

CLL-Derived Exosomes Turn Endothelial Cells into CLL-Supportive Cells

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Abstract

- Hypothesis: via exosomes, CLL cells turn endothelial cells into “CLL supportive cells”.
- We transfected vein-derived (HUVECs) and arterial-derived (HAOEC) endothelial cells with 49 naïve- CLL- patients- derived exosomes
- Endothelial cells took-up CLL-exosomes in a dose- and time-dependent manner.
- Endothelial cells exposed to CLL-exosomes induced a 3.5 fold increase in IL-6 secreted levels.
- We then filtered out this growth medium and added CLL cells to this IL-6 enriched medium. After 15 minutes, STAT3 became phosphorylated and there was ~40% decrease in the rate of apoptosis of these cells, indicating that IL-6 activated STAT3-dependent antiapoptotic pathway.
- Phosphor-proteomic analysis of endothelial cells that were loaded with CLL-exosomes revealed 23 phosphor-proteins that were upregulated. Annotation analysis unraveled the central role of phosphor-βcatenin.
- To test whether βcatenin enhances the generation of IL-6 in these cells, we transfected HUVECs with βcatenin containing plasmid. We found by ELISA xxx fold increase in levels of IL-6 in the culture medium and by CHIP cells.
- through exosomes that they release. Once these exosomes are taken up by EC, they turn them into IL-6 producing cells.

Background

- CLL cells are not simply the seeds that grow on the supportive soil of the microenvironment but play an active role in shaping its Surrounding.
- Secreted by all types of cells, exosomes are nano-scaled particles that travel in blood and carry a cargo which they deliver to cells that engulf them.
- Given the appropriate stimulation, endothelial cells produce IL-6.
- Therefore, we hypothesized that CLL-exosomes actively recruit endothelial cells to become IL-6-secreting cells.

Objective

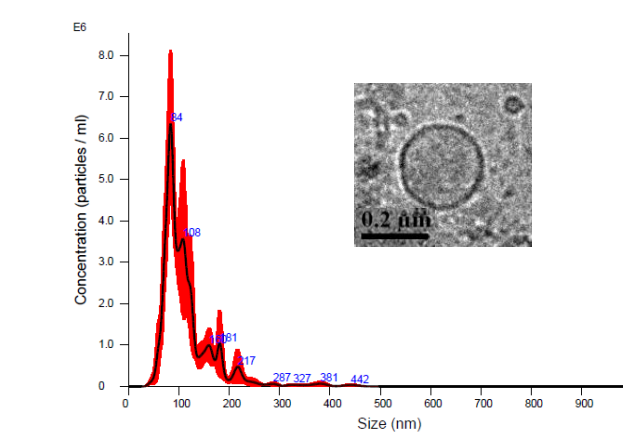
To study the putative role of CLL- derived exosomes in educating endothelial cells to become CLL- supportive cells

Materials & Methods

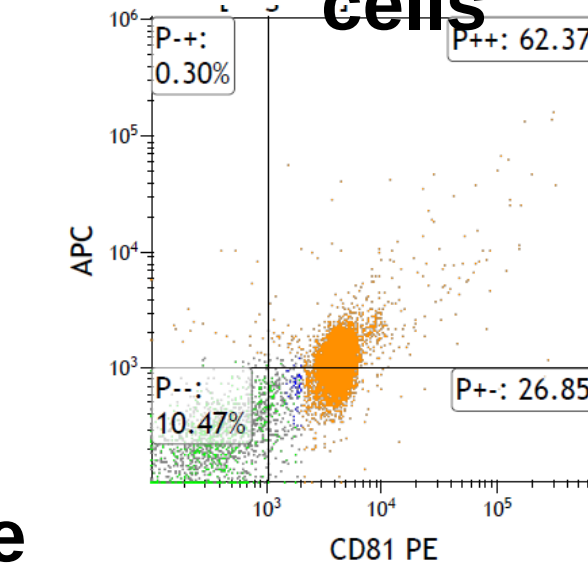
- Patients' characteristics: Peripheral blood samples were obtained from 49 treatment naïve patients with CLL.
- Cell lines: HUVECs and HEAOCwere purchased from PromoCell, (Heidelberg, Germany). The cells were cultured in exosomal free growth medium supplemented with 2% low serum growth supplements, penicillin and streptomycin (PromoCell).
- Isolation and characterization of exosomes: was carried out by serial ultracentrifugation and analyzed by Nano-Sight Tracking device and Electron Microscopy.
- Labelled exosomal uptake: was followed by flow cytometry and fluorescent microscopy
- Western Immunoblotting: was performed as previously described
- Cell transfection: to ectopically express β catenin, CTNNB1 expressing plasmid was done by using the jetPEI® transfection reagent (polyplus transfection Strasbourg (France)
- Chromatin immunoprecipitation (ChIP) assay: was conducted by using the EZ-ChIPkit (Millipore)
- ELISA was conducted with the Human IL-6 Quantikine ELISA Kit (R&D, Minneapolis, MN, USA).
- Apoptosis: was conducted by the Annexin V assay kit MEBCYTO® Apoptosis Kit (MBL, Nagoya, Japan).
- Statistical Analysis: was conducted by using the GraphPad Prism 8 version.

