Functional heterogeneity of umbilical cord blood CD34 positive cells

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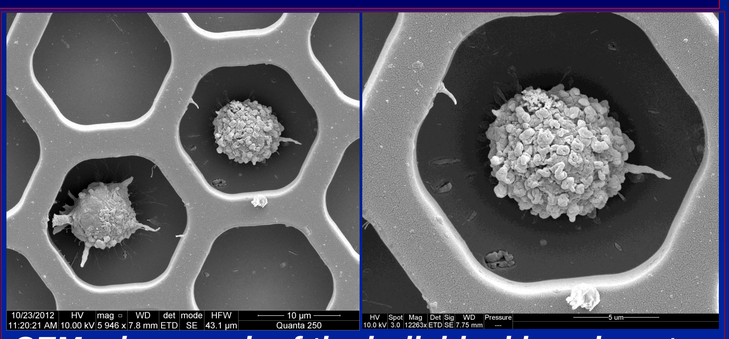
Abstract

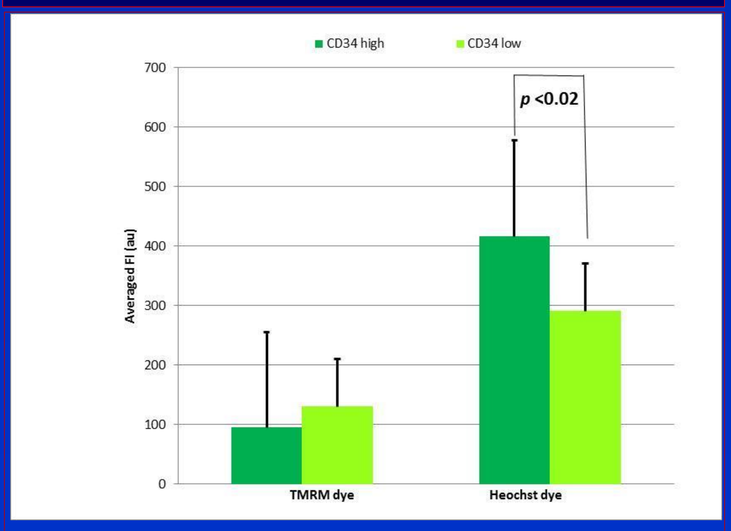
Background. The great heterogeneity in living cells provides the stable spatiotemporal function of the multicellular organism. UCB contains hematopoietic stem cells pool belonging simultaneously to both adult/mother and embryo/infant organisms. Study of morphological and functional heterogeneity in the CD34 positive UCB cells at the single cell level may provide sufficiently detailed information which can contribute to the understanding of the biological activity of these cells and their potential use.



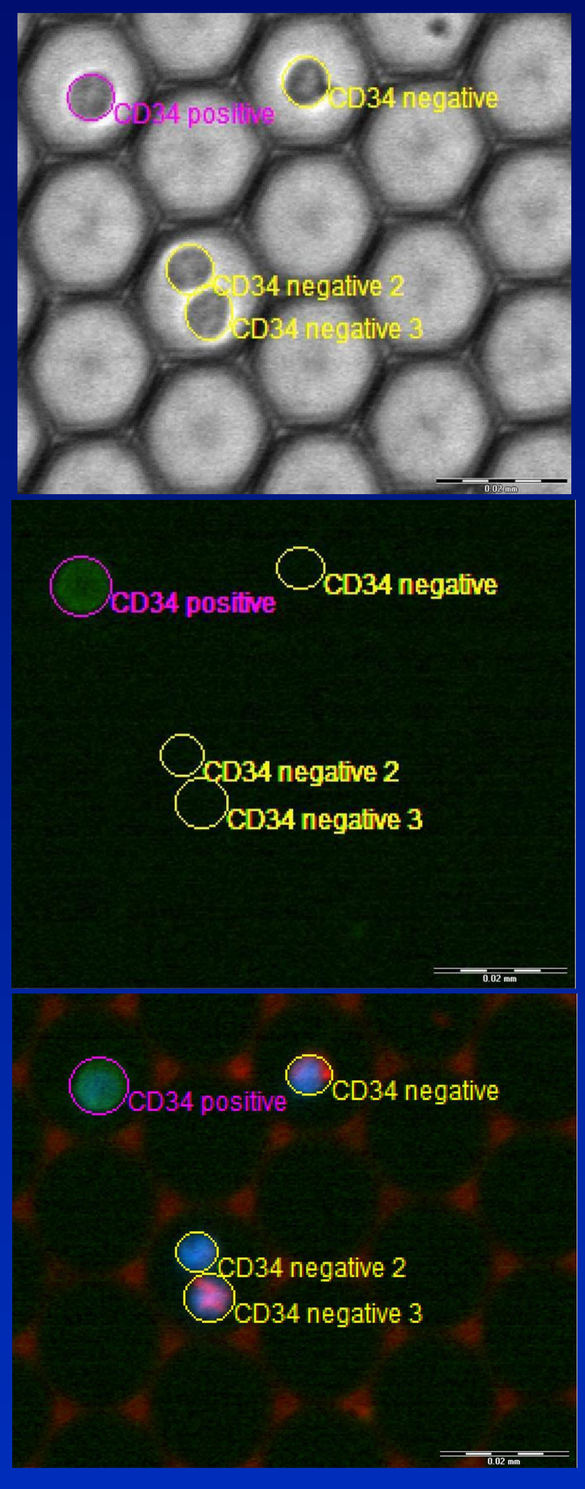
SEM micrograph of the individual lymphocytes in single microchambers (20 µm pitch and 12.5 µm depth). Each microchamber is designed to accommodate a single cell.

