Reviewer's report

Title: Peripheral T-lymphocytes express WNT7A and its restoration in leukemia-derived lymphoblasts inhibits cell proliferation

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Reviewer: Philipp Hemmati

Reviewer's report:

In their manuscript “Peripheral T-lymphocytes express WNT7A and its restoration in leukemia-derived lymphoblasts inhibits cell proliferation” Ochoa-Hernandez et al. investigate the expression of WNT7A in normal peripheral blood mononuclear cells (PBMCs), leukemia cell lines, and blood samples from patients with acute lymphoblastic leukemia (ALL) by quantitative real-time PCR (qPCR). They observed an increased expression of WNT7A in CD3+ cells (as opposed to CD19+ cells) from healthy control individuals, which can be markedly decreased by stimulation with PHA. Leukemic cell lines, i.e. Jurkat, CEM, K562 and HL60, display low levels of WNT7A mRNA. Likewise, samples obtained from patients with ALL exhibit low levels of WNT7A as compared to PBMCs obtained from healthy control individuals. Finally, the authors show that the forced expression of WNT7A by a lentiviral vector in Jurkat cells or the culture of Jurkat cells with rhWNT7A slows proliferation and triggers a decrease in c-Jun and Fra-1 mRNA expression.

The manuscript is well written and the data are presented in a clean manner. The finding that leukemic cell lines as well as leukemic cells isolated from patients with ALL display remarkably low levels of WNT7A mRNA is interesting. Strikingly, re-expression of WNT7A by lentiviral gene transfer into Jurkat cells or addition of recombinant human WNT7A slows cellular proliferation. The authors conclude that this may point out to a potential role of WNT7A in the regulation of leukemic cell growth, as has been proposed for lung cancer cells by others before (Winn et al., JBC 2005 & 2006).

However, the study has some drawbacks: Firstly, the authors exclusively investigated WNT7A. Secondly, analyses were performed at the mRNA level by qPCR for the most part. Thirdly, the mechanism by which WNT7A blocks cellular proliferation is hardly addressed and remains enigmatic.

Major remarks:

1. It would be interesting to know whether the expression of other WNT family members, such as WNT3, WNT4, WNT5A, or WNT10B, is unaltered in leukemia cell lines or primary samples from patients with ALL. This would strengthen the author’s hypothesis that WNT7A plays a prominent role in regulating proliferation of lymphocytes or leukemia-derived lymphoblasts.

2. Figure 1B: I assume that the bars represent data +/- SD obtained by analyzing samples of blood, PBMCs, CD3+ or CD19+ cells of several individuals. If so, it
would be more adequate to show box (as in Figure 3A) or scatter plots. How many individuals were analyzed?

3. Figure 5A: The authors use the optical density (OD) of cell cultures as a surrogate for cellular proliferation over a prolonged period of time, i.e. up to 6 days. Was there any difference in cell viability between empty-vector transduced vs WNT7A-expressing cells? Does expression of WNT7A abrogate the clonogenic growth of the cells?

4. Figure 5B: A control, i.e. vehicle-treated PBMCs, is missing in this experiment. Do cells treated with exogenous WNT7A lose viability?

5. Was there a decrease in the expression of c-Jun or Fra-1 at the protein level in Jurkat cells designed to express WNT7A? Is there a change in phosphorylation of c-Jun upon expression of WNT7A or addition of rhWNT7A to the culture medium?

Minor remarks:

1. The authors should provide more information on the ALL samples used in their experiments: Adults or infants? What subtype of ALL, e.g. B vs T lineage? Blast count in peripheral blood? Were the samples taken at initial diagnosis?

2. Figure 5A/B: What pLVX-WNT7A-clone was used in these experiments?

**Level of interest:** An article of importance in its field

**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.