

Characterization of the surfaceome of hematopoietic and leukemic stem cells expanded with UM171 to improve the development of novel therapies for leukemia

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Introduction

Background information - Despite intensive treatment and significant progress in clinical outcome, 30% of all pediatric acute myeloid leukemia (AML) patients will suffer from relapse. Therefore, more efficacious strategies are crucially needed. Hematopoietic stem cell transplantation (HSCT) has become a potent life-saving procedure for both adults and children with various hematological malignancies. Umbilical cord blood (CB) appears as one of the most attractive sources of HSCs for transplantation, due to its immature immune system and widespread availability. Unfortunately, many patients are deprived from this therapeutic strategy because of the low stem cell dose. Sauvageau's group, have recently discovered a small-molecule, UM171, which stimulates the expansion of CB HSCs *ex vivo* and the maintenance of leukemic stem cells (LSCs) *in vitro*. However, the unavailability of specific surface markers that can prospectively identify HSCs and LSCs is still a major hurdle for the optimization of CB grafts and the development of new targeted therapies.

Purpose of the study - The aim of this collaborative research project is to identify new HSC & LSC surface markers using quantitative proteomic methods.

1- To improve HSCT through CB grafts optimization

2- To uncovering new therapeutic targets for leukemic disorders

Methods & Results - To discover novel and reliable cell surface markers, we have adapted a cutting-edge chemoproteomic approach in combination with UM171-induced cell expansion to characterize the surface proteome (surfaceome) of hematopoietic cells. We found that UM171 induces the upregulation of more than 35 cell surface proteins, including EPCR which we recently characterized as a novel surface marker for the HSC population derived from CB expanded with UM171. We then performed a surfaceome analysis of cell populations from UM171-expanded CB. Preliminary results reveal the enrichment in more than 100 surface proteins in the CD34+EPCR+ population, and several of these are currently being tested as potential new markers for HSCs.

Altogether, these results validate the surfaceome approach for the identification of novel HSC- and LSC-specific surface molecules using CB units and primary AML specimens, respectively.