Methods:

In this study we present an imaging approach based on the cell retainer methodology [Deutsch M. et al, 2006] for real-time investigation of the functional intracellular characteristics of UCB cells. This methodology enables repetitive, high-content correlative multi-parametric measurement and image analysis of individual cells within a population, during various manipulations, e.g. drug introduction, staining procedures, etc.



Among CD34 positive cells ~30% exhibited high FI signal, while the rest had a low FI signal. The $CD34^{high\ FI}$ fraction did not efflux HD effectively, in contrast to $CD34^{low\ FI}$ cells which are associated with significantly lower staining ($p<0.02$). On the other hand, these two fractions of CD34 positive cells do not differ significantly with respect to MMP and ability to hydrolyze FDA.



The separated UCB mononuclear cells were loaded into the *LiveCell™ Array (upper panel).* Firstly, cells were stained for CD34 identification and later sorted based on their CD34 FI *(middle panel)*. A series of vital tests including cell death rate (PI staining), mitochondrial membrane potential (TMRM staining) and Heochst dye (HD) efflux was performed at the level of individual cells. Triple staining of one CD34 positive and three CD34 negative cells is presented in *lower panel*. Scale bar: 20 *µm.*

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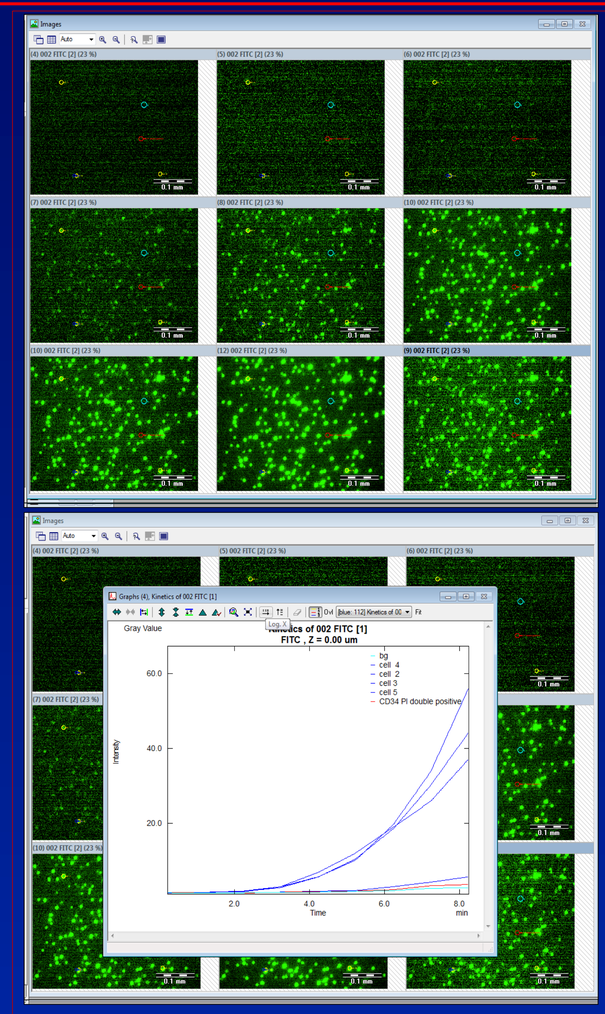
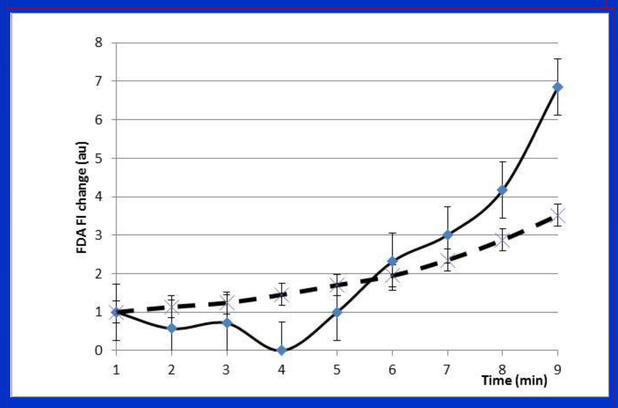


