Reviewer's report

Title: Inhibition of IGF1-R overcomes IGFBP7-induced chemotherapy resistance in T-ALL

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Reviewer: Yusuke Furukawa

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IGFBP-7 is structurally related to the IGFBP family of proteins that have multiple functions via modulation of the abundance and functions of IGF-1 and IGF-2. It has been reported that IGFBP-7 acts as a tumor suppressor in some solid tumors, whereas the increased expression of IGFBP-7 is associated with therapy resistance and poor prognosis in patients with T-ALL and Ph-negative B-ALL. In the present study, the authors investigated the mechanisms by which IGFBP-7 overexpression confers the drug resistance to T-ALL cells and found that IGFBP-7 destabilizes IGF-1R and an IGF1-R inhibitor overcomes IGFBP-7-mediated resistance to vincristine in Jurkat cells. They concluded that IGFBP-7 could serve as a prediction marker for the response of T-ALL patients to IGF-1R inhibitors.

Although this study is dealing with an interesting subject, there are several concerns that should be addressed by additional experiments.

1) Upon IGFBP-7 overexpression with identical levels (Fig. 1), only Jurkat cells acquired the resistance to starvation-induced cell death (Fig. 2), despite the fact that IGFBP-7 induced G0/G1 arrest and reduced the size of sub-G1 fractions, corresponding to spontaneous apoptosis, in both Jurkat and MOLT-4 cells (Fig. 3). This deserves some comments and explanation. This reviewer recommends the authors to investigate the response of the two cell lines to several pro-apoptotic stimuli in addition to nutrient deprivation and anti-leukemia agents.

2) The overexpression of IGFBP-7 conferred the resistance to vincristine and L-asparaginase but not etoposide and cytosine arabinoside in Jurkat cells (Fig. 4). The same experiments should be performed in MOLT-4 cells to clarify whether the resistance to vincristine is dependent on IGF1-R down-regulation and the resistance to L-asparaginase is dependent on IGFBP-7 overexpression itself as the authors discussed in the manuscript.

3) The authors evaluated the resistance to each drug at fixed doses (Fig. 4), which should be done at IC50 levels.

4) In Fig. 5A, the authors should superimpose the peaks of isotype-matched controls in each panel to indicate whether surface IGF-1R is disappeared by IGFBP-7 overexpression as implied by the data shown in Fig. 5C. This is an important point given the combined effect of IGFBP-7 overexpression and the IGF-R1 inhibitor NVP-AEW541 shown in Fig. 6 (see below).
5) Although the use of small molecular compounds is feasible and practical when future clinical application is considered, the combined effect of IGFBP-7 overexpression and AEW541 is slightly odd given the complete disappearance of IGF-1R in IGFBP-7-overexpressing Jurkat cells (Fig. 5C). As the possibility of off-target effects of AEW541 cannot be ruled out, the authors should confirm these results using siRNA against IGF-1R.

6) Using GO analysis, the authors clearly showed the role of IGF-1R as a negative regulator of cell death in T-ALL cells (Tables 1 and 2). The authors should show the inverse correlation of IGFBP-7 and IGF-1R-regulated genes in the same data set.

**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.