Poster #: 1221

XXI IWCLL International Workshop on CLL

The Integrin Adaptor Kindlin-3 Regulates Chronic Lymphocytic Leukemia Development and Progression

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OBJECTIVES

- To study the role of the integrin adaptor kindlin-3 (gene name *Fermt3*) in chronic lymphocytic leukemia (CLL) development and progression using several TCL1 transgenic based mouse models.

CONCLUSIONS

- B cell specific loss of kindlin-3 abrogates CLL onset in peritoneal cavity primary TCL1 transgenic mice.
- At later disease stages, leukemic spread to organs is lost.
- Inducible kindlin-3 deletion at overt leukemia in transplantation settings with low to medium tumor aggressivity severely reduces the tumor and restores lymphoid organ architecture.
- Inducible kindlin-3 deletion at end stage of aggressive tumor transplants cannot revert the disease but induces apoptosis in a part of the CLL cells.
- Kindlin-3 deficient CLL cells display integrin activation defects.

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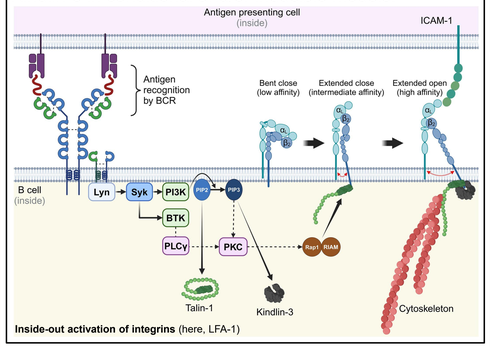
INTRODUCTION

Survival and proliferation of chronic lymphocytic leukemia (CLL) cells are highly dependent on their interaction with the lymphoid microenvironment. Integrins function as mechanoreceptors on B cells, facilitating homing to and retention within lymphoid tissues upon being activated by microenvironmental cues such as chemokines or antigens. Kindlin-3 (gene name *Fermt3*) is an essential adaptor protein that binds to the integrins and is mandatory for integrin activation in B lymphocytes.
In this study, we investigated the effects of loss of overall integrin activation in CLL by B cell-specific deletion of Kindlin-3 in conditional and/or inducible models.



Inside-out activation of integrins (here, LFA-1)

METHODS

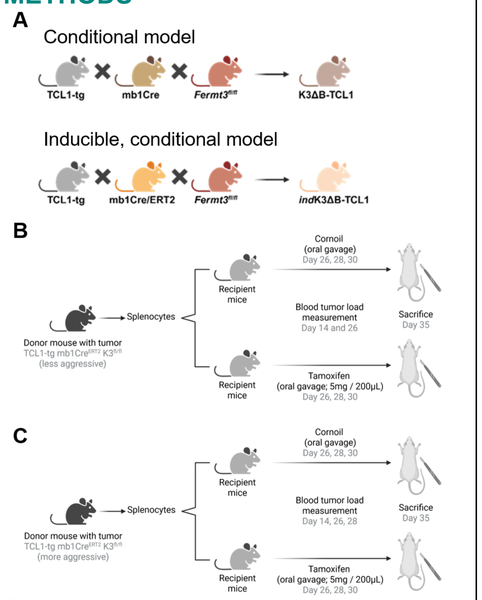


Figure Materials and Methods:
(A) Schematic illustrating the cross used to generate the B-cell specific conditional kindlin-3 knockout (K3ΔB) TCL1-tg mouse strain *(top)* and the inducible, B-cell specific conditional knockout (indK3ΔB TCL1-tg) mouse strain *(bottom)*. **(B)** Schematic illustration of transplant of less aggressive tumors from indK3ΔB TCL1-tg mice into recipient wildtype and the treatment regiment carried out to induce kindlin-3 knockout. **(C)** Schematic illustration of transplant of highly aggressive tumors from indK3ΔB TCL1-tg mice into recipient wildtype and the treatment regiment carried out to induce kindlin-3 knockout.

