The mechanical properties of CLL cells are linked to the actin cytoskeleton and are target of BTK inhibitors

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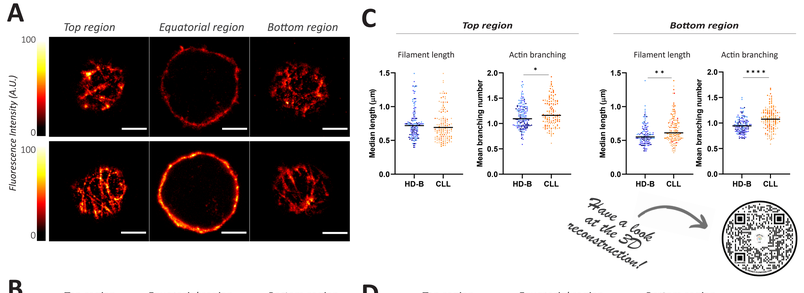
1.Introduction

The ability of lymphocytes to recirculate strongly depends by their capacity to rearrange their cytoskeleton and to adapt to external cues. We recently demonstrated by super resolution microscopy (STED) that HS1 (Cell-Specific LynSubstrate-1) protein, an important cytoskeleton interactor and prognostic factor, is differentially organized in CLL patient's sample [Sampietro et al 2021]. These results prompted us to further study the actomyosin complex, which regulates cell shape and tension at single cell level.

2.Nanoscale cytoskeleton architecture of CLL cells

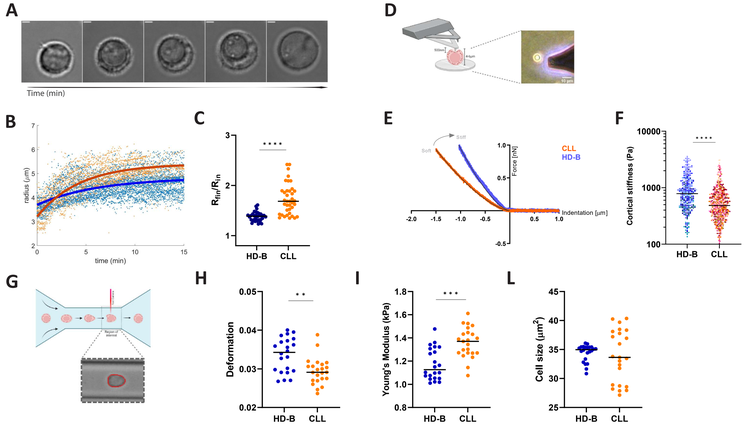
We quantified at nanoscale level in a single cell analysis of actin and myosin organization in primary CLL and healthy B donor (HD-B). We demonstrate that primary CLL cells show longer, and more branched actin filament as compared to the healthy counterpart. Moreover, we also show that myosin density in CLL cells is higher at the top and bottom section compared to HD-B cells. All these results suggest an altered cytoskeleton organization in CLL cells.



(A) *Gallery of images acquired ad the top, equatorial and adhesive section of primary cell* **(B)** *quantification of the signal density % per section* ***(C)*** *Quantification of actin filament morphology at the Top section and bottom section respectively, considering, the length expresses as the median length of all filaments in each single cells and actin branching.* ***(D)*** *Quantification of myosin density percentage (gallery of myosin is not showed)*

3.Different cytoskeletal architecture in CLL cells correlate with specific mechanical

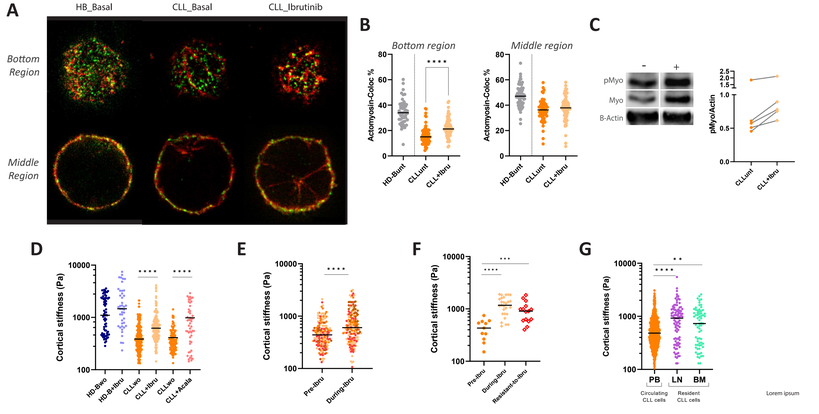
Altered cytoskeletal properties may be responsible for altered mechanical responses. Here we measure CLL cells and HD-B cells mechanical properties using distinct nanomechanical approaches: swelling and atomic force microscopy (AFM) in which cells are in adhesion, resembling a tissue-like condition and real time deformability cytometry (RT-DC), in which cells are in suspension, resembling a blood-like condition. All together these results confirm the differences between healthy and leukemic B cells and suggest that CLL mechanical adaptation properties are very dependent on the specific environment.



