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Hematopoietic stem cell differentiation promotes the release of prominin-1/CD133 - containing membrane vesicles - A role of the endocytic-exocytic pathway

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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.)

1st Editorial Decision 14 December 2010

Thank you for the submission of your manuscript "Hematopoietic stem cell differentiation promotes the release of prominin-1/CD133-containing membrane vesicles - A role of the endocytic-exocytic pathway" to EMBO Molecular Medicine. We have now heard back from the three referees whom we asked to evaluate your manuscript. You will see that they find the topic of your manuscript potentially interesting but they feel that the data need to be strengthened, which should be addressed in a major revision.

While reviewer #1 feels that the study is better suited for a specialized journal, reviewers #2 and #3 are more supportive. However, reviewer #2 raises a number of technical concerns, which should be convincingly addressed. In addition, as implicated by reviewer #1, we feel that the addition of functional data on the role of prominin-1 release would strengthen the manuscript and its medical impact.

Should you be able to address these criticisms convincingly, we could consider a revised manuscript. I should remind you that it is the journal's policy to allow a single round of revision only and that, therefore, acceptance or rejection of the manuscript will depend on the completeness of your responses included in the next, final version of the manuscript. I do realize that addressing all of the referees' criticisms might require a lot of additional time and effort and be technically challenging, and I would also understand it if you were to rather decide to publish the manuscript rapidly and without any significant changes elsewhere.

Should you decide to embark in such a revision, revised manuscripts should be submitted within three months of a request for revision; they will otherwise be treated as new submissions, unless arranged otherwise with the editor.

I look forward to seeing a revised form of your manuscript as soon as possible.

Yours sincerely,

Editor
EMBO Molecular Medicine

REFEREE REPORTS :

Referee #1 - Comments on Novelty/Model system:

The paper describes a new mechanism for the previously reported release of prominin-1 during celluklar differentiation but does not add any new insights into any potential functional importance of theses processes. I feel that the study will not be suitable for a journal with the caliber of EMBO Molecular Medicine and is better suited for a more specialized journal.

Referee #1 - Other Remarks:

Bauer et al describe a new mechnism for the previously reported prominin-1 release from progenitor cells during differentiation. They show that this release is mediated by small membrane vesicles and export via exosomes in hematopoietic progenitor cells.
While the concept of prominin-1 release during differentiation of progenitor cells is well established, the main concern of this reviewer is the lack of any evidence for an actual role of prominin-1 release in stem and progenitor cell function. The data at hand in this study is only descriptive for how prominin-1 CMV may be released by progenitor cells. The authors speculate on several scenarios involving prominin-1 release as a determinant of cell fate. None of these are, however, functionally tested. The biomedical impact of this study is therefore questionable. The study is better suited for a more specialized journal.

Referee #2 - Other Remarks:

In this manuscript (EMM-2010-00531), Bauer et al. study the releasing of small membrane vesicles by stem cells using a co-culture with multipotent mesenchymal stromal cells. They found that prominin-1, a cancer stem cell marker previously shown to be released through plasma membrane budding, is associated with vesicles produced from an endosomal compartment. Moreover, they suggest that quantitative reduction or loss of prominin-1-containing lipid domains from stem cells could be involved in their differentiation.
This manuscript represents a significant and well written piece of work, and I therefore recommend that it be accepted for publication in EMBO Molecular Medicine, with a few minor editions that could be implemented.

Figure1:
Regarding the larger electron dense membrane structures, is there a way to assess if these microvesicles are derived from the plasma membrane? Moreover, it is not clear to me whether the authors think that prominin-1-positive vesicles are strictly constituted by- or enriched with lipid rafts (cf. comment of Fig. S4). In any case, it would be interesting in Fig.1G to compare the lipid composition of prominin-1-positive and negative vesicles using C14-acetate labeling.

Figure 2:
In the manuscript (page 7, two last lines) Fig.2C and 2D are referred as 3C and 3D. In the figure, "d" should be replaced by "D". Even though the EM images are relatively poor in resolution, I would recommend avoiding defining the MVB limiting membrane (dot line). Moreover, it would have been informative to carry out immuno-EM using CD63 to strengthen the IF data.

Figure 3:
As opposed to the vesicular 200,000g pellets situation, the 300g pellets corresponding to cells should have been loaded with the same amount of protein. That needs to be clarified. The presence of prominin-1 positive material in 1,200g and 10,000g pellets at day 14 should be discussed.

Figure S4:
It would be informative to check other exosomal markers to characterize the prominin-1 positive and negative vesicles (e.g. Tsg101, hsc70...). Specifically, the presence of CD63 which is suggested by the authors to induce incorporation of syntenin into prominin-1 vesicles should be assessed.