Methods

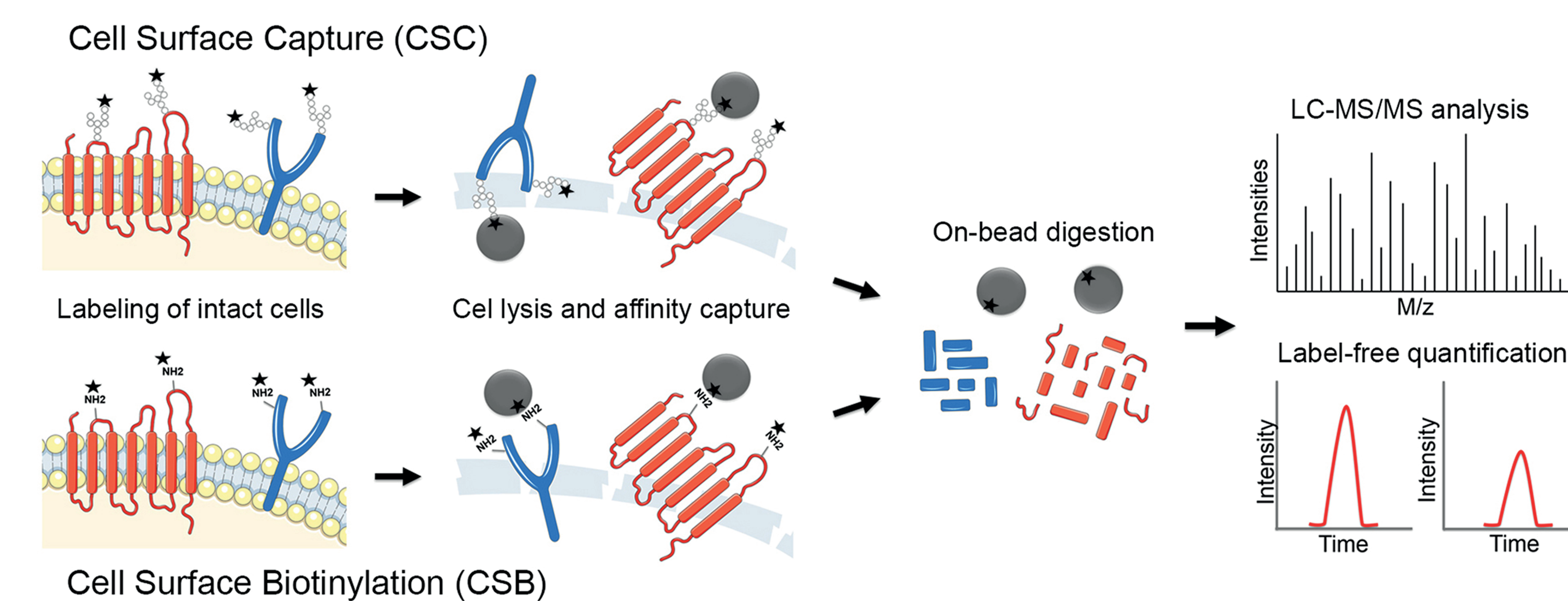


Figure 1: Two chemoproteomics approaches to characterize surfaceome changes

Cell Surface Capture (CSC) - Cell surface sialylated glycoproteins are first oxidized to generate aldehyde groups for coupling with the labeling reagent (Aminooxy-biotin). Cells are lysed and labeled proteins are purified by affinity capture. Following extensive washing, proteins are digested on beads and analyzed by LC-MS/MS for label-free quantification (peak area).

Cell Surface Biotinylation (CSB) - Intact viable cells are biotinylated using a plasma membrane-impermeable reagent (Sulfo-NHS-LC-biotin). Cells are lysed and labeled proteins are isolated by avidin chromatography. Following several stringent wash steps, proteins are digested on beads and analyzed by LC-MS/MS for label-free quantification.

