USP48 Inhibition as a Potential Therapeutic Target for Patients with T-acute Lymphoblastic Leukemia

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Background

- T-acute lymphoblastic leukemia (T-ALL) is a heterogeneous hematopoietic neoplasm of precursor T-cells.
- Ubiquitin is a small protein that marks proteins for degradation at the proteasome.
- Deubiquitinating enzymes (DUBs) remove ubiquitin, allowing targeted proteins to circumvent destruction.
- USP48 is one such DUB and is likely to be overexpressed in patients with T-ALL.
- BRAT1 is a regulator of mTOR that contributes to cell proliferation when present in excess.

Hypothesis

USP48, if overexpressed in T-ALL, interacts with BRAT1 to increase cell proliferation and decrease apoptosis, thus contributing to leukemogenesis.

Methods

- Cell culture: HPB-ALL cells, a T-ALL cell line, were maintained in RPMI-1640 medium supplemented with 4% FBS.
- PR-619 treatment: HPB-ALL cells were treated for 24 hours with varying doses of PR-619 (0 μM, 1.25 μM, 2.5 μM, 5.0 μM), a DUB inhibitor.
- shRNA knockdown: HBP-ALL cells were infected *in vitro* with an shRNA, knocking down the gene that encodes for USP48, and compared with cells infected with a control vector.
- Flow cytometry**:** PR-619-treated and shRNA-knockdown cells were collected to assess the degree of apoptosis after staining with PE Annexin V and 7-AAD by flow cytometry.
- MTT assay: shRNA-treated cells and control cells were assessed for proliferation by MTT assay.
- Western blot: PR-619-treated and shRNA-knockdown cells were collected for a Western blot to assess the presence of USP48 and BRAT1.

Results

- Prior research from this lab has shown that USP48 is overexpressed in T-ALL cell lines.

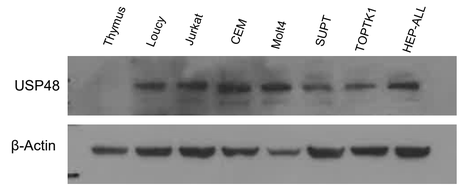

Figure 1. Western blot revealed high USP48 expression in T-ALL cell lines compared to control thymus cells.

- Flow cytometry revealed that cells treated with PR-619 and shRNA showed elevated levels of apoptosis compared to control cells.

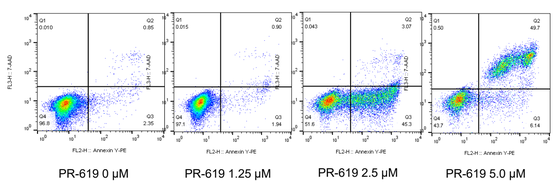

Figure 2. Flow cytometry revealed that apoptosis increased with higher concentrations of PR-619, a DUB inhibitor.

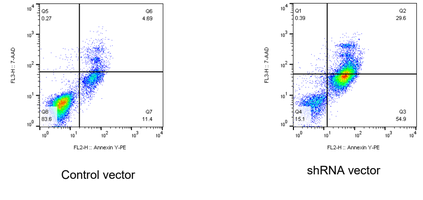

Figure 3. Flow cytometry revealed increased apoptosis in USP48-knockdown cells relative to cells infected with control vector.

Results

- MTT assay displayed decreased proliferation in shRNA-knockdown cells.

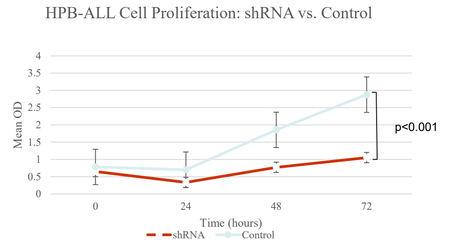

Figure 4. MTT assay revealed decreased proliferation in cells with USP48-knockdown relative to control HPB-ALL cells. Student t-test at 72 hours revealed significant difference in mean OD (p<0.001).

- Western blot revealed that BRAT1 expression correlated with USP48 expression.

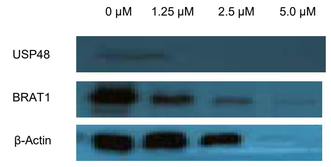

Figure 5. Western blot revealed decreased BRAT1 with decreased level of USP48, suggesting interaction.

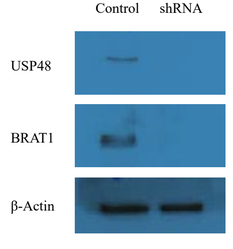

Figure 6. Western blot revealed decreased BRAT1 with USP48-knockdown, suggesting interaction.

Results

- High level of USP48 expression correlated with poor overall survival in patients with T-ALL.

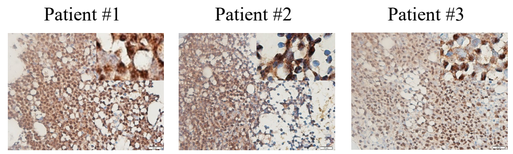

Figure 7. Immunohistochemical staining detected the expression of USP48 in T-ALL bone marrow specimens.

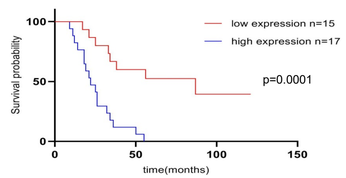

Figure 8. Survival analysis revealed that patients with higher levels of USP48 expression had shorter overall survival (p=0.0001). Median survival: low expression, 87 months (n=15); high expression, 22 months (n=17).

Conclusions

- Our results show a positive relationship between USP48 and BRAT1, suggesting a role in leukemogenesis.
- Higher levels of USP48 expression is correlated with poorer survival outcome.
- Knockdown and inhibition of USP48 shows increased apoptosis and decreased proliferation.
- Inhibition of USP48 may thus present new target therapy for patients with T-ALL.

References

So EY, Ouchi T. The Potential Role of BRCA1-Associated ATM Activator-1 (BRAT1) in Regulation of mTOR. J Cancer Biol Res. 2013 Jul-Aug;1(1):1001.