

ESPS Peer-review Report

Name of Journal: World Journal of Hematology

ESPS Manuscript NO: 6702

Title: Tbl3 encodes a WD40 nucleolar protein with regulatory roles in ribosome biogenesis

Reviewer code: 02446126

Science editor: Ling-Ling Wen

Date sent for review: 2013-10-28 13:34

Date reviewed: 2013-11-26 21:15

CLASSIFICATION	LANGUAGE EVALUATION	RECOMMENDATION	CONCLUSION
<input type="checkbox"/> Grade A (Excellent)	<input checked="" type="checkbox"/> Grade A: Priority Publishing	Google Search:	<input type="checkbox"/> Accept
<input checked="" type="checkbox"/> Grade B (Very good)	<input type="checkbox"/> Grade B: minor language polishing	<input type="checkbox"/> Existed	<input type="checkbox"/> High priority for publication
<input type="checkbox"/> Grade C (Good)	<input type="checkbox"/> Grade C: a great deal of language polishing	<input type="checkbox"/> No records	<input type="checkbox"/> Rejection
<input type="checkbox"/> Grade D (Fair)		BPG Search:	<input type="checkbox"/> Minor revision
<input type="checkbox"/> Grade E (Poor)	<input type="checkbox"/> Grade D: rejected	<input type="checkbox"/> Existed	<input type="checkbox"/> Major revision
		<input type="checkbox"/> No records	

COMMENTS TO AUTHORS

Revision for the World Journal of Hematology Article title: Tbl3 encodes a WD40 nucleolar protein with regulatory roles in ribosome biogenesis. Authors provided a direct evidence that murine transduction-beta-like 3 (tbl3) protein is targeted to compartment of nucleoli and plays an important role in synthesis of 47S pre-rRNA. Thus, this protein is important for regulation of ribosome biosynthesis. Manuscript is well written and a new information about localization of tbl3 protein in nucleolus is very interesting from the complex view on proteome of nucleolus. Nevertheless, I have some remarks and suggestions that authors should take into consideration: 1. Is it possible to also see some level of tbl3 protein in nucleoplasm or is it prominent protein of nucleolus? I guess that certain level of this protein must in be in nucleoplasmic fraction. 2. Exogenous level and localization of tbl3-tagged by EGFP in nucleolus should be verified on endogenous level, by appropriate antibody. This experimental approach is necessary to be sure that result observed in Fig. 3D is not affected by GFP-technology. 3. I would like to recommend overlay of Fig. 3C and 3D to see of how tbl3-positive compartment of nucleoli is surrounded by DAPI-dense chromocenters. 4. I will be nice to see co-localization of tbl3 with other well-known proteins of nucleolus. 5. Western blots (Fig. 3E) should also show endogenous tbl3 when authors will use appropriate antibody. Taken together, I would like to recommend this paper for publication in the World Journal of Hematology, but authors should address above mentioned items. I recommend major revision of this paper.

ESPS Peer-review Report

Name of Journal: World Journal of Hematology

ESPS Manuscript NO: 6702

Title: Tbl3 encodes a WD40 nucleolar protein with regulatory roles in ribosome biogenesis

Reviewer code: 01453224

Science editor: Ling-Ling Wen

Date sent for review: 2013-10-28 13:34

Date reviewed: 2014-01-13 09:25

CLASSIFICATION	LANGUAGE EVALUATION	RECOMMENDATION	CONCLUSION
<input type="checkbox"/> Grade A (Excellent)	<input checked="" type="checkbox"/> Grade A: Priority Publishing	Google Search:	<input type="checkbox"/> Accept
<input type="checkbox"/> Grade B (Very good)	<input type="checkbox"/> Grade B: minor language polishing	<input type="checkbox"/> Existed	<input type="checkbox"/> High priority for publication
<input checked="" type="checkbox"/> Grade C (Good)	<input type="checkbox"/> Grade C: a great deal of language polishing	<input type="checkbox"/> No records	<input type="checkbox"/> Rejection
<input type="checkbox"/> Grade D (Fair)		BPG Search:	<input type="checkbox"/> Minor revision
<input type="checkbox"/> Grade E (Poor)	<input type="checkbox"/> Grade D: rejected	<input type="checkbox"/> Existed	<input type="checkbox"/> Major revision
		<input type="checkbox"/> No records	

COMMENTS TO AUTHORS

Tbl3 is a mammalian protein with thirteen WD40 repeat protein-protein interaction motifs. While the function of its yeast homolog Utp13 in ribosome biogenesis is known, the role of Tbl3 in mammalian cells remains to be studied. In this manuscript, the authors show that Tbl3 is ubiquitously expressed and is localized to nucleoli, a site of ribosome biogenesis, when ectopically expressed. Depletion of Tbl3 by RNAi in MPRO murine promyelocyte cells resulted in increased level of newly-synthesized 47S pre-rRNA without affecting the rate of pre-rRNA processing or cellular level of ribosomes. They further show that proliferation of MPRO cells as well as LAP3 fibroblasts is repressed by depletion of Tbl3. All the experiments are neatly done and therefore convincing. In addition, it is an interesting observation that depletion of Tbl3 upregulates 47S pre-rRNA transcription without affecting rRNA processing, because a previous study showed that yeast Utp13 mutant has a severe defect on rRNA processing. However, the molecular mechanism underlying Tbl3-mediated repression of rRNA transcription is not provided. Specific points: 1. Although the scope of the journal is hematology, it is unclear from INTRODUCTION how this study is related to hematology. The relationship between ribosome biogenesis and hematology, which is explained in the last paragraph of DISCUSSION, should be described in INTRODUCTION. 2. In MATERIALS AND METHODS, the authors describe that they made two independent shRNAs for Tbl3. In RNAi experiments shown in RESULTS, however, they used only one shRNA to deplete Tbl3. Therefore, the possibility of an off-target effect can not be excluded. They should show that similar results can be obtained by a second shRNA. Alternatively, they should test whether the defects in Tbl3 shRNA-transfected cells can be rescued by co-transfection of shRNA-resistant ectopic Tbl3. 3. The

