):

This is very nice work demonstrating a role for Clusterin mediated degradation pathway via binding to HSPG that shuttles targets to the lysosomal system. Overall the work presented is very strong and thoroughly performed. A particular strength of the study is that it uses unbiased screening to arrive at this pathway. The results and validation are very convincing, but the only criticism is whether this is actually specific to Clusterin. For example, would other factors such as apoE that also bind to HSPG also function in a similar manner? This data is not necessary for the current manuscript, but if available it would greatly strengthen this paper. One minor note is that two receptors have been proposed for CLU: LRP2 (aka megalin) and PLXNA4. The authors should be sure to cite these from the literature.

Answer to the comments of referee #1

In this manuscript, Itakura and colleagues convincingly show a novel pathway of degradation of extracellular misfolded proteins by autophagy. The authors developed a novel sensor system to assess the process and detailed thoroughly the molecular mechanism. As the topic is of interest and poorly understood, this Referee recommends its publication with just a few stylistic revisions.

-As the authors mention, how stressed proteins are internalized and degraded is poorly understood, and they nicely show that the CRED pathway is conserved among several cell lines derived from different human tissues. However, the authors didn't check the CRED pathway in primary tissues, all tissues or other model organisms. Thus, the claim that the process is "universal" seems a bit far-fetched. Please remove or rephrase all sentences related to this claim.
<Reply>
We thank the referee for the positive and thoughtful review. As suggested, we have rephrased from "universal" to "general".

-Page 9, line 29: "the CRED pathway is a ubiquitous process in virtually all tissues". Similar to the previous comment, the CRED pathway is conserved in all human tissues tested.
<Reply>
We have rephrased the text accordingly. Page 9, Line 31

-Page 10, lines 11-12: "Our results demonstrated that the CRED pathway results in Aβ degradation (Fig. 6)." The authors only show that AB is internalized with Clusterin and that Clusterin is degraded, but didn't prove Aβ degradation following this pathway. Please rephrase.
<Reply>
We have rephrased the text as follows "Our results demonstrated that the CRED pathway internalizes Clusterin-Aβ complex (Fig. 6)". Page 10, Line 13

Answer to the comments of referee #2

This is very nice work demonstrating a role for Clusterin mediated degradation pathway via binding to HSPG that shuttles targets to the lysosomal system. Overall the work presented is very strong and thoroughly performed. A particular strength of the study is that it uses unbiased screening to arrive at this pathway. The results and validation are very convincing, but the only

criticism is whether this is actually specific to Clusterin. For example, would other factors such as apoE that also bind to HSPG also function in a similar manner? This data is not necessary for the current manuscript, but if available it would greatly strengthen this paper.

<Reply>

We thank the referee for positive recommendation. We have not tested requirement of HSPG to other chaperones and believe such an investigation deserves of its own detailed study in the future, which we will be enthusiastically pursuing. We have included this discussion: "apolipoprotien E is a chaperone for Aβ and also known to interact to HS, implying that HS might be a common receptor for extracellular chaperones." Page 10, line 11.

One minor note is that two receptors have been proposed for CLU: LRP2 (aka megalin) and PLXNA4. The authors should be sure to cite these from the literature.

<Reply>

We have now cited the paper (Please see page 5, line 21).