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Decreased cell stiffness enhances leukemia development and progression

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Emerging evidence has linked cell mechanics to functional behaviors [1]. The biophysical traits of a single cell are inextricably linked to the cytoskeleton. It has become increasingly evident that intrinsic and extrinsic mechanical properties, which describe the resistance to deformation (elasticity) or flow (viscosity) in response to an applied force, regulate cellular activities, such as cell morphology, adhesion, migration, and trafficking. Recent studies have demonstrated that solid tumor cells with higher migratory and invasive potential are softer than cells with lower migration and invasion potential [2-5]. However, how cell intrinsic mechanical properties might affect liquid tumor (leukemia) development and progression remains unclear.

Ptpn21 (protein tyrosine phosphatase, non-receptor type 21), a poorly studied tyrosine phosphatase [6], binds to actin filaments and regulates cytoskeleton-associated cellular processes [7]. We have recently shown that Ptpn21 plays an important role in maintaining cell mechanical properties [8], and that it helps retain hematopoietic stem cells (HSCs) in the bone marrow (BM) niche through a biomechanical mechanism [8]. Knock-out of *Ptpn21* results in impaired retention of HSCs within BM niches. *Ptpn21* knock-out stem cells exhibit enhanced mobility and spontaneous egress into the peripheral blood. These phenotypes were attributable to the decrease in cellular mechanical stiffness and the increase in cell deformability [8]. Mechanistically, Ptpn21 functions by dephosphorylating Spetin1 (Tyr^{246}) [8], a rarely described component of the cytoskeleton. Importantly, missense mutations and

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Author contributions. L.H., F.N., and X. W. conducted mouse experiments and summarized the data. M.E.F. performed microfluidics assays. K.M.Y. performed the Atomic Force Microscope measurements. W.A.L. and T.A.S. provided critical advice. C.K.Q. designed the experiments and directed the entire project. L.H., F.N. and C.K.Q. wrote the manuscript.

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frameshift truncating mutations in *PTPN21* have been identified in chronic lymphocytic leukemia (IntOGen - mutational cancer drivers database) and colon cancer [9-11], respectively. However, the pathogenic effects of *PTPN21* loss of function mutations remain to be determined.

Given the decreased cell mechanical tension and increased deformability displayed by *Ptpn21* knock-out hematopoietic cells [8], we utilized *Ptpn21* knock-out (*Ptpn21*$^{-/-}$) mice as a model to examine the biomechanical regulation of leukemic development and progression. BM lineage negative (Lin$^-$) cells isolated from *Ptpn21*$^{-/-}$ or *Ptpn21*$^{+/+}$ mice (CD45.2$^+$) were transduced with acute myeloid leukemia (AML)-associated oncogene *MLL-AF9* and transplanted into sublethally irradiated congenic BoyJ mice (CD45.1$^+$). Following *in vivo* expansion (to obtain sufficient *MLL-AF9*-transduced cells), leukemic cells were harvested and inoculated into BoyJ mice again (Figure 1A). Surprisingly, although Ptpn21 was reported to play a positive role in cell signaling (c-Src activation) [12, 13], the recipient mice inoculated with *MLL-AF9-Ptpn21*$^{-/-}$ leukemic cells developed AML more quickly than the mice receiving *MLL-AF9-Ptpn21*$^{+/+}$ control leukemic cells. The survival of *MLL-AF9-Ptpn21*$^{-/-}$ cell recipients was shortened (Figure 1B). Leukemic burden, as determined by white blood cell counts, spleen weights, and leukemic cells (GFP$^+$) in the peripheral blood, BM, and spleen, was markedly increased in the transplants inoculated with *MLL-AF9-Ptpn21*$^{-/-}$ cells 15 (Figure 1C-1E) and 25 days (Supplementary Figure 1A-1C) after the inoculation. Moreover, pathological examination of the BM, spleen, liver, and lung revealed more severe leukemic cell infiltration in *MLL-AF9-Ptpn21*$^{-/-}$ compared to *MLL-AF9-Ptpn21*$^{+/+}$ cell recipients (Figure 1F). As *MLL-AF9*-transduced leukemic cells were expanded *in vivo* initially, to rule out the possibility that the observed effects of Ptpn21 deficiency on leukemia was caused by the expansion process, we scaled up retroviral gene transduction and transplanted freshly isolated GFP$^+$ *MLL-AF9* transduced cells into congenic mice. The homing ability of *MLL-AF9-Ptpn21*$^{-/-}$ leukemic cells was similar to that of *MLL-AF9-Ptpn21*$^{+/+}$ leukemic cells (Supplementary Figure 2A). However, *MLL-AF9-Ptpn21*$^{-/-}$ leukemic cells proliferated much faster than *MLL-AF9-Ptpn21*$^{+/+}$ leukemic cells in the transplants, consistent with the data shown in Figure 1. White blood cell counts, and leukemic cells (GFP$^+$) in the peripheral blood, BM and spleen were significantly increased in *MLL-AF9-Ptpn21*$^{-/-}$ cell recipients (Supplementary Figure 2A-2C). Indeed, the cell cycling of *MLL-AF9-Ptpn21*$^{-/-}$ leukemic cells in the transplants was faster compared to that of *MLL-AF9-Ptpn21*$^{+/+}$ leukemic cells (Supplementary Figure 2D).