(A) *Bright field images of a representative 20 min time-lapse record of a single cell swelling as a consequence of osmotic shock. Scale bar = 5 µm.* ***(B)*** *Swelling kinetics of individual cells, where the continuous blue and red lines show global fitted curves on the total pool of HD-B and CLL cells respectively.* ***(C)*** *Scatter plot of the swelling ratio (Rfin/Rin).* ***(D)*** *Schematic representation (BioRender.com) of the AFM-FS basic principle, showing a cantilever pressing on a B cell at the cortical level . The image shows a real snapshot of the AFM cantilever tip approaching a cell. Scale bar = 10 µm.* ***(E)*** *Representative force-indentation curves from an AFM experiment in the force spectroscopy mode for HD-B (blue dots) and CLL (orange dots) cells. Black lines are the fitting curves according to the Hertz-Sneddon model.* ***(F)*** *Scatter plot of the cortical stiffness expressed as Young's Modulus (Pa) of HD-B and CLL cells.* ***(G)*** *Biorender scheme of RT-DC, showing a cell passing through a microfluidic channel. Inset exemplifies bright-field image of a cell within region-of-interest, where deformation is obtained from red contour.* ***(H, I, L)*** *Quantification of deformation and Young's mosulus and cell size, each dot represents the median or mean value of each individual experiment.mean cell size of each individual measurement.*

4.CLL cells mechanical response can be tuned in vitro and in vivo

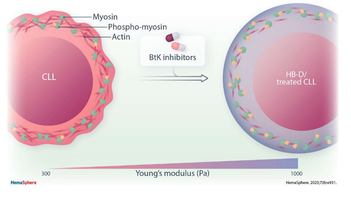
We further assessed to which extent the mechanical properties of the cells could be modulated in vitro and vitro by specific stimuli as BTK inhibitors. We treated CLL cells, and we quantified the level of colocalization between actin and myosin, and the level of phosphorylated myosin, as a possible sign of more active and contractile actomyosin complex. Moreover, we measure by AFM the cortical stiffness of CLL and HD-B cells before and after treatment with Ibrutinib, showing that the drug restores the CLL cells mechanical properties to a healthy phenotype and activates the actomyosin complex. We also confirmed this result in vivo in patients undergoing treatment. Moreover, we observed at the time of the onset of Ibrutinib resistance a trend of decreasing stiffness in CLL cells, suggesting a reversal to the initial leukemic phenotype.



(A) *Representative images of co-stained anti-myosin-Alexa532 (green) and anti-actin-Alexa568 (red) of HB untreated, CLL untreated (unt) and CLL+Ibru upon treatment with brutinib. Scale bar = 2 µm.* ***(B)*** *Quantification of the colocalization of myosin and actin by Manders' coefficient expressed as percentage of colocalized actin pixels on myosin pixels for the two analysed sections.* ***(C)*** *Western Blot (WB) analysis of CLL cells before (CLLunt) and after 4h treatment with 10 µM Ibrutinib (CLL+Ibru). Bands represent respectively phospho myosin, total myosin, and ß-actin. On the right the corresponding quantification of p-myosin increases upon treatment with ibrutinib.* ***(D)*** *Scatter plot of the cortical stiffness measured by AFM-FS of primary HD-B and CLL cells before (unt = untreated) and after drugs treatment (+Ibru= ibrutinib treatment; +Acala= acalabrutinib treatment).* ***(E)*** *Cortical stiffness distribution of patient before and under going clinical treatment with Ibrutinib.* ***(F)*** *Scatter plot of cortical stiffness of 1 patient at 3 time point during the course of the disease: Pre-treatment (before starting the treatment), during ibrutinib treatment and Resistant to ibrutinib (once the patient relapse during the therapy).* ***(G)*** *Scatter plot of the cortical stiffness of circulating CLL cells from Peripheral Blood (PB) and resident CLL cells from lymph nodes (LN) and bone marrow (BM) of patients.*

5.Conclusion

- ***Mechanical properties of CLL cells correlate with their cytoskeleton organization at different levels***
- ***CLL and HB cells respond differently depending on the imposed mechanical forces and the external environment***
- ***CLL mechanical properties can be tuned by target therapies both in vitro and in vivo***
- ***Altered cellular stiffness of resident B cells could be a possible mechanism of cellular retention within the infiltrated tissues and could be associated with drug resistance***



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Thank you fot your attention!

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