THE STEM CELL DERIVED MICROPARTICLES FROM UMBILICAL CORD BLOOD AFFECT THE VIABILITY, THE APOPTOSIS AND THE CLONOGENICITY OF HEMATOPOIETIC CELLS

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INTRODUCTION

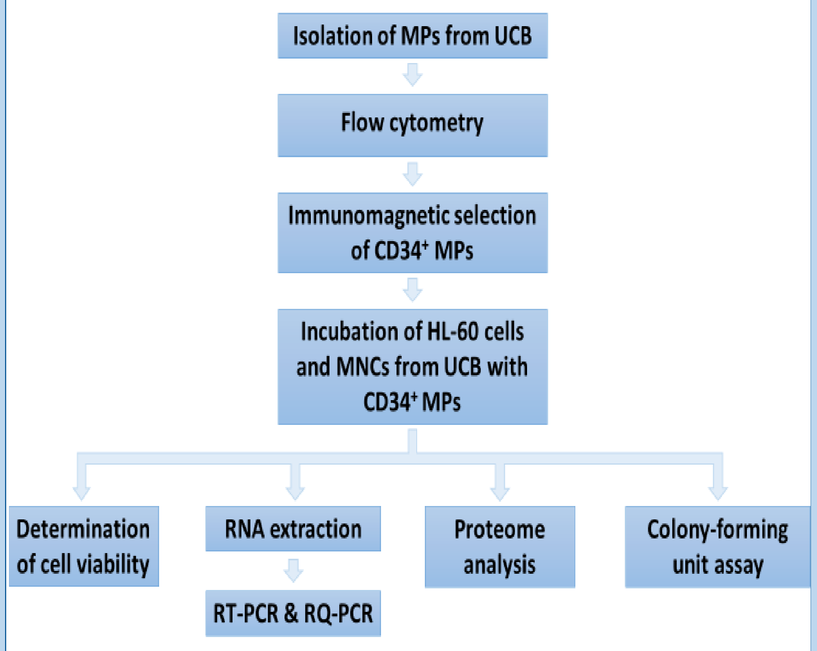
Microparticles (MPs) are cell-derived vesicles, sized 100nm-1μm[1]. MPs play role in intercellular communication that is associated with physiological and pathological conditions. MPs have been detected in the blood in many pathological conditions associated mainly with endothelial injury, thrombosis and inflammation[1,2]. Our previous work has be shown that MPs derived from hemopoietic stem cells (CD34+MPs) contained selected small single-stranded non-coding RNA molecules (miRNAs) implying their participation in trans-cellular communication[3].

AIM

The aim of this work was to study the effect of CD34+MPs on apoptosis of leukemia cell line HL-60 and mononuclear cells (MNC) from umbilical cord blood (UCB).

METHODS

CD34+MPs were isolated from the plasma of umbilical cord blood units (UCBs) after centrifugation and immunomagnetic selection using MACS technology (Miltenyi Biotec, Germany). The number of CD34+MPs was estimated by flow cytometry using CD34-PE and Annexin V-FITC Abs. Mononuclear cells (MNCs) were collected by density gradient centrifugation using lymphoprep. MNCs and HL60 cells were cultured in liquid culture without growth factors for 24hrs with or without CD34+MPs (800 CD34+MPs/0,9x10^{6}cells). After incubation, determination of cell viability, RT-PCR and RQ-PCR, as well as proteome analysis has been performed. Furthermore, MNCs were cultured in liquid culture without growth factors for 1 or 24 hours with or without CD34+ MPs and then were seeded in semisolid cultures in the presence of a cocktail of growth factors for colony-forming units growth (CFUs). After 14 days of incubation, enumeration of colonies has been performed.