Ptpn21 has been shown to be a positive regulator of c-Src signaling[12, 13]. We therefore compared cell signaling activities in leukemic cells with and without *Ptpn21*. c-Src activity in *MLL-AF9-Ptpn21*$^{-/-}$ leukemic cells was marginally decreased relative to that in *MLL-AF9-Ptpn21*$^{+/+}$ counterparts. The impact of Ptpn21 deficiency on other signaling pathways, such as Erk, Stat5, mTOR, and NF-κB activities, was undetectable (Figure 2A). In agreement with these results, *MLL-AF9-Ptpn21*$^{-/-}$ leukemic cells did not show any growth advantages in culture dishes (Figure 2B). Apoptosis in these cells was also comparable to that in *MLL-AF9-Ptpn21*$^{+/+}$ control cells (Figure 2C). These data apparently did not account for the enhanced leukemia development and progression in *MLL-AF9-Ptpn21*$^{-/-}$ cell transplants. Atomic force microscopy measurements of cell stiffness showed that *Ptpn21*-deleted leukemic cells were softer than control leukemic cells (Figure 2D), similar

to "normal" *Ptpn21*$^{-/-}$ hematopoietic cells. A consequence of decreased cell stiffness can be enhanced cell deformability [1]. To further examine the relative deformability of *Ptpn21*–deleted leukemic cells, we utilized a microfluidic device, an established bioengineering instrument with multiple microchannels (5.9 ± 0.8 μm in width) mimicking capillary beds, to determine relative cell deformability based on transit time [14, 15]. Compared to control cells, *Ptpn21*-deleted leukemic cells traversed the microfluidic channels faster and a lower proportion of the cells obstructed the device (>10 sec transit time) (Figure 2E, 2F), consistent with the important role of Ptpn21 in cell mechanics and motility [8]. Importantly, in the transplants, *Ptpn21*-deleted leukemic cells proliferated much faster than control cells (Figure 2G). Taken together, these data suggest that the cell mechanical changes (decreased tension and increased deformability) rather than the impact on cell signaling, are likely responsible for the accelerated leukemia progression in *MLL-AF9-Ptpn21*$^{-/-}$ cell transplants.

