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SET8 promotes epithelial-mesenchymal transition and confers TWIST dual transcriptional activities

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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 01 June 2011

Thank you very much for submitting your research manuscript on the Twist/Set-8 interplay for consideration to The EMBO Journal editorial office.
I do enclose the comments from three scientists below that you will recognize are overall supportive of your study. However, they still demand some clarifications and extensions, particularly to corroborate the functional relevance and co-operativity between Twist and Set8. Specifically, ref#1 strongly encourages the inclusion of xenograft experiments and requests expansion of the statistical as well as IHC-analyses towards Twist. Together with addressing the other issues raised by the referees (image quality, endogenous ChIP, statistical presentation), we would be delighted to receive an adequately revised version for final scientific assessment.

Please be reminded that it is EMBO Journal policy to allow a single round of revisions only and that the ultimate decision on acceptance depends on the content and strength of the final version of your manuscript.

Yours sincerely,

Editor
The EMBO Journal

REFEREE REPORTS:

Referee #1:

In the present manuscript, Yang and colleagues highlight SET8 as a novel transcriptional co-regulator essential for Twist in promoting EMT and thereby cell motility. They demonstrate that the

chromatin modifier is recruited by Twist to the promoting sequences of its two CDH1 and CDH2 target genes.
1. Based on IP and GST binding assays, the authors first clearly demonstrate that the two partners directly interact and that are both essential for the H3K4 methylation and activation of both Twist target genes. Experiments using either ectopic expression or knock-downs (through RNA interference) have been combined. It would be judicious to additionally include a comparison of the expression levels of the endogenous Twist1 and SET8 in the two studied breast cell lines. This will be of particular help to appreciate the observed moderated or more pronounced variations in gene expression.
2. MDA-MB231 cells are already known to display invasive properties. The choice of the cell line is therefore questionable. Xenograft experiments employing the MDA-MB231 derivatives (expressing either SET8 and/or Twist) should be included to further support a cooperative effect of both proteins in providing cells with invasive or metastatic capabilities.
3. While authors defend a cooperative effect between SET8 and Twist in regulating target gene expression and EMT, they restrict their statistical analysis to SET8.. Although they convincingly demonstrate a relationship between SET8 expression and the metastatic behaviour, this observation does not support a cooperative effect between the two partners. Including Twist expression as an additional parameter is required. Additionally, IHC experiments could be performed to evaluate a potential co-expression of both proteins at the invasive front of tumours.

Minor point: please clarify whether experiments were performed with either Twist1 or Twist2

Referee #2:

Yang and colleagues provide a succint and complete description of an association (physical and functional) between SET8 and TWIST that impacts gene regulation and is deemed important to EMT in breast cancer.
The authors provide compelling biochemical evidence for interaction of TWIST with Set8 in vivo and in vitro. Manipulation of TWIST and Set8 in breast cancer cell lines is utilized to explore the functional impact of this association. the authors document that overexpression of TWIST and Set8 in MCF7 cells leads to phenotypic and molecular alterations consistent with EMT and that manipulation of the same factors in MDA231 alters invasive growth properties.
Molecular analysis of targets suggests that TWIST can serve both as an activator (N-cadherin) and repressor (E-cdherin) of transcription - amazingly both processes are dependent on Set8 and on catalytic function of Set8.
A small cohort of primary breast tumors analyzed for SET8 expression yields correlations of Set8 with metastasis that are entirely consistent with the biochemical and molecular analyses.

I offer the authors the following comments:
1. Several polypeptides are described as copurifying with FLAG-TWIST. Some appear reasonable in that there stoichiometry is consistent with that of TWIST. Others would appear at first glance to be contaminants that are not present at stoichiometric levels with TWIST (ie LATS2, DDEF2). The authors should either not refer to these as copurifying to a substantial extent OR explain why they are not in stoichiometry with TWIST.
2. A bit more detail on methods of preparation of nuclear extract would assist interested readers in evaluating the biochemistry presented.
3. The IF images in Figure 3A are simply not visible in the version of this manuscript I received. Please submit images in which the fluorescence is readily visible to the reader.
4. Quantitative RT-PCR would be useful in the experiments in figures 3B and 3C
5. The invasion assay depicted in Figure 4 is poorly described both in the text and figure legends. it would greatly assist the reader if a bit more detail were added to the figure legend.
6. finally, Vertino and colleagues have described methylation of ER-alpha in MCF7 modulating its activity (Molecular Cell, vol 30, pp 336-347). this important result should be discussed in light of the current findings.