Figure 1: Conditional *Fermt3* deletion in B cells leads to tumor abrogation in the TCL1-tg mouse model.

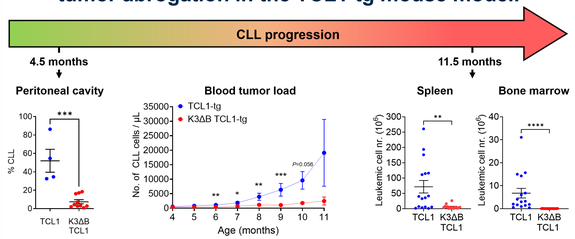


Figure 1: K3ΔB TCL1-tg and TCL1-tg mice were sacrificed at 4.5 months of age, and tumor load in the peritoneal cavity was analyzed by flow cytometry using anti-CD5/CD19 antibodies. Blood tumor load of mice was monitored monthly from 4 to 11 months. Tumor load in the spleen and bone marrow of K3ΔB TCL1-tg and TCL1-tg mice sacrificed at 11.5 months of age was analyzed. Groups were compared using unpaired t-test or Mann-Whitney test. *: P < 0.05; **: P < 0.01; ***: P < 0.001 ; ****: P < 0.0001.

Figure 2: Induction of conditional Kindlin-3 deletion impairs disease progression.

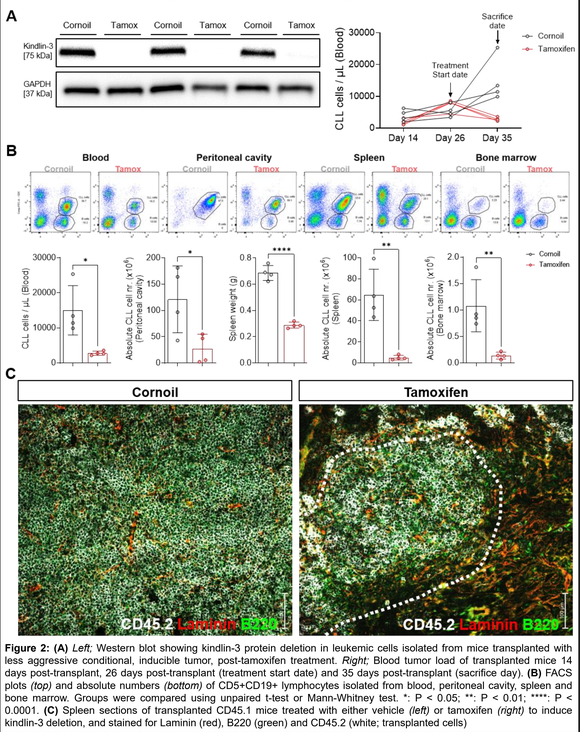


Figure 2: (A) *Left;* Western blot showing kindlin-3 protein deletion in leukemic cells isolated from mice transplanted with less aggressive conditional, inducible tumor, post-tamoxifen treatment. *Right;* Blood tumor load of transplanted mice 14 days post-transplant, 26 days post-transplant (treatment start date) and 35 days post-transplant (sacrifice day). **(B)** FACS plots *(top)* and absolute numbers *(bottom)* of CD5+CD19+ lymphocytes isolated from blood, peritoneal cavity, spleen and bone marrow. Groups were compared using unpaired t-test or Mann-Whitney test. *: P < 0.05; **: P < 0.01; ***: P < 0.001. ****: P < 0.0001. **(C)** Spleen sections of transplanted CD45.1 mice treated with either vehicle *(left)* or tamoxifen *(right)* to induce kindlin-3 deletion, and stained for Laminin (red), B220 (green) and CD45.2 (white; transplanted cells)