Results

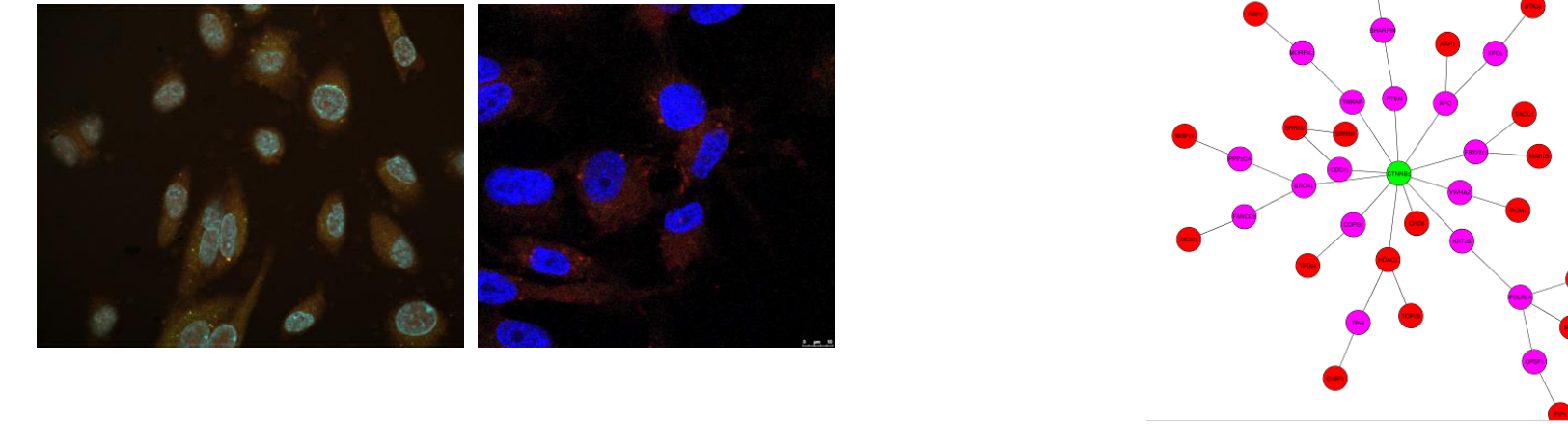
1. CLL- derived exosomes