General comments:
Would it be possible to use lysosomal inhibitors during cell culture to assess the relative involvement of the exosomal pathway vs. lysosomal degradation during the differentiation process? What is the dynamics of the internal pool of prominin-1? Does it vary with differentiation and could it be possible to affect its presence by blocking endocytosis (e.g. dynasore...)?

Referee #3 - Other Remarks:

The paper by Bauer et al. reports on the mechanism of release of prominin-1/CD133- containing membrane vesicles upon hematopoietic stem/progenitor cell differentiation, employing an in vitro culture system of hematopoietic stem/progenitor cells on mesenchymal stroma cells as feeder.

Prominin-1/CD133 represents a surface marker that has been used in numerous studies for isolation and characterization of hematopoietic stem cells, yet prominin-1/CD133 function and its impact on stem cell phenotype is not understood. Thus, elucidating the molecular mechanism of prominin-1/CD133 action in stem cells is expected to open new avenues of influencing stem cell phenotype and function.

The Corbeil laboratory has worked for many years on prominin-1/CD133 and its potential function in stem cell biology. The results reported here on the release of prominin-1/CD133-containing exosomes membrane vesicles during hematopoietic stem/progenitor cell differentiation are surprising given that prominin-1/CD133 release in neural stem cells occurs by a budding mechanism of plasma membrane protrusions and yields vesicles that are distinct from exosomes. The authors also show that prominin-1/CD133-containing exosome release occurs concomitantly with hematopoietic stem/progenitor cell differentiation. This observation is in support of the concept of release of stem/progenitor cell-affiliated proteins upon differentiation.

This is an interesting paper and the conclusions reached are supported by the data shown. The experiments involve a number of demanding techniques that are well performed.

I have only minor points:

The sub headings "Primary antibodies derived from the same species" and "Primary antibodies derived from different species" of the "Immunoflourescence and confocal microscopy" section in the "Materials and Methods" should be deleted, since they do not add to the intention of the study.

If required parts of the "Materials and Methods" could be included as "Online Supporting Information", which would significantly shorten the body of the manuscript.

1st Revision - Authors' Response 25 March 2011

Reviewer 1 - Comments on Novelty/Model system:

The paper describes a new mechanism for the previously reported release of prominin-1 during cellular differentiation but does not add any new insights into any potential functional importance of

theses processes. I feel that the study will not be suitable for a journal with the caliber of EMBO Molecular Medicine and is better suited for a more specialized journal.

Authors' response:

We are pleased to read that this reviewer appreciates the novelty of the described mechanism employed by hematopoietic stem and progenitor cells to release selectively the widely used stem cell marker prominin-1 during the process of differentiation. We understand his/her concern regarding the functional importance of these processes and, in answer to it, we present additional experiences (see below) bringing out the functional impact of our work with regard to the cell biology of the bone marrow niche, the comprehension of which is clinically important in order to improve the success rate of bone marrow transplantation.

Reviewer 1 - Other Remarks:

Bauer et al describe a new mechanism for the previously reported prominin-1 release from progenitor cells during differentiation. They show that this release is mediated by small membrane vesicles and export via exosomes in hematopoietic progenitor cells.

While the concept of prominin-1 release during differentiation of progenitor cells is well established, the main concern of this reviewer is the lack of any evidence for an actual role of prominin-1 release in stem and progenitor cell function. The data at hand in this study is only descriptive for how prominin-1–CMV may be released by progenitor cells. The authors speculate on several scenarios involving prominin-1 release as a determinant of cell fate. None of these are, however, functionally tested. The biomedical impact of this study is therefore questionable. The study is better suited for a more specialized journal.

Authors' response:

We thank this reviewer for his/her stimulating remarks that prompt us to further investigate the relevance of the release of prominin-1–CMV.

First, the process of cell differentiation is an important issue in stem cell biology and understanding its mechanism might allow us to manipulate clinically the fate of primitive cells opening thus novel modalities for stem cell therapy. For this reason, we undertook the present investigation, and could demonstrate that, in contrast to neuro-epithelial progenitors, hematopoietic stem and progenitor cells released the stem cell marker prominin-1 via membrane vesicles that are derived from the endocytic pathway. To substantiate the initial set of data, we now provide additional evidence that the release of these vesicles can be stimulated by the provoked differentiation of hematopoietic stem and progenitor cells using phorbol ester, which results in a net depletion of the intracellular pool of prominin-1 (new Figs 5, 6). Therefore, manipulating the endocytic-exocytic pathway in vitro, and eventually in vivo, might help to modulate the fate (proliferation versus differentiation) of rare stem cells (e.g. those derived from cord blood), which might be clinically valuable.

