Chronic Lymphocytic Leukemia B-cells are rescued from apoptosis by extracellular vesicles from bone marrow mesenchymal stromal cells

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Introduction

Chronic Lymphocytic Leukemia (CLL) is a malignant disease with a variable progression; survival after diagnosis can range from months to decades. Currently, CLL remains incurable, and there is a need for earlier prediction of patient outcomes. The interaction between the microenvironment, notably composed of **Bone Marrow Mesenchymal Stromal Cells** (BM-MSC), and leukemic cells plays an important role in promoting the increased survival of leukemic B cells. **Extracellular vesicles** (EVs) produced by leukemic cells and the microenvironment may be implicated in these interactions. EVs, including microparticles and exosomes, are small plasma membrane fragments with sizes ranging from 0.01 to 1µm, and contain products specific to the original cell, such as microRNA, mRNA and proteins. EVs are expelled by stimulated cells or after stress signals and can deliver their content to the target cell. It has been shown that these vesicles have many implications in biological processes such as intercellular communication, coagulation activity, immunosuppression, and tumor growth. Our objectives are first to confirm the uptake of EVs in CLL B-cells and to assess their role in the **cross-talk** between malignant cells and their microenvironment.

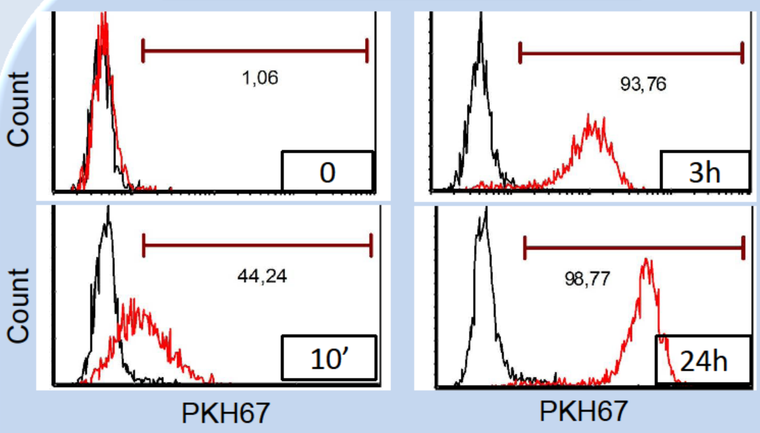
Methodology

Serial centrifugations (400g-5min/150000g-1h) were used to isolate EVs from BM-MSC. EVs were characterized by flow cytometry (Navios calibrated with megamix beads PLUS) and were also analyzed by Transmission (TEM) and Scanning Electron Microscopy (SEM).

A study of integration capacity of EVs in CLL-B cells was performed using PKH67 Kit and qRTPCR (1). EVs were added during 24h to CLL B-cells and analysis of apoptosis in CLL cells was assessed by flow cytometry using Annexin/7AAD staining (2). Finally, we studied the effect of EVS on the proliferation of CLL B-cells by CFSE (Carboxyfluorescein succinimidyl ester) staining (3).

Results

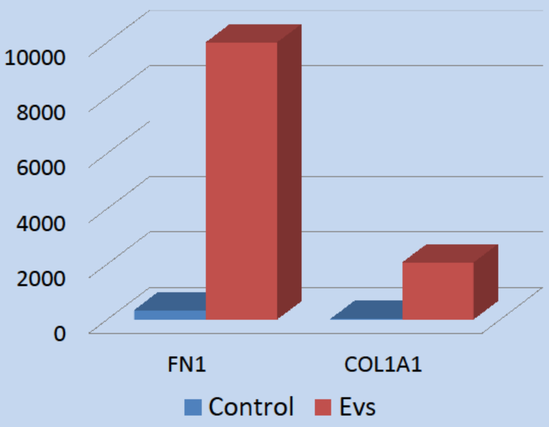
1. Proof of EV integration



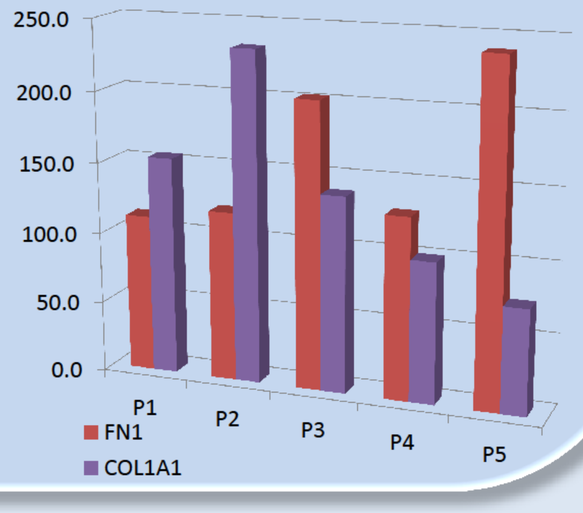
EVs from MSC were labeled with PKH67 and incubated with CLL B-cells during several times. After 3 hours the majority of cells had **integrated fluorescent vesicles**.