This work raises an intriguing possibility, that is, cell mechanical rigidity is a tumor suppressing mechanism. The biomechanical regulation by Ptpn21 appears to have an anti-leukemic effect. Consistent with this notion, AML patients with lower *PTPN21* expression levels in leukemic cells had poorer prognosis compared to those with higher *PTPN21* levels (Supplementary Figure 3). Nevertheless, it remains unclear how *Ptpn21*-deleted leukemic cells proliferated faster than control cells in the *in vivo* setting. Since the *in vitro* growth rate of *Ptpn21* deleted leukemic cells was comparable to that of control leukemic cells, conceivably many factors in the *in vivo* microenvironment that the leukemic cells were associated with might have contributed to the overall elevated growth of the softer leukemic cells lacking *Ptpn21* in mice, such as the viscosity of the plasma, the shearing forces, and/or potential immune evasion. However, the possibility that other unknown effects caused by Ptpn21 deficiency on signaling or cellular physiology might promote increased proliferation cannot be fully excluded. Further studies are required to address these possibilities. Finally, given the leukemia-suppressing role of Ptpn21 identified in this study, we would predict that the loss-of-function mutations of *PTPN21* found in leukemia (IntOGen - mutational cancer drivers database) and colon cancer [9-11] might promote tumor development and/or progression through impacting biomechanics. A deeper understanding of the relationship between the cell mechanical alterations and tumor cell activities might ultimately lead to rational design of more effective therapies to inhibit *PTPN21*-mutated malignancies.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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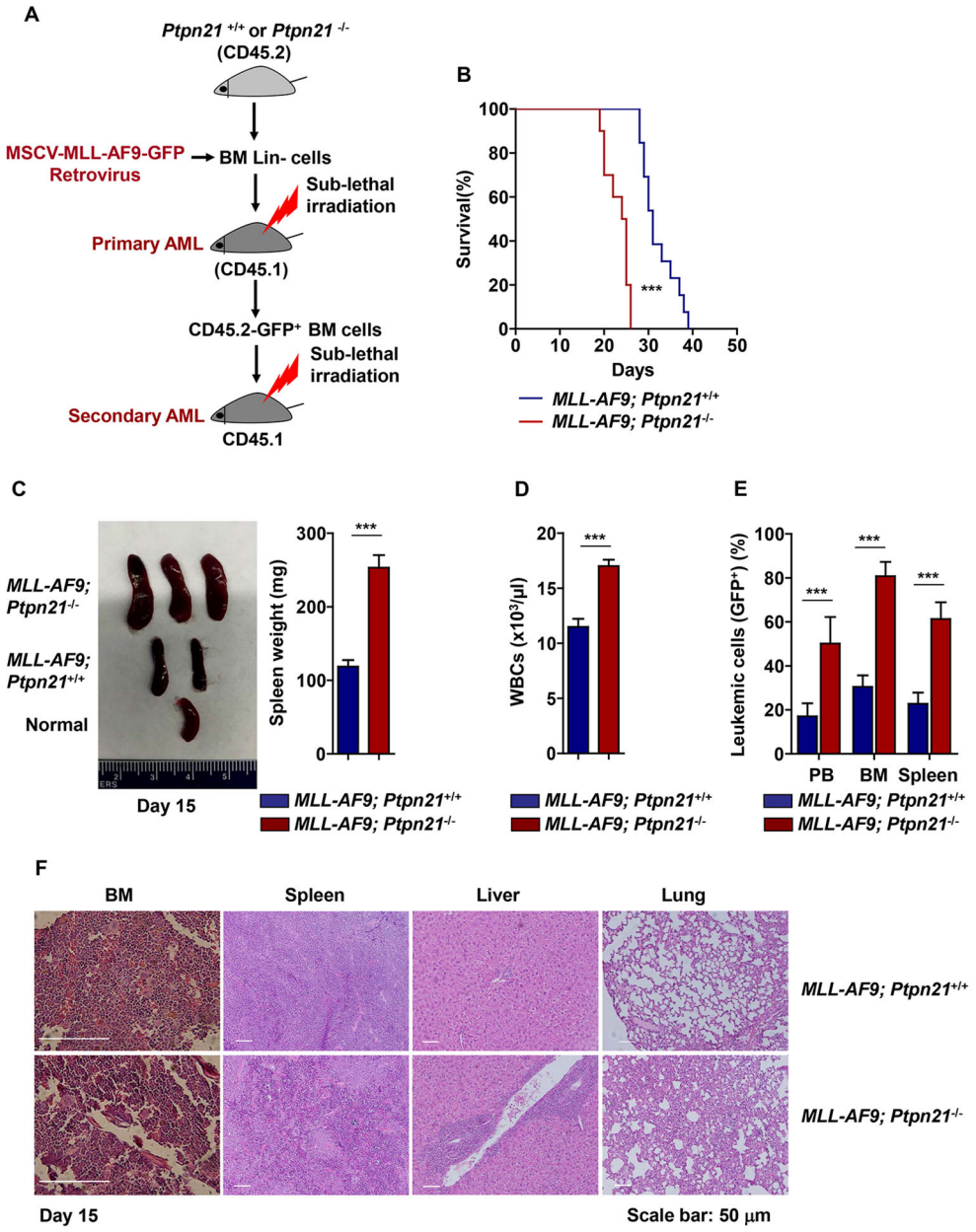