REFERENCES

1. Pethe A, Hartmann TN. The cytoskeletal control of B cell receptor and integrin signaling in normal B cells and chronic lymphocytic leukemia. FEBS Lett. Published online April 17, 2025. *doi:10.1002/1873-3468.70045*
2. Härzschel A, Li L, Krenn PW, et al. Kindlin-3 maintains marginal zone B cells but confines follicular B cell activation and differentiation. *J Leukoc Biol.* 2022;111(4):745-758. doi:10.1002/JLB.1HI0621-313R

ACKNOWLEDGMENTS AND DISCLOSURES

Lighthouse Core Facility
DFG Deutsche Forschungsgemeinschaft
Deutsche Krebshilfe HELFEN. FORSCHEN. INFORMIEREN.

Figure 3: Kindlin-3 deletion leads to increased apoptosis of aggressive leukemic cells in mice.

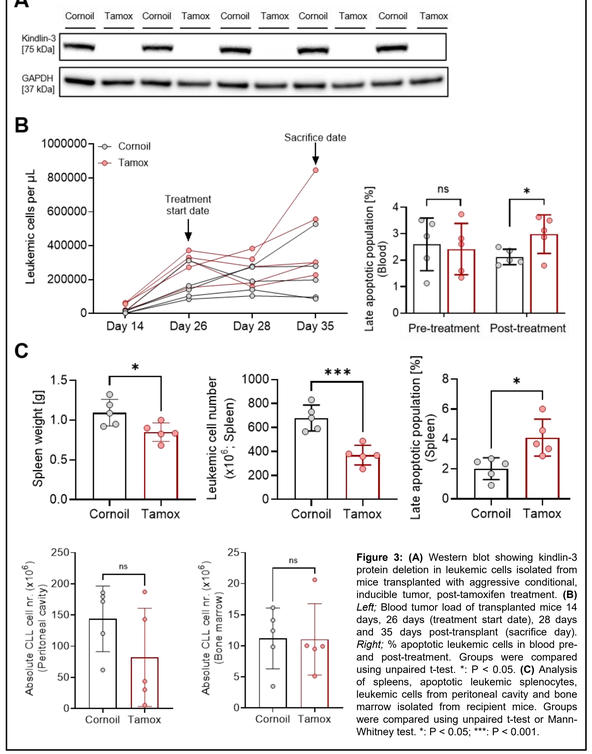


Figure 3: (A) Western blot showing kindlin-3 protein deletion in leukemic cells isolated from mice transplanted with aggressive conditional, inducible tumor, post-tamoxifen treatment. **(B)** *Left;* Blood tumor load of transplanted mice 14 days, 26 days (treatment start date), 28 days and 35 days post-transplant (sacrifice day). *Right;* % apoptotic leukemic cells in blood pre- and post-treatment. Groups were compared using unpaired t-test. *: P < 0.05. **(C)** Analysis of spleens, apoptotic leukemic splenocytes, leukemic cells from peritoneal cavity and bone marrow isolated from recipient mice. Groups were compared using unpaired t-test or Mann-Whitney test. *: P < 0.05; ***: P < 0.001.

Figure 4: Loss of kindlin-3 leads to loss of integrin function in leukemic cells.

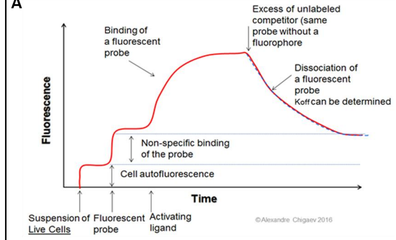


Figure 4: (A) Scheme of the LDV-assay carried out to analyze VLA-4 activation and ligand affinity. **(B)** % VLA-4 activation *(left)* and % Ligand affinity of VLA-4 *(right)* post different stimulus in TCL1-tg and K3ΔB TCL1-tg leukemic cells. Mn^{2+} stimulus artificially forces the integrin into an active conformation and is considered to be an artificially induced 100% activation.
Groups were compared using unpaired t-test or Mann-Whitney test. **: P < 0.01; ****: P < 0.0001.