-> Modification of gene expression by qRTPCR :

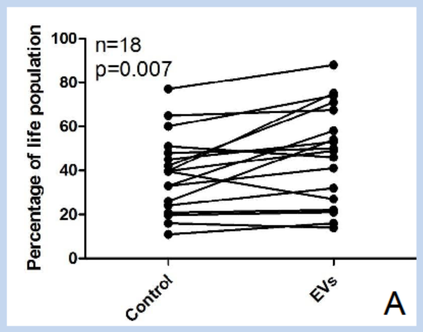
The two first genes increased in CLL cells after contact with MSC were fibronectin (FN1) and collagen 1A (COL1A1) which are known to be highly expressed in MSC, as demonstrated by microarray analysis (data not shown). FN1 and COL1A1 expression were studied after 24h incubation, with PBS (negative control) and EVs from BM-MSC.



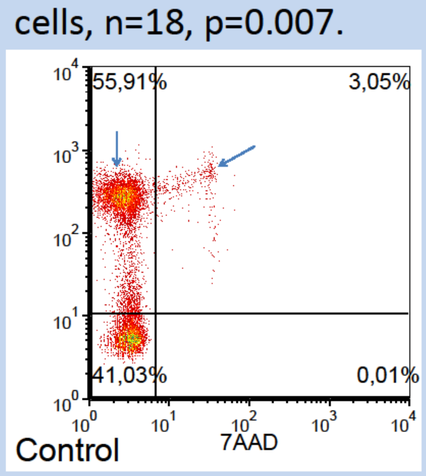
To prove the **RNA transfert** in CLL B-cells by vesicles, RNA expression was directly characterized in EVs. FN1 and COL1A1 RNAs were found in all samples.



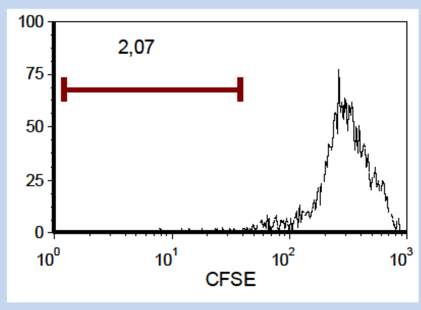
2. Protection against apoptosis



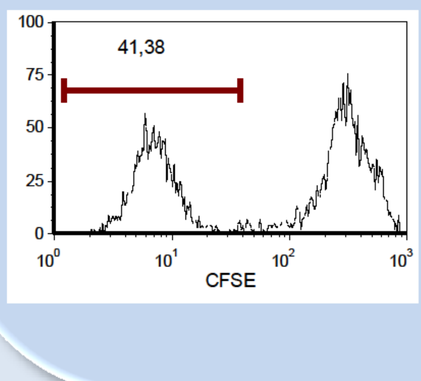
Addition of different concentrations of EVs showed an **increased survival** of CLL B-cells (A). This protective effect is due to a decrease of apoptosis (B). Mean decrease of 9.9% of apoptotic cells, n=18, p=0.007.

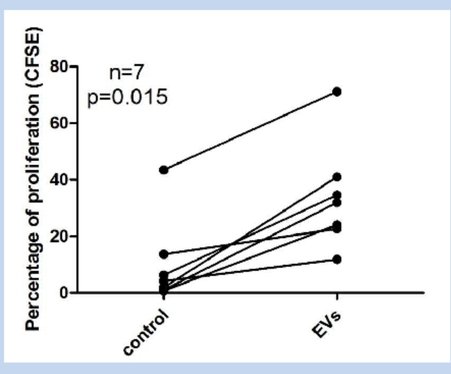


3. Effect of EVs on CLL B-cell proliferation



Proliferation of CLL B-cells was defined by CFSE labeling. Addition of different concentrations of **BM-MSC EVS on CLL B-cells induced proliferation**. This modification is dose-dependent (not shown).





Conclusion

We showed that **EVs from BM-MSC protect CLL B-cells from apoptosis and increase their proliferation**. It highlighted the **importance of microenvironment and EVs** in the context of CLL progression. This study will help to understand EVs role in the interactions between leukemic cells and microenvironment for better understanding of disease physiopathology but also to find new treatment targets for CLL patients.

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