Reviewer’s report

Title: Indirubin inhibits Wnt/β-catenin signal pathway via promoter demethylation of WIF-1

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Reviewer's report:

The publication with the title "Indirubin inhibits Wnt/β-catenin signal pathway via promoter demethylation of WIF-1" from Lui and colleagues describes the effect of indirubin on the Wnt/β-catenin signaling in HaCaT cells. The topic is interesting; however there are some major points that are problematic concerning the interpretation of this work.

1) Although the title is neutral already the background of the abstract refers to psoriasis. However the effect was only tested on HaCaT cells that are not good models for psoriasis (Soboleva et al. Genetically predetermined limitation in HaCaT cells that affects their ability to serve as an experimental model of psoriasis. Russian J of Genetics, 2014.10:1081-1089) and the effect was not tested on psoriasis-like keratinocytes, so that a statement to psoriasis is not possible. Especially the sentence in the conclusion on page 17 that "these results suggest the factors by indirubin to ameliorate psoriasis, and they provide experimental evidence for indirubin treatment for psoriasis" cannot be drawn with these data, because no psoriasis model was used for the studies. Furthermore in the background as part of the introduction it is mentioned that the underlying cause of psoriasis is unclear and the treatment is largely still in exploratory stages. However nothing is mentioned on IL-17 and other cytokines as leading cytokine of psoriasis (e.g. Gaffen et al. The IL-23-IL-17 immune axis: from mechanisms to therapeutic testing. Nature Reviews Immunology.14:585-600) and there are many treatment optioned concerning systemic therapy of antiinflammatory targets as well as biologics and topic therapies. So many different treatment options are approved (Rendon et al Psoriasis pathogenesis and treatment. Int J Mol Sci. 2019.20(6):1475).

2) Some essential details in the M&M section are missing, e.g. the transfection method for the luciferase reporter gene assay, especially as HaCaT cells are not that easy to transfect.

The test principle of the cell viability assay should be added on page 7.

The information to the HaCaT cells that stand for human, adult, low calcium, high temperature, human adult skin keratinocytes are written with some capital letters. This cell line was characterized in the following publication: Boukamp et al. Normal keratinization in a spontaneously immortalized aneuploid human keratinocyte cell line The Journal of Cell Biology.1988 106 (3): 761-771. These cells cannot be bought by ATCC but for example by CLS (cell line services). If they are bought by CLS this cell line has already passage 38, therefore it is not possible to use the first three passages for all experiments. There are some fundamental errors.
3) The first sentence in the results section sounds confusing and should for example be changed to "we previously detected a reduced expression of…

Concerning figure 1b in the legend it is described that mRNA expression is shown however the description of the y-axis shows gray scale value for protein versus GAPDH. So it seems that the density of the bands of the Western blot in figure 1a are measured.

In figure 1c it is mentioned that the mRNA expression of wif-1 was promoted in HaCat cells by ELISA. If an ELISA was performed then not the mRNA was measured. It would be easier to change promote to increase.

In figure 1d the description low, medial and high and in figure 1e and f the description of A; B; C; D should be explained.

4) In the results section DNMT1 as DNA methyltransferase should be explained again.

On page 12 the first sentence is misleading: "To analyze the relationship between wif-1 and indirubin, DNMT1, a luciferase reporter assay was carried out" leads to the impression that DNMT1 is a luciferase reporter assay.

On page 13 the description that cells in the G0/G1 phase was large should be mentioned more precisely in addition of the percentage the cells that are in the G0/G1 phase.

5) The headline that indirubin treatment reduces the expression of key proteins on HaCat cells should be explained in more detail. Why are involucrin, keratin 17, TGase I chosen as key proteins in HaCaT cells? What do they detect? Why was keratin 17 tested? Involucrin are early and loricrin late differentiation markers. This should be outlined and explained.

6) In the discussion line 6 it is stated "to explore the potential mechanism of psoriatic keratinocytes hyperproliferation, we explored the role of wif-1 in the pathology of psoriasis…

This is not true as HaCaT cells are no psoriatic keratinocytes but an immortalized normal keratinocyte cell line (see arguments under point 2). Primary keratinocytes should ideally be treated with cytokines to create a psoriasis-like cell type to be able to make a statement to psoriasis.

7) In the discussion on page 16 line 6 it is stated that one of the most important signaling pathway to induce psoriasis is the wnt signaling pathway. As reference the paper written by Lin et al (Gene 2017) is mentioned (reference 20), however this publication deals with gallbladder cancer and not psoriasis. Furthermore at least IL-17 as key cytokine of psoriasis should be discussed in this section.

8) on page 15 in the first sentence of the conclusion it is mentioned that the expression level of wif-1 was lower in HaCaT cells. "Lower" indicates a comparison but it is not mentioned with what HaCaT cells were compared.

In general the central theme that the data provide evidence for psoriasis treatment and might be relevant for scientists or medicines are missing. Several experiments were described such as western blot, luciferase reporter assays, gene knock-down experiments but the explanation of the experiments and the conclusions from the experiments are insufficient.
Minor effects:
In the abstract there is a typing error in line 39 NDMT1 instead of DNMT1

In page 3 is stated that indirubin can be found in both healthy and disease-affected urine. If indirubin can be found in the uterine, the person is not healthy there is an infection with bacteria, isn't it.

In the background section of the introduction the principal wnt-signaling pathway should be described a bit in detail

JAK/STAT signal pathway is written in capital letters
Page 13 line 28 inolucrine should be changed in involucrin.
Page 12 line 26 or page 15 line 15 "when" should be written in small letters.

Are the methods appropriate and well described?
If not, please specify what is required in your comments to the authors.

No

Does the work include the necessary controls?
If not, please specify which controls are required in your comments to the authors.

Yes

Are the conclusions drawn adequately supported by the data shown?
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