RESULTS

Effect of CD34+ microparticles on cell viability in HL-60 cells

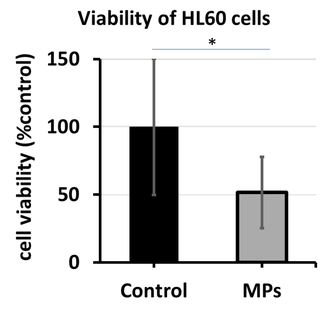


***Figure 1**. Cell viability of HL-60 cells after 1 day (24 hours) and 3 days incubation with 800 CD34+ MPs (p<0,05).*

Effect of CD34+ microparticles on expression of apoptosis-related genes in HL-60 cells

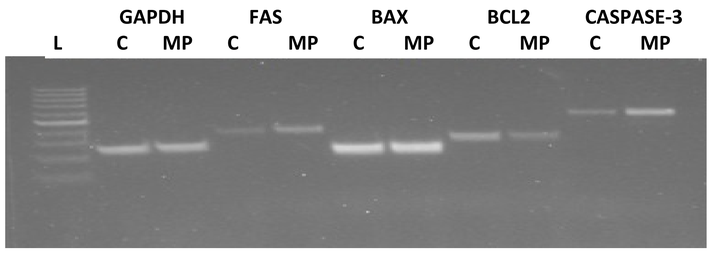


***Figure 2.** Semiquantitative PCR analysis of the expression of FAS, BAX, BCL-2 and CASPASE-3 genes in HL-60 after 24 hours incubation with 800 CD34+ MPs.*

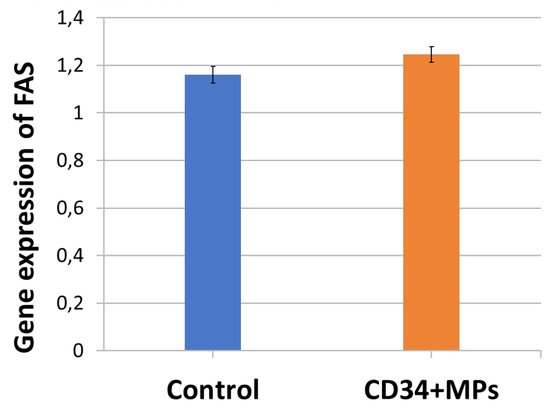


***Figure 3**. Real-time PCR analysis of the expression of pro-apoptotic gene FAS in HL-60 after 24 hours incubation with 800 CD34+ MPs*

Effect of CD34+ microparticles on cell viability in MNCs from UCB

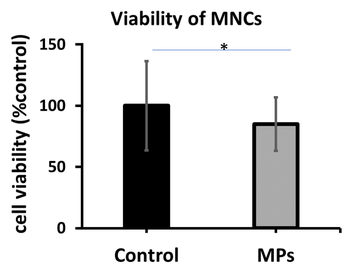


***Figure 3**. Cell viability of MNCs after 24 hours incubation with 800 CD34+MPs (p<0,05).*

Effect of CD34+ microparticles on expression of apoptosis-related genes in MNCs from UCB

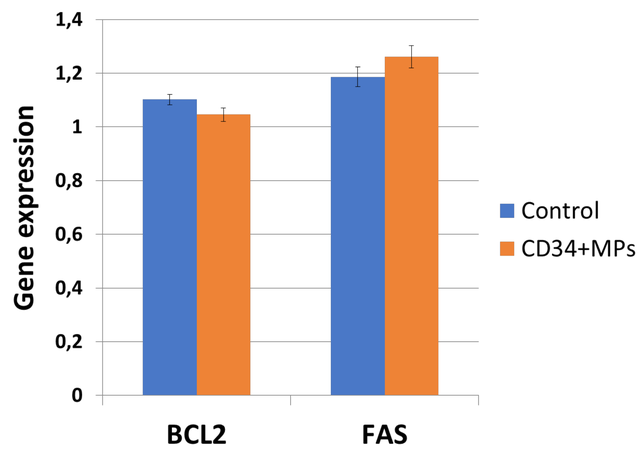


***Figure 3.** Real-time PCR analysis of the expression of anti-apoptotic gene BCL-2 and pro-apoptotic gene FAS in MNCs from UCB after 24 hours incubation with 800 CD34+ MPs.*

RESULTS

Effect of CD34+ microparticles on protein levels of expression of apoptosis-related proteins in HL-60 and MNCs from UCB