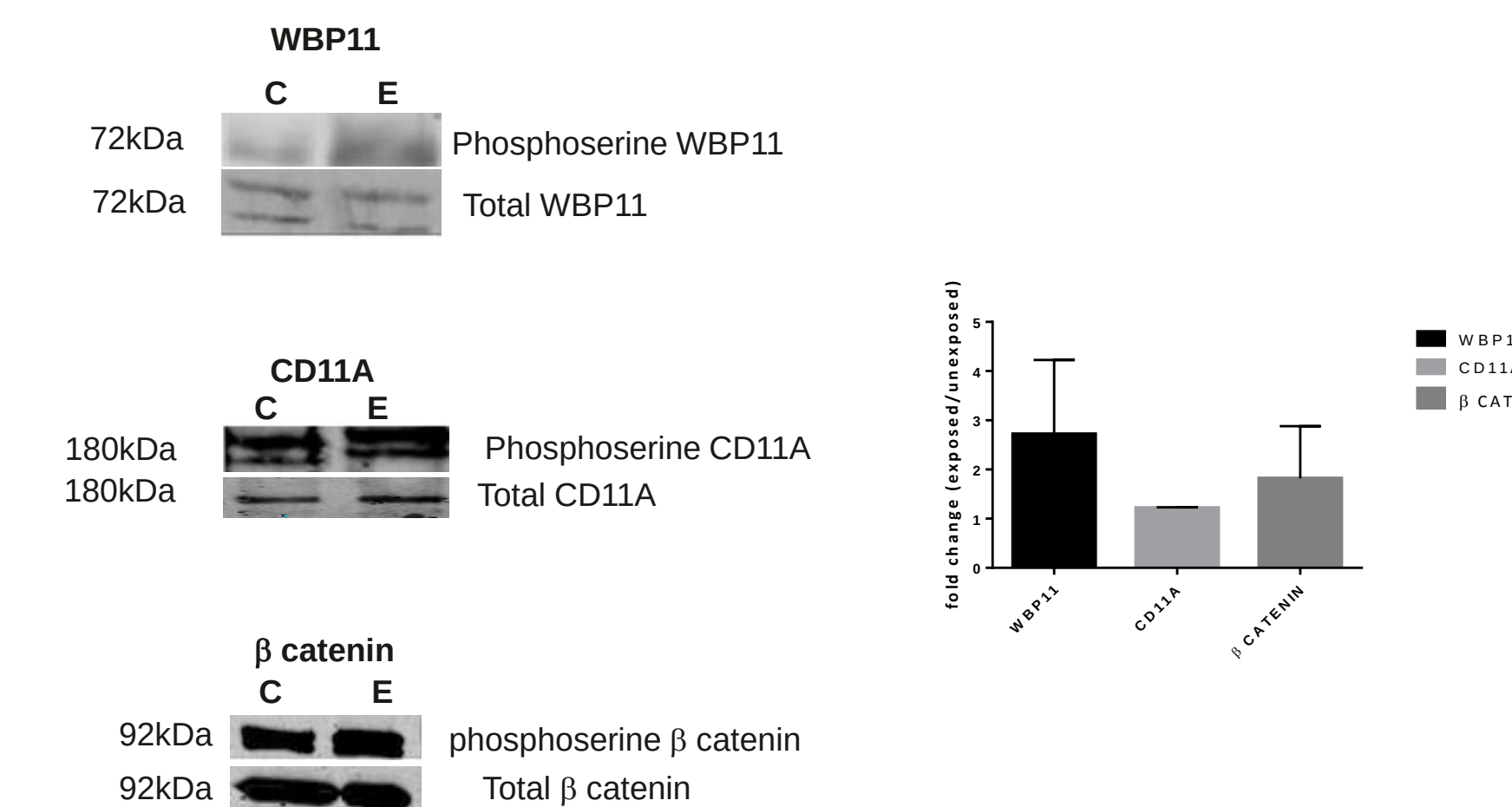
2 b. CLL- derived exosomes are Taken up by Endothelial cells