Second, our new data (Fig 7) demonstrating that the prominin-1–CMV released by hematopoietic stem and progenitor cells are internalized by mesenchymal stromal cells, are functionally important to understand the intercellular communication occurring within the bone marrow niche. Such phenomenon is particularly relevant nowadays as recently highlighted: "Mesenchymal and haematopoietic stem cells form a unique bone marrow niche" (Méndez-Ferrer et al. (2010) Nature, 466:829-34).

Thus, we believe that the present work constitutes a significant contribution to our understanding of the molecular events underlying both, proliferation/differentiation of hematopoietic stem cells and their communication with neighbouring cells within the bone marrow niche, which is obviously medically important.

Reviewer 2 – Other Remarks:

In this manuscript (EMM-2010-00531), Bauer et al. study the releasing of small membrane vesicles by stem cells using a co-culture with multipotent mesenchymal stromal cells. They found that prominin-1, a cancer stem cell marker previously shown to be released through plasma membrane budding, is associated with vesicles produced from an endosomal compartment. Moreover, they suggest that quantitative reduction or loss of prominin-1-containing lipid domains from stem cells could be involved in their differentiation.

This manuscript represents a significant and well written piece of work, and I therefore recommend that it be accepted for publication in EMBO Molecular Medicine, with a few minor editions that could be implemented.

Authors' response:

We are delighted that this reviewer appreciates our efforts, and thank him/her for the minor comments and suggestions.

Figure1:

Regarding the larger electron dense membrane structures, is there a way to assess if these microvesicles are derived from the plasma membrane? Moreover, it is not clear to me whether the authors think that prominin-1-positive vesicles are strictly constituted by- or enriched with lipid rafts (cf. comment of Fig. S4). In any case, it would be interesting in Fig.1G to compare the lipid composition of prominin-1-positive and negative vesicles using C14-acetate labeling.

Authors' response:

Theoretically, an exhaustive proteome analysis of the large dense membrane structures recovered in the pellet of the sucrose gradient (Fig. 1B, fraction P) could be performed. Such particles could be immuno-isolated based on prominin-1 as we have performed for the smaller ones. The gained information might be instructive with regards to membrane constituents, and eventually tell us whether they are enriched in proteins that are solely expressed at the plasma membrane. However, such investigation would require a significant amount of material. With regard to lipid rafts, it is premature for us to claim that prominin-1–CMV are strictly composed or not of lipid raft constituents. We agree with the reviewer that it would be interesting to know the lipidome of prominin-1–CMV, and compare it to that of prominin-1–negative ones, but at the moment, we do not have such information. We are now concentrating our effort on the proteome (see below).

Figure 2:

In the manuscript (page 7, two last lines) Fig.2C and 2D are referred as 3C and 3D. In the figure, "d" should be replaced by "D". Even though the EM images are relatively poor in resolution, I would recommend avoiding defining the MVB limiting membrane (dot line). Moreover, it would have been informative to carry out immuno-EM using CD63 to strengthen the IF data.

Authors' response:

First, we are sorry for the wrong labelling, which is now corrected in the revised manuscript. Likewise, we replaced the EM images of prominin-1 by better ones, and removed the dot lines. As requested, we have performed the immuno-EM for CD63 for both, cells and membrane vesicles, and these new data are presented in the modified Fig 2 and Fig S1, respectively.

Figure 3:

As opposed to the vesicular 200,000g pellets situation, the 300g pellets corresponding to cells should have been loaded with the same amount of protein. That needs to be clarified. The presence of prominin-1 positive material in 1,200g and 10,000g pellets at day 14 should be discussed.

Authors' response:

In some experiments, we normalized the amount of non-differentiated versus differentiated cells (300g pellet), and consequently the corresponding volume in other fractions prior to loading. This is now illustrated by the comparable amounts of actin and a-tubulin detected in 300g pellet fractions (new Fig 5, panel A versus B). However, in order to be accurate when we compare one culture condition to another, we always evaluated the ratio of prominin-1 immunoreactivity found in 200,000g versus 300g pellets in a given condition.

The tiny amount of prominin-1 in 1,200 and 10,000 x *g* pellets observed after spontaneous differentiation of hematopoietic stem and progenitor cells (Fig 3A, bottom panel), which is interestingly also detected upon the provoked differentiation using phorbol ester (see new Fig 5B) might derive from either fragmentation of plasma membrane protrusions or relics of cytokinesis. We added in the revised manuscript (Results section) the following: "In contrast to prominin-1, neither actin nor a-tubulin were recovered in the 200,000 x *g* pellet (Fig 5), which rules out both fragmentation of plasma membrane protrusions (e.g. actin-base microvillus) and relics of cytokinesis (tubulin-based) in this fraction. The tiny amount of prominin-1 in 1,200 and 10,000 x *g* pellets might nevertheless derive from such membranes (see also Fig 3A) (Dubreuil et al, 2007; Giebel & Beckmann, 2007)".