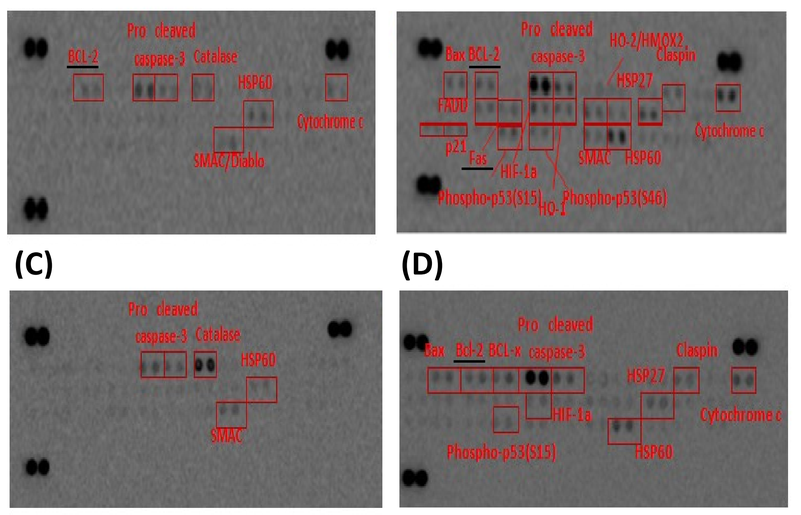


***Figure 4.** Representative proteome profiler showing the presence of apoptotic factors in HL-60 cells and MNCs after 24 hours incubation with CD34+MPs. **(A)** HL60 Control, **(B)** HL60 MPs, **(C)** MNCs Control, **(D)** MNCs MPs (A1,A2,A23,A24,E1,E2: positive markers).*

CFU assay of stem and hematopoietic progenitor cells in MNCs from UCB after incubation with different concentrations of CD34+MPs for 1 and 24 hours

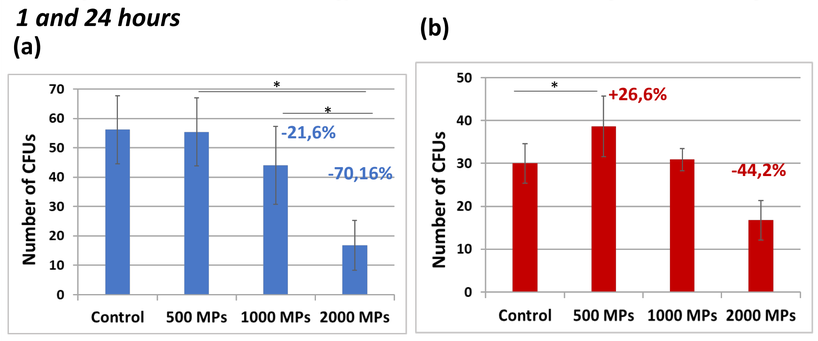


***Figure 5.** Total number of colonies derived from MNCs after incubation with or without (Control) 500, 1000 and 2000 CD34+ MPs for 1 hour **(a)** and 24 hours **(b)**.*

The number of leukemic HL60 cells was decreased significantly in the presence of CD34+MPs in liquid culture. The number of UCB-MNCs in the presence of the CD34+MPs was decreased significantly compared to the control. The expression of anti-apoptotic Bcl2 was decreased, whereas the expression of pro-apoptotic Fas was increased in both HL-60 cells and MNCs. CFUs were decreased after incubation of MNC for 1 h with 2000 CD34+MPs compared to 500 and 1000 (CFUs 500 CD34+MPs vs. CFUs 2000 CD34+MPs p=0,022 rho=0,978 and CFUs 1000 CD34+MPs vs. CFUs 2000 CD34+MPs p=0,049 rho=0,951) (Fig5a). CFUs obtained from cells incubated with 500 CD34+MPs for 24 hrs were shown increase compared to control (p=0,017 rho=-1,00) (Fig 5b).

CONCLUSIONS

In summarizing we have identified the inhibitory effect of stem cell–derived MPs (CD34+MPs) in the viability of leukemic cells and MNCs with concominant increase of the apoptotic FAS gene expression. In parallel CD34+MPs affect the growth of CFU in a dose- and time-dependent manner.

ACKNOWLEDGEMENT

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