Recognition of nuclear export signals by CRM1 carrying the oncogenic E571K mutation

Jordan Baumhardt, Janek Walker, Yoonji Lee, Binita Shakya, Chad Brautigam, Rosa Lapalombella, Nick Grishin, and Yuh Min Chook

Corresponding author(s): Yuh Min Chook, University of Texas Southwestern Medical Center

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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.)

RE: Manuscript #E20-04-0233
TITLE: "Recognition of nuclear export signals by CRM1 carrying the oncogenic E571K mutation"

Dear Dr. Chook:

You will see that the reviewers are very supportive of your paper and only have minor comments. Referee 1 asks you to address several issues by changes to the text. I ask you to please incorporate these changes in a final revised version.

Sincerely,
Tom Misteli
Monitoring Editor
Molecular Biology of the Cell

Dear Dr. Chook,

The review of your manuscript, referenced above, is now complete. The Monitoring Editor has decided that your manuscript requires minor revisions before it can be published in Molecular Biology of the Cell, as described in the Monitoring Editor's decision letter above and the reviewer comments (if any) below.

A reminder: Please do not contact the Monitoring Editor directly regarding your manuscript. If you have any questions regarding the review process or the decision, please contact the MBoC Editorial Office (mboc@ascb.org).

When submitting your revision include a rebuttal letter that details, point-by-point, how the Monitoring Editor's and reviewers' comments have been addressed. (The file type for this letter must be "rebuttal letter"; do not include your response to the Monitoring Editor and reviewers in a "cover letter.") Please bear in mind that your rebuttal letter will be published with your paper if it is accepted, unless you have opted out of publishing the review history.

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In preparing your revised manuscript, please follow the instruction in the Information for Authors (www.molbiolcell.org/info-for-authors). In particular, to prepare for the possible acceptance of your revised manuscript, submit final, publication-quality figures with your revision as described.

To submit the rebuttal letter, revised version, and figures, please use this link (please enable cookies, or cut and paste URL): Link Not Available

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MBoC Editorial Board will provide guidance as you prepare your video. Information about how to prepare and submit a video abstract is available at www.molbiolcell.org/science-sketches. Please contact mboc@ascb.org if you are interested in creating a Science Sketch.

Thank you for submitting your manuscript to Molecular Biology of the Cell. Please do not hesitate to contact this office if you have any questions.

Sincerely,

Eric Baker
Journal Production Manager
MBoC Editorial Office
mbc@ascb.org

Reviewer #1 (Remarks to the Author):

In the manuscript by Baumhardt et al. entitled "Recognition of nuclear export signals by CRM1 carrying the oncogenic E571K mutation", the authors employ a combination of a structural approaches, biochemistry, and cell biology to explore the consequences of an oncogenic mutation in the Nuclear Export Sequence receptor protein, CRM1. The studies reveal some potential consequences of this amino acid substitution, which could contribute to cell growth changes observed; however, the also present information with broader implications for conjecture about NES binding.

For this study, the authors used CRIPSR/Cas to produce a cell line with the CRM1 E157K allele (either heterozygous or homozygous). The authors then go on to solve ten new crystal structure and perform biochemical analyses to bring their total number of NES sequences analyzed to 27. This work has important implications for understanding how an oncogenic change could alter cell physiology. Importantly, the authors also analyze some patient cells to extend the implication of their studies to patient samples.

Some minor changes to the presentation would help to clarify some of the important results that emerge.

Specific Comments:

The Abstract does not mention that the authors used CRISPR/Cas to model the patient mutation in cells and identified a cell cycle defect. This seems like an important experiment, which sets the stage for the functional studies. This point could at least be mentioned in the abstract.

The authors should emphasize in the text the result that the NES of Mek1 kinase binds to the CRM1 variants with higher affinity. This result is in striking contrast to the other results described and follows that sentence earlier in the paragraph stating "Most of the NES peptides bind CRM1(E571K) with lower affinity than WT CRM1." Instead of one sentence stating the two results together "The two NESs that bind WT and mutant CRM1 very differently are the NESs of the eIF4E Transporter (4E-T; also known as EIF4ENIF1), which bind CRM1(E571K) 10-fold weaker

(Supplemental Figure 1E), and the NES of the Mek1 kinase, which binds CRM1(E571K) 14-fold stronger (Supplemental Figure 2A)."