experiments in Tbl3-depleted cells suggested that in the absence of Tbl3, either 47S pre-rRNA transcription is upregulated or pre-rRNA processing is repressed. The authors prefer the former possibility because the ratio of pre-rRNA and mature rRNAs (e.g., 28S and 18S rRNAs) is similar between control and shRNA-transfected cells. To confirm this possibility, they should examine whether RNA polymerase inhibitors (e.g., actinomycin D) block the increase in the level of 47S pre-rRNA in Tbl3-depleted cells. 4. Depletion of Tbl3 in promyelocytes and fibroblasts resulted in a reduced number of cells after several days of culture. Is it due to decreased rate of cell proliferation as the authors suggest, or increased rate of apoptotic cell death? 5. In Fig. 4, the subcellular localization pattern of some typical Tbl3 mutants which did not localize to nucleoli should be shown in the figure or described in the text. In addition, although deletion mutants of Tbl3 with intact WD40 repeats failed to localize to nucleoli, the authors suggest in RESULTS and DISCUSSION that Tbl3 is possibly localized to nucleoli through WD40 repeats. This does not make sense to this reviewer.

ESPS Peer-review Report

Name of Journal: World Journal of Hematology

ESPS Manuscript NO: 6702

Title: Tbl3 encodes a WD40 nucleolar protein with regulatory roles in ribosome biogenesis

Reviewer code: 01573121

Science editor: Ling-Ling Wen

Date sent for review: 2013-10-28 13:34

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CLASSIFICATION	LANGUAGE EVALUATION	RECOMMENDATION	CONCLUSION
<input type="checkbox"/> Grade A (Excellent)	<input type="checkbox"/> Grade A: Priority Publishing	Google Search:	<input type="checkbox"/> Accept
<input type="checkbox"/> Grade B (Very good)	<input checked="" type="checkbox"/> Grade B: minor language polishing	<input type="checkbox"/> Existed	<input type="checkbox"/> High priority for publication
<input type="checkbox"/> Grade C (Good)	<input type="checkbox"/> Grade C: a great deal of language polishing	<input type="checkbox"/> No records	<input type="checkbox"/> Rejection
<input checked="" type="checkbox"/> Grade D (Fair)		BPG Search:	<input type="checkbox"/> Rejection
<input type="checkbox"/> Grade E (Poor)	<input type="checkbox"/> Grade D: rejected	<input type="checkbox"/> Existed	<input checked="" type="checkbox"/> Minor revision
		<input type="checkbox"/> No records	<input type="checkbox"/> Major revision

COMMENTS TO AUTHORS

The manuscript "Tbl3 encodes a WD40 nucleolar protein with regulatory roles in ribosome biogenesis" addresses the structural and partly functional characterization of the protein tbl3. This protein is the ortholog of the yeast utp13 protein shown to be part of the SSU processome involved in rRNA processing. The report includes data on general expression and subcellular localization that clearly indicated the similarity with the yeast protein. The following attempt to identify a nucleolar localization sequence failed to distinguish a specific region. The authors then proceeded to address the possible function of tbl3 in rRNA processing. To do so, they knocked down tbl3 expression by transfecting shRNA and analyzing rRNA synthesis and processing. The results showed that, unexpectedly, tbl3 depletion caused an increase in rRNA synthesis and did not affect processing. Further analysis revealed a general growth inhibitory effect. The experimental approach of the study is a classical characterization of a previously not studied (in mammals) protein, carried out with high standard technical expertise (mostly). For this reason, the results could be helpful for the scientific community and deserve dissemination. It is, however, disappointing that the authors did not attempt to better define the results they obtained. Especially because tbl3 depletion led to two unexpected findings: 1) no effect on rRNA processing, 2) increase of rRNA transcription. In combination with the observed cell growth inhibition, these results points to unusual scenarios that would require a little more convincing data. For instance the authors observe an increase of 47S level, no change in rRNA processing and no change in the steady state level of mature rRNA. The implication (not clearly stated) is that there is an increase of rRNA turnover. This could be easily confirmed with the available experimental setup (see below). I think that the manuscript would be

substantially improved if the authors could address at least some of the following suggestions: 1) The authors should provide more quantitative data on the lack of an effect of *tbl3* depletion on rRNA processing. For instance with a graphical representation of the ratio between processing intermediates, possibly as averages of multiple quantifications. 2) The authors should measure the decay rate of 47S (using the data of Fig. 5) to confirm that it is not affected by *tbl3* depletion. 3) They should also measure the decay rate of mature rRNA by extending the chase time of the experiment shown in Fig. 5. This should confirm an increase of rRNA turnover caused by *tbl3* depletion. 4) The authors mention that longer time of selection of transfectants allows the recovery of growth rate. They should check if this is due to the recovery of 47S level. This analysis could give strength to the proposed causal correlation between 47S overexpression and cell growth inhibition. 5) The steady state level of rRNA after depletion of *tbl3* should be analyzed by a more quantitative technique (Northern, qRT-PCR) other than ethidium bromide staining