Results

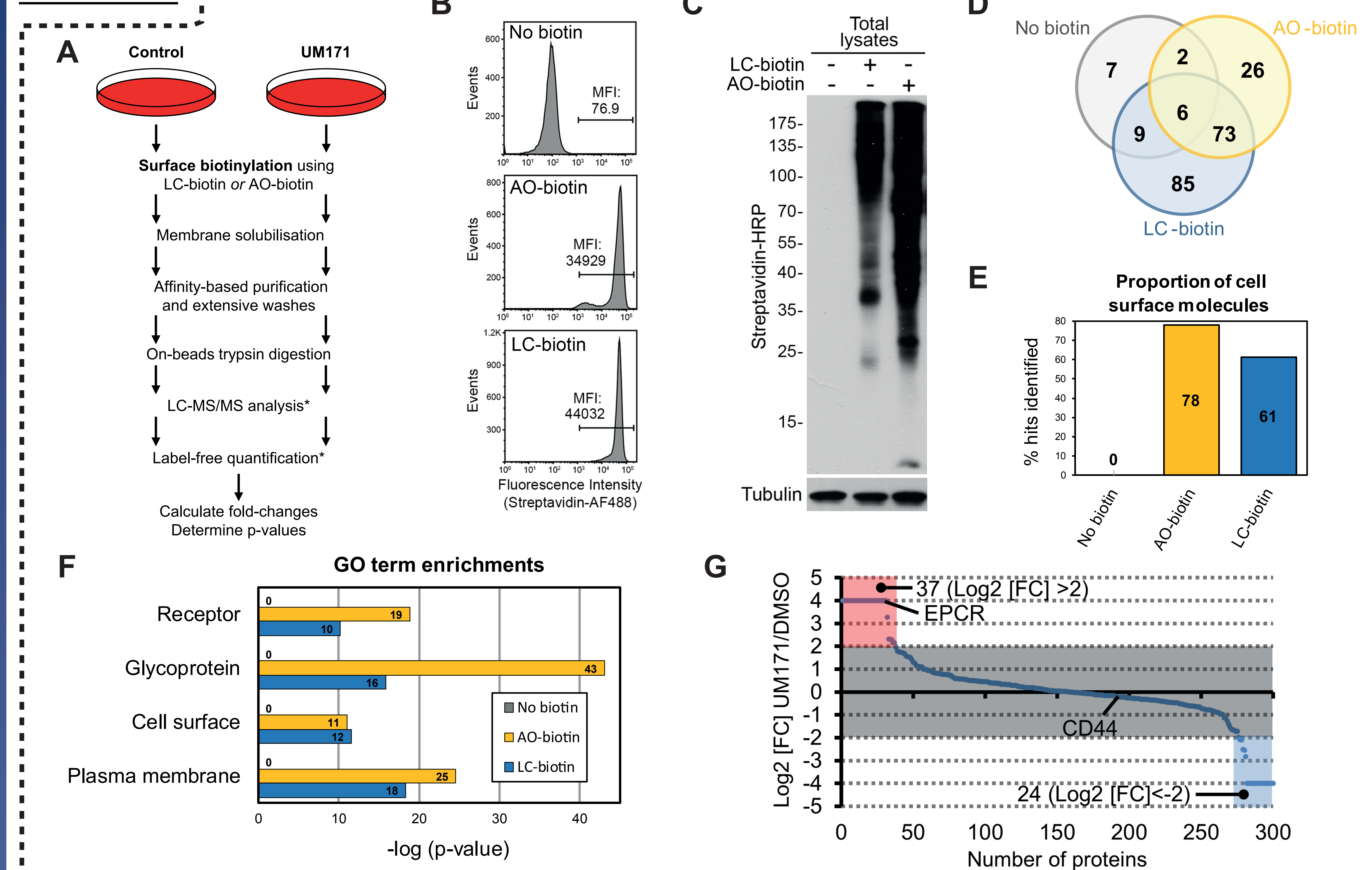


Figure 2: Optimization of surface proteomic method in OCI-AML5 cell line

(A) Schematic diagram of the workflow for the identification and quantification of surfaceome changes associated with UM171 treatment. (*LC-MS/MS analyses were performed by Dr. Eric Bonneil, Center for Advanced Proteomics Analyses, IRIC, Université de Montréal)

(B) FACS analysis showing the efficiency of cell surface labeling with aminooxybiotin (AO-biotin) or sulfo-NHS-LC-biotin (LC-biotin).

(C) Immunoblot analysis of whole cell lysates showing the biotinylation patterns of cell surface proteins purified with either CSC or CSB.

(D) Venn diagram of proteins identified by mass spectrometry from surfaceome of OCI-AML5 isolated using either AO-biotin or LC-biotin.

(E) Proportion of identified proteins that corresponds to cell surface molecules.

(F) Most significant Gene Ontology (GO) term enrichments associated with purified proteins shows a striking bias towards cell surfaceome.

(G) Fold-change in surface proteins as a function of UM171 treatment (expressed in Log2).