Figure S4:

It would be informative to check other exosomal markers to characterize the prominin-1 positive and negative vesicles (e.g. Tsg101, hsc70...). Specifically, the presence of CD63 which is suggested by the authors to induce incorporation of syntenin into prominin-1 vesicles should be assessed.

Authors' response:

Indeed, we are currently performing a proteomic analysis of prominin-1–containing membrane vesicles. Interestingly, our preliminary data confirm the presence of syntenin in these vesicles (unpublished data). However, we are not claiming that the incorporation of syntenin within prominin-1–positive vesicles is due to its selective interaction with CD63. We have modified the text accordingly. With the proteome, we hope to make a follow-up story.

General comments:

Would it be possible to use lysosomal inhibitors during cell culture to assess the relative involvement of the exosomal pathway vs. lysosomal degradation during the differentiation process? What is the dynamics of the internal pool of prominin-1? Does it vary with differentiation and could it be possible to affect its presence by blocking endocytosis (e.g. dynasore...)?

Authors' response:

This is an interesting issue that we have not investigated yet. Indeed, we are evaluating whether phorbol ester-induced differentiation of hematopoietic stem and progenitor cells provokes the ubiquitination of prominin-1, which might drive it to endocytosis and sorting to the luminal vesicles

of multivesicular bodies for lysosomal degradation. In another cellular system, we could demonstrate that a fraction of prominin-1 might be the target of such post-translational modification (P. Janich, D.C., unpublished data).

Concerning the dynamics of the internal pool of prominin-1, we could demonstrate by confocal microscopy its preferential release concomitant to the cell differentiation induced by phorbol ester (see new Fig 6 and Fig S6, respectively, in the revised manuscript). However, the addition of 80 mM dynasore (Macia et al. (2006) Developmental Cell, 10:839; Rana et al. (2011) Inter J Biochem Cel Biol. 43:106) to hematopoietic stem and progenitor cells resulted in cell death under our experimental conditions (data not shown). We are currently investigating the dynamics of the intracellular transport of prominin-l in these cells by metabolic labelling and cell-surface biotinylation as reported for tetraspanin membrane proteins (Abache et al. (2007) J Cell Biochem, 102:650), and we hope to make another story with such detailed analyses.

Reviewer 3 – Other Remarks:

The paper by Bauer et al. reports on the mechanism of release of prominin-1/CD133- containing membrane vesicles upon hematopoietic stem/progenitor cell differentiation, employing an in vitro culture system of hematopoietic stem/progenitor cells on mesenchymal stroma cells as feeder.

Prominin-1/CD133 represents a surface marker that has been used in numerous studies for isolation and characterization of hematopoietic stem cells, yet prominin-1/CD133 function and its impact on stem cell phenotype is not understood. Thus, elucidating the molecular mechanism of prominin-1/CD133 action in stem cells is expected to open new avenues of influencing stem cell phenotype and function.

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This is an interesting paper and the conclusions reached are supported by the data shown. The experiments involve a number of demanding techniques that are well performed.

Authors' response:

Again, we are very glad to read that this reviewer also highly appreciates work.

I have only minor points:

The sub headings "Primary antibodies derived from the same species" and "Primary antibodies derived from different species" of the "Immunoflourescence and confocal microscopy" section in the "Materials and Methods" should be deleted, since they do not add to the intention of the study.

Authors' response:

These sub headings were removed.

If required parts of the "Materials and Methods" could be included as "Online Supporting Information", which would significantly shorten the body of the manuscript.

Authors' response:

We agree with the reviewer, and consequently we have significantly shortened the Materials and Methods section in the main body of the revised manuscript. However, in order to keep all necessary information we added them in a detailed Materials and Methods section in the Online Supporting Information.

2nd Editorial Decision 11 April 2011

Please find enclosed the final reports on your manuscript. We are pleased to inform you that your manuscript is accepted for publication and will be sent to our publisher to be included in the next available issue of EMBO Molecular Medicine if or once we have received your licenses (see below).

Congratulations on your interesting work.

Yours sincerely,

Editor
EMBO Molecular Medicine

REFEREE REPORTS:

Referee #2 - Other Remarks:

suitable for publication

Referee #3 - Other Remarks:

I only had minor points which the authors have now addressed.