The authors could state, "The two NESs that bind WT and mutant CRM1 very differently are the NESs of the eIF4E Transporter (4E-T; also known as EIF4ENIF1) and Mek1 kinase. The 4E-TNES shows a striking decrease in binding affinity for CRM1(E571K) compared to WT CRM1 with 10-fold weaker binding. In contrast, the Mek1NES showed increased binding affinity for CRM1(E571K) compared to WT CRM1 with 14-fold stronger binding.

These different results for these NES motifs represent some of the most important findings of the biochemistry and the difference is striking so a few sentences are merited. On first read through, I missed that one bound stronger to CRM1(E571K) than WT CRM1.

Although the Discussion is already pretty long, it would be interesting to hear the authors thoughts on why such a mutation would be most prevalent in hematologic cancers and also to have them speculate on oncogenic mechanism- decreased affinity for a protein that confers oncogenic properties in the nucleus? Increased affinity for a cargo that alters physiology (either decreasing nuclear pool or increasing cytoplasmic pool?) or most likely the totality of a number of misregulated cargoes. Another function of CRM1? The authors do briefly speculate but a bit more would be interesting.

Really Minor Points:

In the Abstract, the 4E-T abbreviation appears without being defined earlier.

Human embryonic kidney 293 cells, also often referred to as HEK 293, HEK-293, 293 cells, or less precisely as HEK cells, are a specific cell line originally derived from human embryonic kidney cells grown in tissue culture. They are not usually abbreviated Hek293 cells as the authors employ here.

There is a typo in the following sentence on Page 9: 'has' should be 'have'

Wrong: Several NES peptides, including the PKINES, has negatively charged β-strand side chains but show little difference in affinity for E571K vs. WT CRM1.

Correct: Several NES peptides, including the PKINES, have negatively charged β-strand side chains but show little difference in affinity for E571K vs. WT CRM1.

Reviewer #2 (Remarks to the Author):

In this paper, Baumhardt et al. investigate how a point mutation (E571K) in the nuclear export receptor CRM1 highly prevalent in cancers affects the mechanism of tumorigenesis.
Overall the paper is well written, and is very rich in experimental design. I particularly cherish the way the authors combine biophysical and cellular methods to dig into the complexity of this apparently kryptic mutation in CRM1.
The observation that NES peptides don't fully recapitulate the complexity of the karyopherin:cargo complex is completely in line with what we have observed with NLS-cargos. Overall, I don't have any major comments. This is an excellent paper that fit well in MBC. I recommend its publication as is.

Minor typos:

- Page 15 "the endogeneous 4E-T protein..." should be 'endogenous'

- Page 17 "have altered localizations in that study..." I think it should singular 'localization'

- Page 22 "expressed in in E. coli ..." remove 'in'

Response to reviewers' comments (Edits are marked in the manuscript version below)

Reviewer #1 (Remarks to the Author):

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Some minor changes to the presentation would help to clarify some of the important results that emerge.

Specific Comments:

The Abstract does not mention that the authors used CRISPR/Cas to model the patient mutation in cells and identified a cell cycle defect. This seems like an important experiment, which sets the stage for the functional studies. This point could at least be mentioned in the abstract.

Authors' response:

Thank you for pointing out this omission. We added this information to the abstract, lines 4-7, page 2 of the revised manuscript.

The authors should emphasize in the text the result that the NES of Mek1 kinase binds to the CRM1 variants with higher affinity. This result is in striking contrast to the other results described and follows that sentence earlier in the paragraph stating "Most of the NES peptides bind CRM1(E571K) with lower affinity than WT CRM1." Instead of one sentence stating the two results together "The two NESs that bind WT and mutant CRM1 very differently are the NESs of the eIF4E Transporter (4E-T; also known as EIF4ENIF1), which bind CRM1(E571K) 10-fold weaker (Supplemental Figure 1E), and the NES of the Mek1 kinase, which binds CRM1(E571K) 14-fold stronger (Supplemental Figure 2A)."

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with 10-fold weaker binding. In contrast, the Mek1NES showed increased binding affinity for CRM1(E571K) compared to WT CRM1 with 14-fold stronger binding.