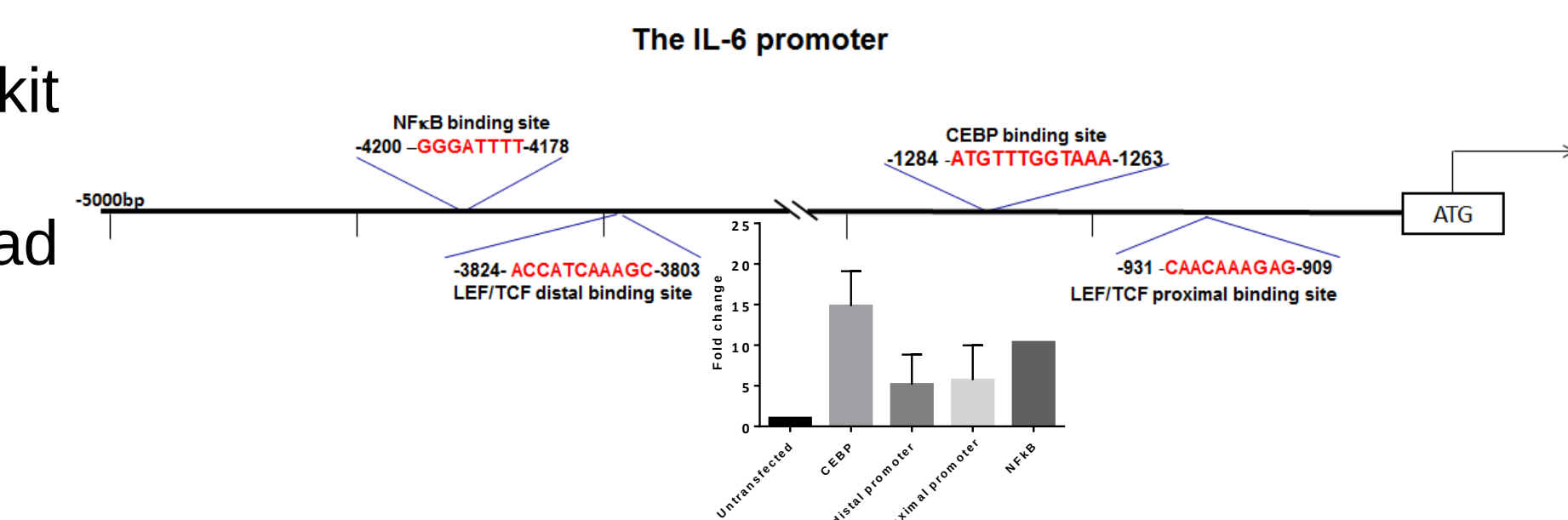
2a. CLL- derived exosomes are Taken up by Endothelial cells



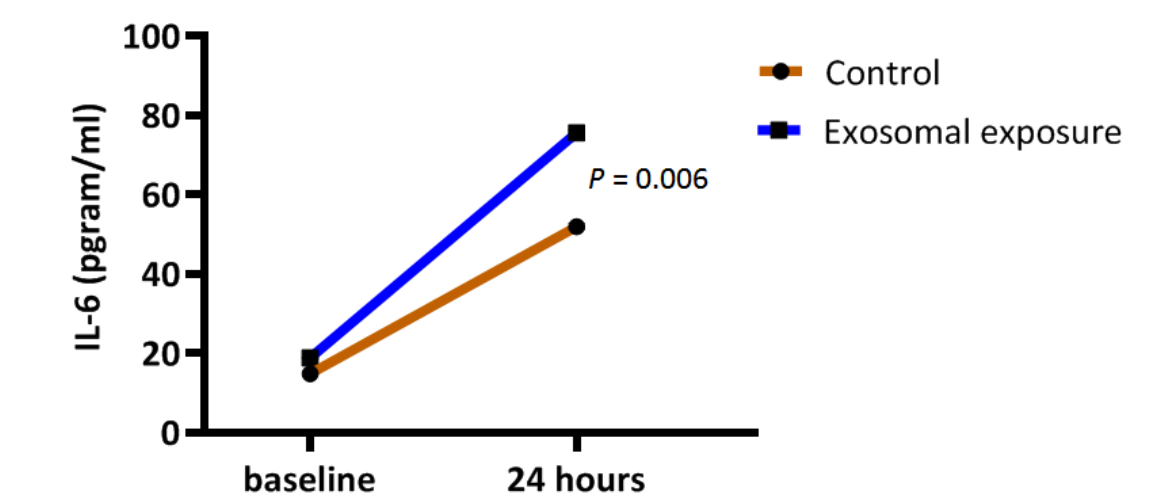
3. Validation of the phosphor-proteome analysis results



