Reviewer's report

Title: Potential cancer-related role of circadian gene TIMELESS suggested by expression profiling and in vitro analyses

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Reviewer: Filippo Tamanini

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Mao et. al. explore the role of the TIMELESS protein in cancer by analyzing its expression profile in online available gene expression databases and by characterizing the impact of its downregulation by siRNA on the rate of proliferation of breast (MCF-7) and cervical (HeLa) cancer cells.

The description of a priori chosen gene (Timeless) that is found differentially expressed in cancer specimens, does not say much about its functional and biological relevance relative to cancer, unless it is supported by strong experimental work. The required functional support is partly provided here by Timeless siRNA knockdown experiments, which however remain not sufficient and rather superficial in the interpretation of the results. Finally, some experiments presented in this manuscript are not properly controlled as detailed below.

Major Issues:

1. The question about the expression level of TIMELESS relative to cancer prognosis is interesting, but overall it has been already addressed by the same authors with a similar approach and conclusion (Fu et. al Mol Carcinog. 2012). Therefore, the gene expression analysis is not novel, although it has been extended here to more cancer studies.

2. In the present manuscript the authors perform experiments using only one siRNA against TIMELESS. This is problematic, because any loss-of-function study requires to be performed with two independent siRNAs to compensate for background effects caused by off targeting.

3. The cell proliferation experiments are not convincing. Please, show whether, or not, the low level of proliferation may be caused simply by increased apoptosis in absence of TIMELESS. Moreover, it would be great for the article to extend this part of the study with more experiments that link the downregulation/overexpression of TIMELESS to in vitro cancer properties (cell mobility, invasion, colonies formation in soft agar, comet assays, etc), as it was shown previously by the authors for CRY2.

4. We know already by other studies (see Engelen et al PlosOne 2013) that TIMELESS expression is particularly elevated in cell/tissues undergoing active proliferation. Therefore, it is somehow not surprising that TIMELESS expression
is high in cancer tissues, which are by nature highly proliferative. Please, provide some comments and references on this point.

Minor issues

1. Please provide the sequences of the siRNA oligos used. Including the standard deviation and significance of Timeless expression by qPCR after downregulation by siRNA.

2. More discussion and potential experiments should be elaborated around the genes that are found differentially expressed after Timeless knockdown (which in my opinion is the most relevant and novel part of the study). Please, validate those genes by qPCR including standard deviations.

**Level of interest:** An article whose findings are important to those with closely related research interests

**Quality of written English:** Acceptable

**Statistical review:** No, the manuscript does not need to be seen by a statistician.

**Declaration of competing interests:**

I declare that I have no competing interests’