Figure 1. Deletion of *Ptpn21* promotes development and progression of *MLL-AF9*-induced leukemia.

(A) Lin^{-} cells isolated from *Ptpn21*$^{-/-}$ and *Ptpn21*$^{+/+}$ mice were infected with *MSCV-MLL-AF9-IRES-GFP* retrovirus. Entire cell populations (5×10^5 cells/mouse) were transplanted into sublethally irradiated (700 rad; with a cesium irradiator) BoyJ mice for *in vivo* expansion of leukemic cells. GFP^{+} leukemic cells sorted from the recipient mice were transplanted into sublethally irradiated BoyJ mice. (B) Kaplan-Meier survival curve of the secondary mice receiving 1×10^5 GFP^{+} *MLL-AF9*-tranduced *Ptpn21*$^{-/-}$ (n=10 mice) or *MLL-AF9*-tranduced *Ptpn21*$^{+/+}$ (n=13 mice) cells. (C-E) Fifteen days after leukemic cell inoculation, recipient mice (n=5 mice per genotype) were sacrificed. Spleens were weighted (C), white blood cell counts in the peripheral blood were determined by a hematology

analyzer (D), and leukemic cells (GFP^+) in the peripheral blood, BM, and spleen (E) were assessed by FACS analyses. (F) Histopathological examination of the BM, spleen, liver, and lung dissected from the recipient mice transplanted with *MLL-AF9-Ptpn21*$^{-/-}$ or *MLL-AF9-Ptpn21*$^{+/+}$ cells. Representative images from 5 mice per genotype are shown. Data shown in (C) – (E) are mean ± standard deviation of all mice examined. *** $P < .001$.