These different results for these NES motifs represent some of the most important findings of the biochemistry and the difference is striking so a few sentences are merited. On first read through, I missed that one bound stronger to CRM1(E571K) than WT CRM1.

Authors' response:

The reviewer made a great point. We followed the suggestion and emphasized that the Mek1's affinity for CRM1(E571K) is 14-fold higher than for WT CRM1 (lines 9-14, page 7).

Although the Discussion is already pretty long, it would be interesting to hear the authors thoughts on why such a mutation would be most prevalent in hematologic cancers and also to have them speculate on oncogenic mechanism- decreased affinity for a protein that confers oncogenic properties in the nucleus? Increased affinity for a cargo that alters physiology (either decreasing nuclear pool or increasing cytoplasmic pool?) or most likely the totality of a number of misregulated cargoes. Another function of CRM1? The authors do briefly speculate but a bit more would be interesting.

Authors' response:

We thank the reviewer for interest in our thought about why E571K is prevalent in blood cancers and in our speculation on the oncogenic mechanisms. We added additional discussion points to address the reviewer's comments (lines 15-25, page 19). In short, we discuss engineering CRM1(E571K) into several B-cell lines to compare functional changes across cell types. These studies may shed light on why the mutation is found at a very high frequency in B-cell malignancies compared to solid tumors. As far as oncogenic mechanism speculation, we think a select few cargos have altered subcellular localizations that contribute to oncogenesis. It is too early to tell whether oncogenic properties are due to increased or decreased nuclear/cytoplasmic concentrations. We speculate in the discussion that 4E-T-mediated mRNA translational repression may be relieved with decreased 4E-T levels in the cytoplasm, leading to translation and upregulation of oncogenes. Alternatively, there may be an enhancement of potential nuclear functions of 4E-T (currently not defined), which could also result in upregulation of oncogenes.

Really Minor Points:

In the Abstract, the 4E-T abbreviation appears without being defined earlier.

Authors' response:

We defined the 4E-T abbreviation in lines 19-20, page 4 and then again in line 11, page 7.

Human embryonic kidney 293 cells, also often referred to as HEK 293, HEK-293, 293 cells, or less precisely as HEK cells, are a specific cell line originally derived from human embryonic kidney cells grown in tissue culture. They are not usually abbreviated Hek293 cells as the authors employ here.

Authors' response:

We corrected our abbreviation to HEK 293 (many places in the manuscript).

There is a typo in the following sentence on Page 9: 'has' should be 'have'
Wrong: Several NES peptides, including the PKINES, has negatively charged β-strand side chains but show little difference in affinity for E571K vs. WT CRM1.

Correct: Several NES peptides, including the PKINES, have negatively charged β-strand side chains but show little difference in affinity for E571K vs. WT CRM1.

We made the suggested correction (line x, page y).

Reviewer #2 (Remarks to the Author):

In this paper, Baumhardt et al. investigate how a point mutation (E571K) in the nuclear export receptor CRM1 highly prevalent in cancers affects the mechanism of tumorigenesis.
Overall the paper is well written, and is very rich in experimental design. I particularly cherish the way the authors combine biophysical and cellular methods to dig into the complexity of this apparently kryptic mutation in CRM1.
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- Page 15 "the endogeneous 4E-T protein..." should be 'endogenous'

- Page 17 "have altered localizations in that study..." I think it should singular 'localization'

- Page 22 "expressed in in E. coli ..." remove 'in'

Authors' response:

We thank the reviewer for the great feedback! We fixed all three errors.

RE: Manuscript #E20-04-0233R
TITLE: "Recognition of nuclear export signals by CRM1 carrying the oncogenic E571K mutation"

Dear Dr. Chook:

I have now looked over the revised manuscript and find that your changes address the reviewers' minor points. I am pleased to proceed with publication of this very interesting study. Thank you for sending it to MBoC.

Sincerely,
Tom Misteli
Monitoring Editor
Molecular Biology of the Cell

Dear Dr. Chook:

Congratulations on the acceptance of your manuscript.

A PDF of your manuscript will be published on MBoC in Press, an early release version of the journal, within 10 days. The date your manuscript appears at www.molbiolcell.org/toc/mboc/0/0 is the official publication date. Your manuscript will also be scheduled for publication in the next available issue of MBoC.

Within approximately four weeks you will receive a PDF page proof of your article.

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