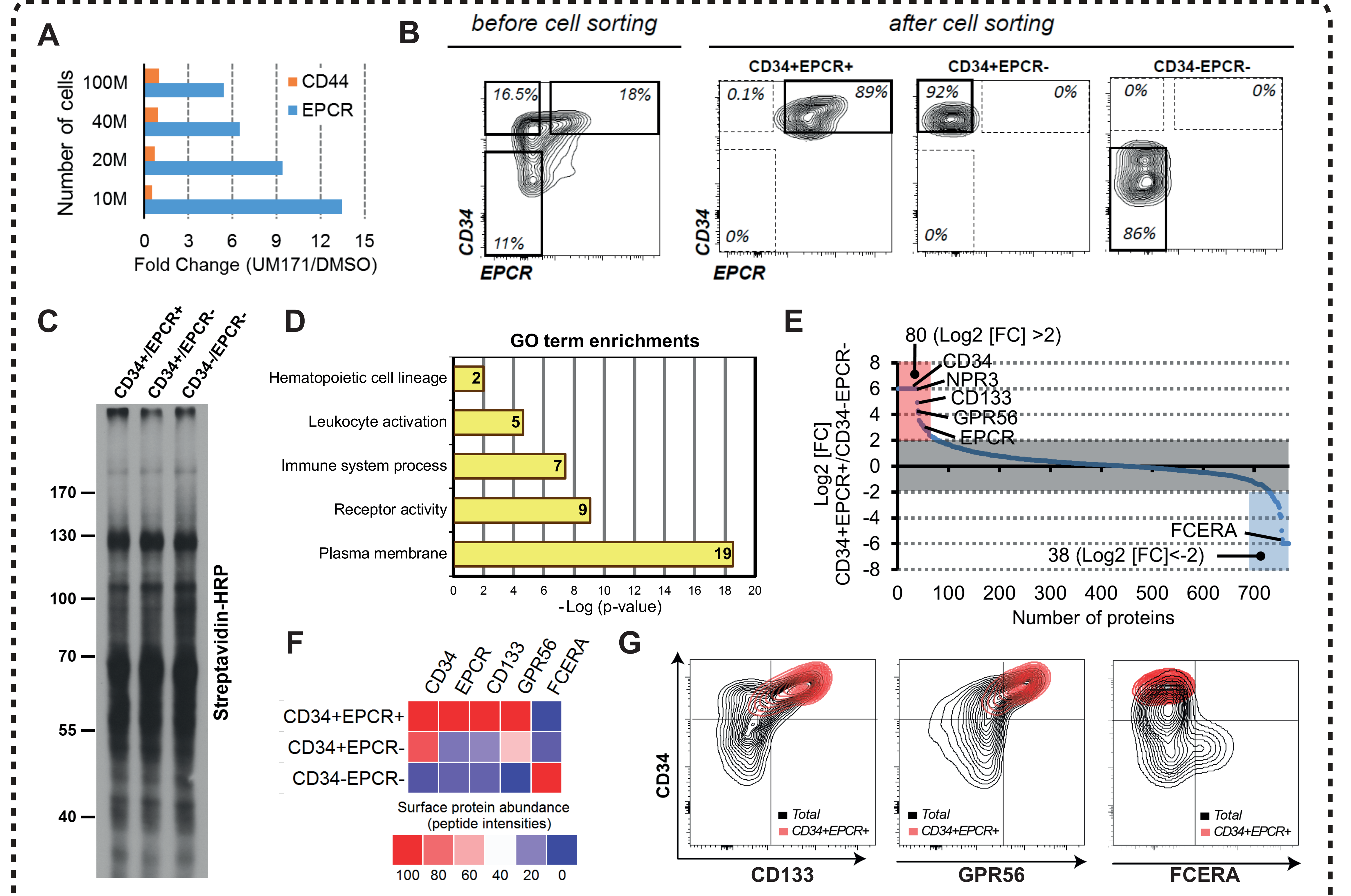


Figure 4: Surfaceome analysis of hematopoietic stem cells from UM171-expanded cord blood

(A) Cell titration showing that EPCR induction can be identified in LC-MS/MS using as low as 10 million OCI-AML5 cells.

(B) CB derived CD34+ cells were expanded for 7 days in the presence of UM171 (38 nM), and sorted based on CD34 and EPCR.

(C) Biotinylation patterns of cell surface proteins isolated from CD34+EPCR+, CD34+EPCR- and CD34-EPCR- populations.

(D) The surface proteomics method was applied to sorted populations, and identified proteins were queried for GO terms enrichment using g:Profiler database.

(E) Hits were quantified using mass spectrometry. Fold-change in surface proteins comparing CD34+EPCR+ with CD34-EPCR- cells.

(F) Heatmap representing the abundance of identified surface proteins as quantified by mass spectrometry.

(G) Surface expression of hits identified by the surfaceome approach on the CD34+EPCR+ population by flow cytometry.