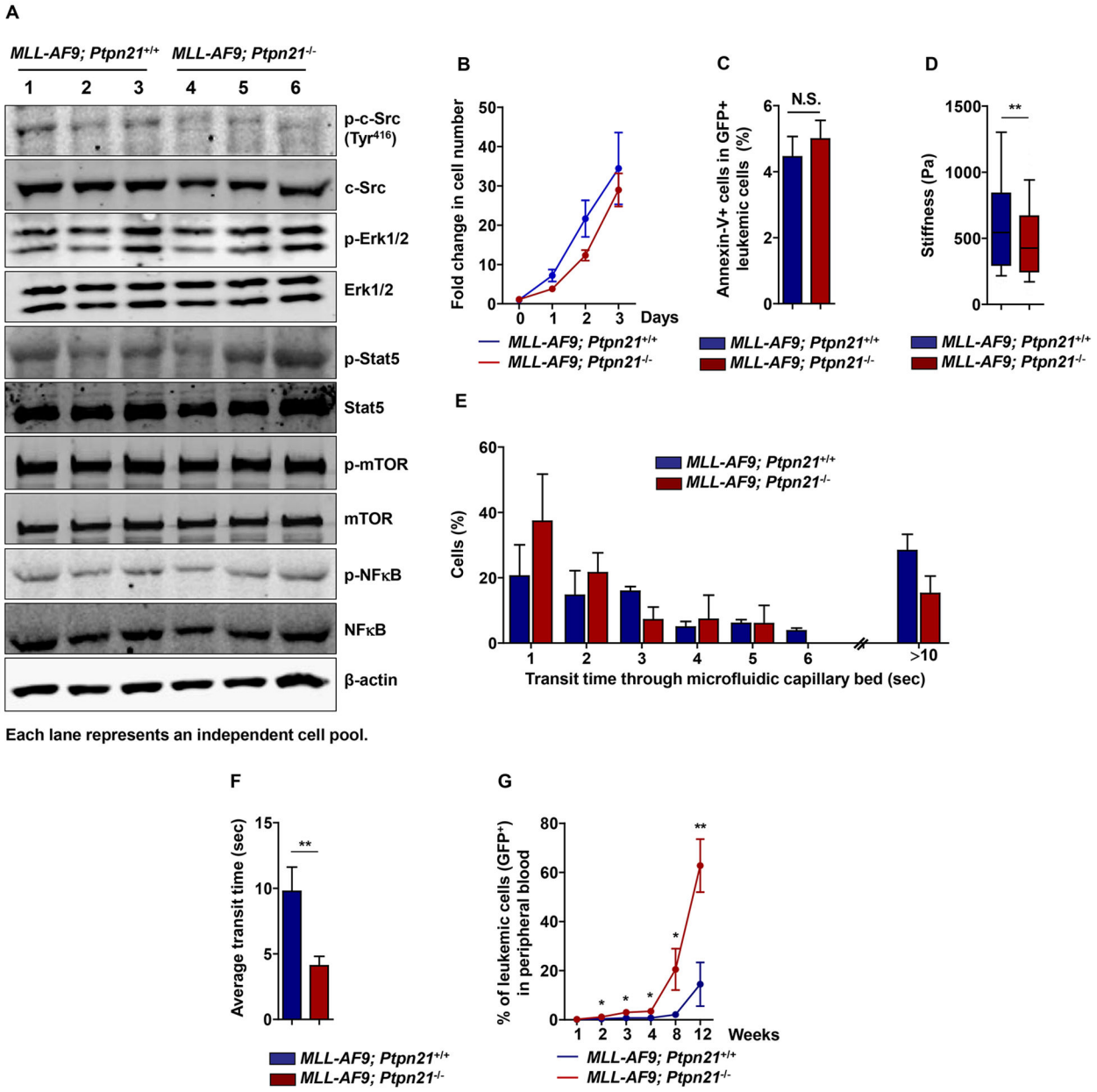

Figure 2. Cellular mechanical properties but not cell signaling activities are impacted in *Ptpn21* deleted MLL leukemic cells that proliferate faster than control cells *in vivo*.

(A) Lin$^-$ cells isolated from *Ptpn21*$^{-/-}$ and *Ptpn21*$^{+/+}$ mice were infected with *MSCV-MLL-AF9-IRES-GFP* retrovirus. GFP$^+$ *MLL-AF9*-transduced cells were sorted by FACS. Sorted cells were lysed and subjected to immunoblotting analyses with the indicated antibodies (n= 3 mice per genotype). (B, C) Sorted GFP$^+$ *MLL-AF9*-transduced cells were cultured in IMDM medium containing 10%FBS, 50 ng/mL SCF, 50 ng/mL Flt3L, 20 ng/mL IL-3, and 20 ng/mL IL-6, for the indicated periods of time. Cell growth rates were determined (n=4-6 mice per genotype) (B). Apoptosis in the cells cultured for 24 hours were assayed by FACS (n=4-6 mice per genotype) (C). (D) Leukemic cells isolated were measured for mechanical stiffness by atomic force microscopy (n= 3 mice per genotype; 108 cells examined per

mouse sample). (E, F) Leukemic cells isolated were assayed by the microfluidic system (n=3 mice per genotype, 30 cells examined per mouse sample). Cells in transit were recorded using a bright-field microscopy, transit times were manually calculated (E) to determine average transit time through the device (F). Data in (B) – (F) are shown as mean ± standard deviation. (G) Sorted GFP^+ *MLL-AF9*-tranduced cells (1×10^5 cells/mouse) were transplanted into sublethally irradiated (200 rad with an X-ray irradiator) BoyJ mice (n=6-7 mice per group). GFP^+ leukemic cells in the peripheral blood were assessed at the indicated time points before all recipient animals receiving *MLL-AF9-Ptpn21*$^{-/-}$ leukemic cells died. * $P < .05$, ** $P < .01$; N.S., not significant.