Referee #3:

In "SET-8 promotes EMT and confers TWIST dual transcriptional regulation activities", Yang et al.

demonstrate that TWIST associated with interacting proteins, including SET-8. The authors validate the interactions and demonstrate that TWIST recruits SET-8, which impacts invasion and gene expression (positively and negatively). Overall, the paper is very logical and the data is straightforward. There are a couple minor points that should be addressed. Otherwise, this paper is suited for EMBO J.

Minor points:
1. Why whole cell extract. The HDAC repressor complex was not present and other cytosolic proteins were there. This should be addressed in the discussion.
2. Figure 3A is hard to see. Better images.
3. Need to include the paretnal in panel 3C.
4. Since TWIST can be ChIPPed, include an endogenous co-IP.
5. Why are there different outcomes at targets with these two components. Please discuss the possibiliites.
6. Need to compare histone modification ChIPs to corresponding parent histone (H4K20me1 to H4).
7. Need to indicate error bars and stats on the figures throughout the manuscript.

1st Revision - Authors' Response 27 August 2011

Response to Referees' comments

Referee #1:

1. Based on IP and GST binding assays, the authors first clearly demonstrate that the two partners directly interact and that are both essential for the H3K4 methylation and activation of both Twist target genes. Experiments using either ectopic expression or knock-downs (through RNA interference) have been combined. It would be judicious to additionally include a comparison of the expression levels of the endogenous Twist1 and SET8 in the two studied breast cell lines. This will be of particular help to appreciate the observed moderated or more pronounced variations in gene expression.

Authors: We appreciate the referee for this comment. We did examine the endogenous mRNA and protein levels of TWIST1 and SET8 in two breast cancer cell lines with different invasive capacity. The results showed that the invasive cell line MDA-MB-231 has a slight higher SET8 protein expression than the non-invasive MCF-7 cells, and TWIST protein level is high in MDA-MB-231 cells and extremely low in MCF-7 cells. qPCR analysis of TWIST and SET8 expression is consistent with their protein expression patterns in two cell lines. The data have been added to the revision as Figure 3A.

2. MDA-MB231 cells are already known to display invasive properties. The choice of the cell line is therefore questionable. Xenograft experiments employing the MDA-MB231 derivatives (expressing either SET8 and/or Twist) should be included to further support a cooperative effect of both proteins in providing cells with invasive or metastatic capabilities.

Authors: Following the referee's suggestion, xenograft experiments have been conducted by orthotopically inoculating MDA-MB-231-Luc-D3H2LN with either overexpression and/or depletion of SET8 and/or Twist into the abdominal mammary fat pad of 6-week-old immunocompromised female BALB/c mice. The results showed that SET8 cooperates with TWIST in enhancing the lung metastasis and cell intravasation in the model. The data have been added to the revision as Figure 6.

3. While authors defend a cooperative effect between SET8 and Twist in regulating target gene expression and EMT, they restrict their statistical analysis to SET8. Although they convincingly demonstrate a relationship between SET8 expression and the metastatic behaviour, this observation does not support a cooperative effect between the two partners. Including Twist expression as an additional parameter is required. Additionally, IHC experiments could be performed to evaluate a potential co-expression of both proteins at the invasive front of tumours.