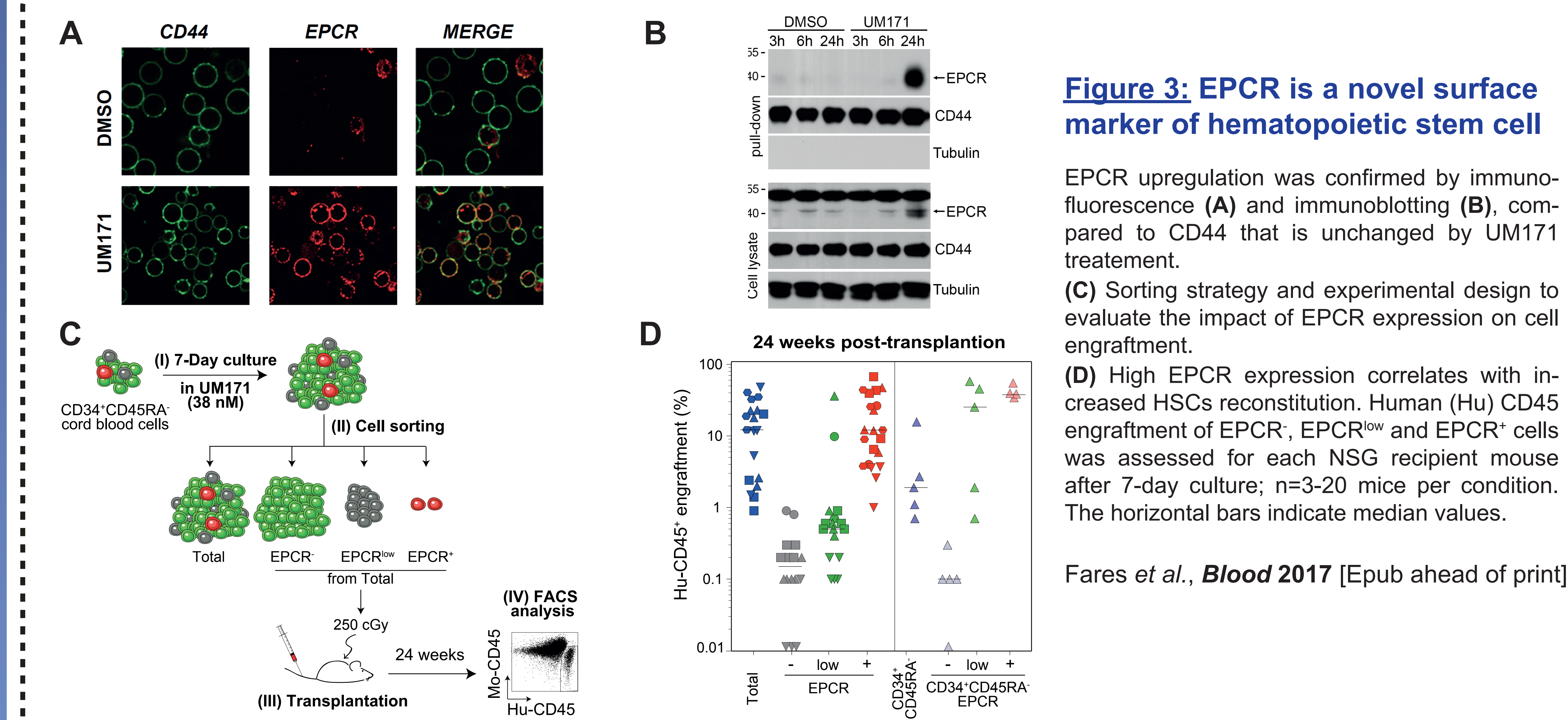


Figure 3: EPCR is a novel surface marker of hematopoietic stem cell

EPCR upregulation was confirmed by immunofluorescence (A) and immunoblotting (B), compared to CD44 that is unchanged by UM171 treatment.

(C) Sorting strategy and experimental design to evaluate the impact of EPCR expression on cell engraftment.

(D) High EPCR expression correlates with increased HSCs reconstitution. Human (Hu) CD45 engraftment of EPCR, EPCR^{low} and EPCR⁺ cells was assessed for each NSG recipient mouse after 7-day culture; n=3-20 mice per condition. The horizontal bars indicate median values.

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Conclusion

- Development of a state-of-the-art chemoproteomics approach for the characterization and quantification of surfaceome changes in UM171-expanded CB cells.
- Identification of more than 100 surface molecules that are potential new HSC markers

Work in progress

- FACS analyses + transplantation experiments on a reduced list of several candidates (preliminary data support the discovery of a new marker which could allow the purification of HSCs to near homogeneity with a frequency of 1 in 2 cells)
- Surfaceome analyses on several primary AML specimens

Perspectives

Finding reliable HSC and LSC-specific markers to improve CB grafts and uncover potent therapeutic targets, respectively