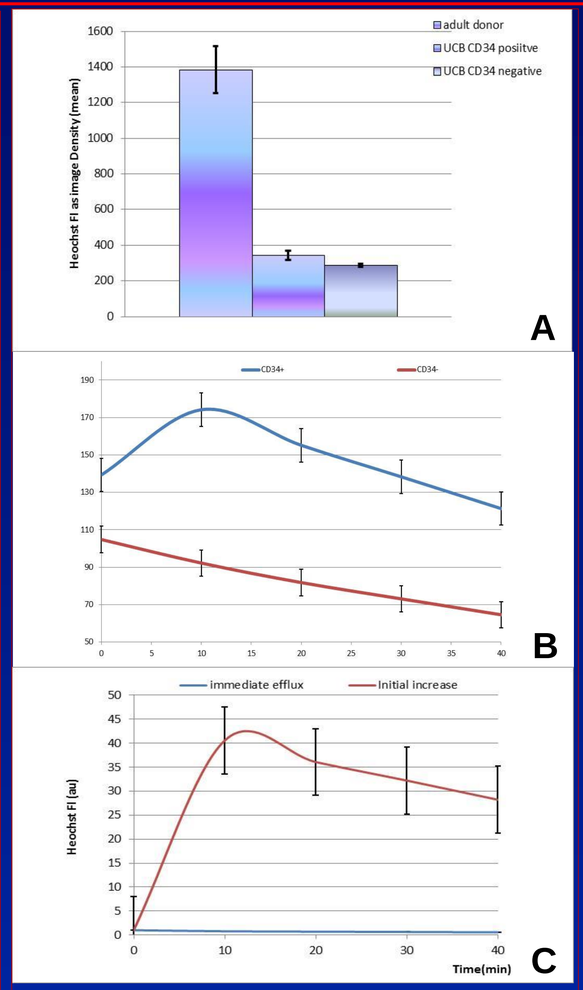
Cytoplasm membrane integrity and intracellular metabolism (the rate of FDA hydrolysis by non-specific esterases) were calculated from repeated periodic measurements for each single cell.

The FI change in individual cord blood cells, stained with FDA and scanned nine times is presented in upper panel.

5 representative cells are indicated by colored circles: yellow – three live CD34-positive cells, red – one dead PI-positive CD34-positive cell, indigo – one CD34 negative cell. The rest cells are CD34 negative. Additionally, the blue circle indicates the empty microchamber serviced for background evaluation. Time dependent FI levels in indicated cells are presented in the graph inserted in lower panel. Scale bar: 100 *µm*



The increase of the FDA FI within the UCBC (smoothed line) is delayed for about four minutes in comparison to peripheral mononuclear cells of the healthy donor (dashed line). Nevertheless, after the FDA hydrolysis have been started the slope is higher in UCBCs.



Hoechst dye extrusion was used as a test for stemness in single cells. The averages FI in UCBCs in comparison to peripheral mononuclear cells from adult donor are presented (A). The kinetics of dye efflux was measured in each individual cell by imaging. The FI change in cell populations of CD34-positive and CD34-negative cells separately (B) and two subsets of CD34-positive cells (C) are presented as averages for series of images.

Conclusions

Freshly isolated individual mononuclear cells derived from UCB reveal a certain level of heterogeneity with respect to intracellular functional characteristics. The intracellular FDA hydrolysis is delayed in comparison to adult cells. Two subsets of the CD34 positive cells divided according to CD34 FI exhibited different kinetics of HD efflux.

The results obtained may be important for further basic investigations, and to applied research as well.