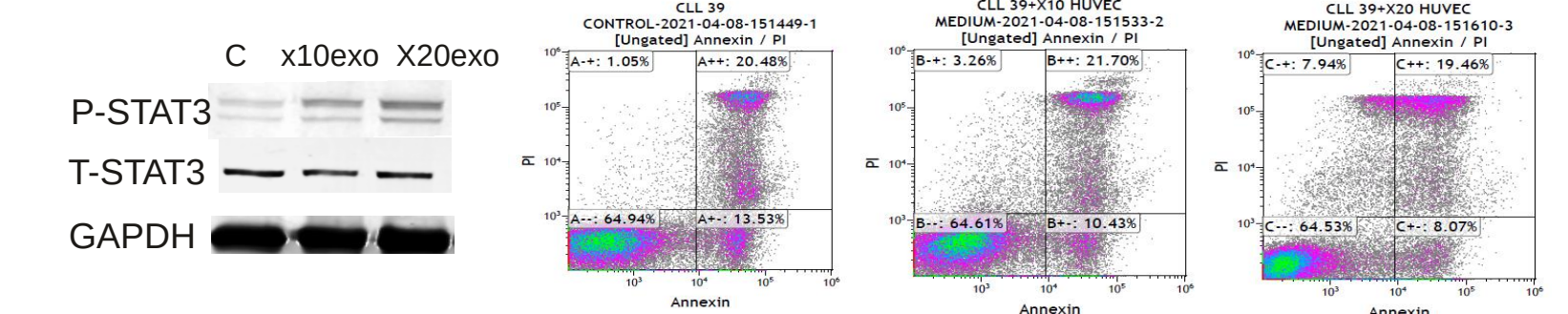
4. β catenin increase the binding of 3 transcription factors to the IL-6 promoter



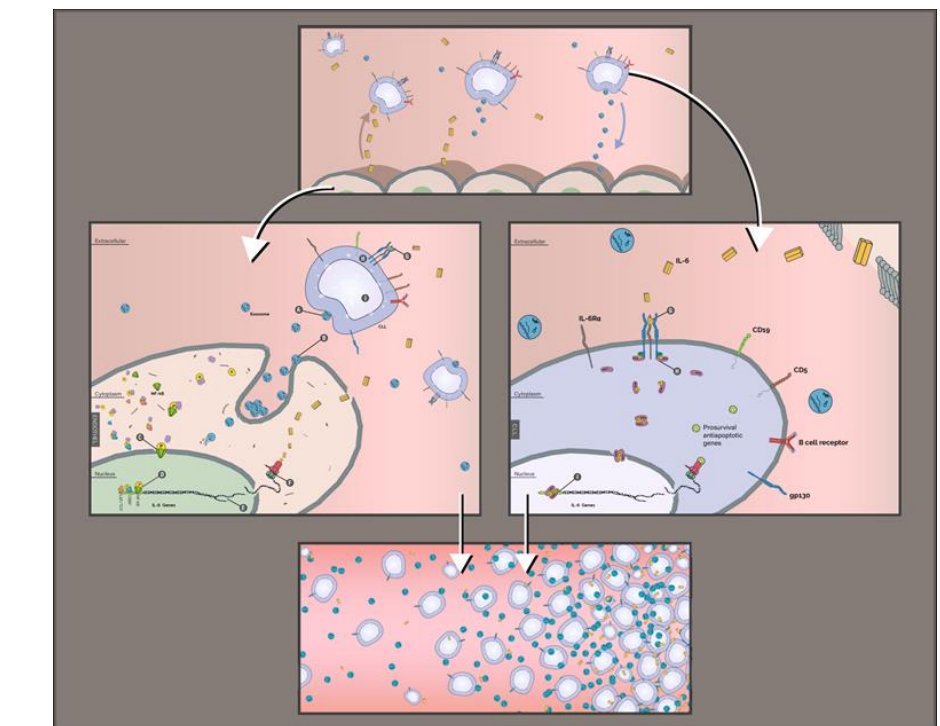
5. CLL- derived exosomes induce IL-6 secretion by endothelial cells



6. IL-6 induce the phosphorylation of STAT3 and protects from apoptosis endothelial cells



7. CLL-derived exosomes turn endothelial cells into CLL-supportive cells



Summary & Discussion

Taken together, we found that CLL cells communicate with endothelial cells through exosomes that they release. Once these exosomes are taken up by endothelia, they turn them into IL-6 producing cells.