Reviewer's report

Title: Targeting of human interleukin-12B by small hairpin RNAs in xenografted psoriatic skin

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Reviewer: Patrick Zeeuwen

Reviewer's report:

This manuscript entitled “Targeting of human interleukin-12B by small hairpin RNAs in xenografted psoriatic skin” by Bak et al. is a clear and well-written report, which followed up their previous study on therapeutic applicability of targeting TNF-alpha mRNA for RNAi-mediated knockdown by anti-TNF-alpha shRNAs delivered by lentiviral vectors to xenografted psoriatic skin (Mol Ther 2009, 17:1743-1753).

In this current report the authors describe the development of a novel lentiviral vector that can be used for cloning of shRNA oligonucleotides, which is a valuable tool for knockdown studies using lentiviral shRNA delivery. Furthermore they tested whether targeting of IL12B mRNA by RNAi-mediated degradation in xenografted human psoriatic skin is therapeutically relevant. They showed stable knockdown of IL12B and a reduced epidermal thickness in the xenografted skin. However, anti-IL12B shRNAs did not improve the psoriatic phenotype, which was evaluated by only a semi-quantitative clinical scoring. The authors concluded that the therapeutic potential of targeting IL12B at the RNA level in psoriasis is questioned.

I think the authors sell themselves short with this conclusion, as other hallmarks of the psoriatic lesion (see comment#2) that possibly could show an (pre)improvement were not tested in this study (or were they?)

Discretionary revisions

1) In the introduction/background of this paper the authors describe the role of a dysregulated immune system as a main cause of psoriasis. However, recent publications have reported that genetic factors contribute to susceptibility for psoriasis (e.g. HLA-Cw6, IL12B, IL23R, LCE3B/C). The authors should include these data in the introduction.

2) shIL12B-encoding lentiviral vectors did not seem to have an effect on the clinical phenotype of the psoriatic skin. However, a reduction of epidermal thickness in grafts injected with shIL12B-encoding lentiviral vectors was shown. In the discussion the authors note that “the clinical observation is only a superficial score, and cannot reflect the disease status in the deeper layers” and “a scale that has yet not sloughed off may hide a more alleviated disease state of the skin, but is not registered in the clinical score”. I suggest that the authors could compare the untreated skin grafts with the grafts treated by
shIL12B-encoding lentiviral vectors for other hallmarks of psoriasis, like proliferation status (Ki-67) and epidermal gene expression (keratin 16, elafin, hBD2), using immunohistochemistry or real-time quantitative PCR, as both paraffin-embedded grafts as well as material for qPCR is available. Why didn’t they? It is also known that anti-IL12 downregulates mRNA expression of type 1 cytokines in psoriasis (Toichi, et al. 2006. J. Immunol. 177: 4917–4926). This could also be studied to strengthen or invalidate the conclusions about the questioned therapeutic potential of targeting IL12B mRNA.

3) As a positive control in their xenograft study the authors use Betnovat, which also did not show a significant improvement of the psoriatic phenotype. Is this the right control for this experiment? Why didn’t they use the monoclonal antibody against IL12B (Ustekinumab) as a positive control?

4) There is no discussion why potent shRNAs from literature (for example shIL12B#2, Roth et al. ref 30) do not work in the present model.

5) Figure 5 should be improved by adding immunohistochemical pictures of the in vivo knockdown of IL12B and TNF-alpha in xenografted psoriatic skin.

Minor essential revisions

• Page 5, line 17+18: “Our research……xenografted psoriatic skin” could be removed (same sentence in text above: page 5, line 6 + 7).
• Page 11, line 17: PBS/-?
• Page 18 line 3: ref[49] doesn’t exist
• There is no reference to figure 4a in the text

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