Authors: Based on the referee's suggestion, we have included *TWIST* expression analysis in the experiment and the data have been added to the revision as Figure 7B and 7D. *TWIST* expression by qPCR in samples from the breast normal tissue, non-metastatic breast cancer (NMBC), and metastatic breast cancer (MBC) showed that *TWIST* expression is up-regulated in metastatic breast tumors (Fig. 7B), and there appeared to be a progressive increase in *TWIST* mRNA levels from normal to metastatic samples, suggesting that *TWIST* is positive correlation with metastatic capacity. Further, we also tested the relationship between *SET8* and *TWIST* expression in non-metastatic or metastatic breast tumors. We revealed that a positive correlation of *SET8* and *TWIST* expression in MBCs with a Pearson correlation coefficient of 0.5936 ($p<0.0001$) and a Spearman correlation coefficient of 0.5800 ($p<0.0001$), and that both have no statistical correlation in NMBCs (Fig. 7D). Meanwhile, complying with referee's instruction, we conducted immunohistochemistry (IHC) staining of endogenous SET8 and TWIST proteins in sample sections of NMBCs, MBCs, and metastatic lymph nodes (MLNs), and found that these two proteins are highly expressed in MBCs (i.e. vascular tumor embolism) and MLNs compared to those in NMBCs. The data have been added to the revision as Figure 7E.

Minor point:

Please clarify whether experiments were performed with either Twist1 or Twist2.

Authors: Our experiments were performed with TWIST1. TWIST1 is also referred to as TWIST (see "NCBI"-"Search catalog"-"Gene"-"TWIST1, homo sapiens"-"Other Aliases"-"TWIST"). We have added this annotation in "Introduction" of the revision.

Referee #2:

1. Several polypeptides are described as copurifying with FLAG-TWIST. Some appear reasonable in that there stoichiometry is consistent with that of TWIST. Others would appear at first glance to be contaminants that are not present at stoichiometric levels with TWIST (i.e. LATS2, DDEF2). The authors should either not refer to these as copurifying to a substantial extent OR explain why they are not in stoichiometry with TWIST.

Authors: We have deleted the reference to LATS2 and DDEF2.

2. A bit more detail on methods of preparation of nuclear extract would assist interested readers in evaluating the biochemistry presented.

Authors: A more detailed procedure for preparation of cell nuclear extracts has been added to the revision.

3. The IF images in Figure 3A are simply not visible in the version of this manuscript I received. Please submit images in which the fluorescence is readily visible to the reader.

Authors: The experiments have been repeated and the images have been replaced.

4. Quantitative RT-PCR would be useful in the experiments in figures 3B and 3C.

Authors: Quantitative RT-PCR results corresponding to Western blot in Figures 3B and 3C of the previous version have been added to the revision as Figure 2B (right panel) and 2C (right panel).

5. The invasion assay depicted in Figure 4 is poorly described both in the text and figure legends. it would greatly assist the reader if a bit more detail were added to the figure legend.

Authors: Detailed descriptions of the invasion assay have been added to the revision.

6. Finally, Vertino and colleagues have described methylation of ER-alpha in MCF7 modulating its activity (Molecular Cell, vol 30, pp 336-347). This important result should be discussed in light of the current findings.

Authors: The issue has been discussed in the "Discussion".
RE: Manuscript EMBOJ-2011-78079

Response to Referees' comments

Referee #3:

Minor points:

1. Why whole cell extract. The HDAC repressor complex was not present and other cytosolic proteins were there. This should be addressed in the discussion.

Authors: The issue has been discussed in the revision.

2. Figure 3A is hard to see. Better images.

Authors: The experiments have been repeated and the images have been replaced.

3. Need to include the paretnal in panel 3C.

Authors: We have added the parental control to panel 2C in the revision.

4. Since TWIST can be ChIPPed, include an endogenous co-IP.

Authors: We performed endogenous co-IP with the TWIST antibodies (Figure 1D, lower panel in the revision).

5. Why are there different outcomes at targets with these two components. Please discuss the possibilities.

Authors: The issue has been discussed in the revision.

6. Need to compare histone modification ChIPs to corresponding parent histone (H4K20me1 to H4).

Authors: The data have been presented according to the suggestion.

7. Need to indicate error bars and stats on the figures throughout the manuscript.

Authors: We have added error bars and stats in the revision.

2nd Editorial Decision 09 September 2011

The paper has been re-reviewed by one original referee with no further comments.

