Define the surfaceome landscape of hematopoietic stem cells and pediatric leukemia specimens to improve the development of novel therapies for hematological diseases

Léo Aubert, Elisa Tomellini, Unain Ansari, Jalila Chagraoui, Simon Girard, Marie-Eve Bordeleau, Guy Sauvageau and Philippe P. Roux

Institute for Research in Immunology and Cancer (IRIC), Université de Montréal, Montreal, QC, Canada

Introduction

Background information - Despite major advances in understanding the pathogenesis of various types of hematological diseases, **30% of all pediatric acute myeloid leukemia (AML) patients will suffer from relapse**. ***Therefore, more efficacious strategies are crucially needed.*** Over the past decades, hematopoietic stem cell transplantation (HSCT) isolated from umbilical cord blood (CB) has evolved as a potent life-saving procedure for both adults and children with different types of blood disorders. Unfortunately, many patients are deprived of access to such therapies due to the low stem cell dose in CB units. Dr Guy Sauvageau's group and their collaborators have recently discovered a small-molecule, UM171, which promotes the *ex vivo* expansion of CB HSCs. However, the lack of reliable surface markers that can prospectively identify HSCs 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 cord blood grafts optimization

2- To uncovering new therapeutic targets for leukemic disorders

Methods & Results - To discover novel and reliable cell surface markers, we have optimized and adapted a surface proteomic approach to simultaneously identify and quantify surface proteins in UM171-expanded CB cells. Using the recently discovered HSC marker, EPCR, we have revealed the enrichment in more than 100 surface proteins in the CD34$^+$EPCR$^+$ population. In addition to well-known HSC markers (CD34, EPCR, CD133 and GPR56), we identified several surface proteins that may further subdivide the EPCR-positive population. To determine if these surface molecules are specific to HSC or other hematopoietic populations, their surface expression are being evaluated by flow cytometry when antibodies are available. One such candidate could be GPA33, which appears to further subdivide the HSC population from CB cells. We are currently performing transplantation experiments in NSG mice to determine if GPA33 expression correlates with better engraftments. We are also producing unavailable antibodies against the other surface proteins to analyze their expression and evaluate the engraftment of positive populations.

Methods

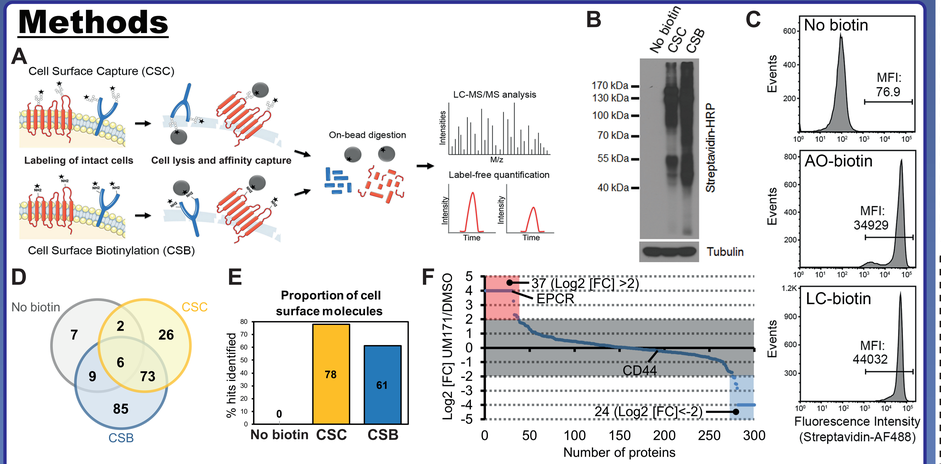


Figure 1: Optimization of a proteomic method for identification and quantification of the cell surface proteins (surfaceome) in OCI-AML5 cell line

(A) Schematic outline of the two surfaceomic approaches optimized for mass spectrometry analysis: **C**ell **S**urface **C**apture (**CSC**) and **C**ell **S**urface **B**iotinylation (**CSB**).

(B) Biotinylation patterns of cell surface proteins labeled either with Aminooxy-biotin (CSC) or Sulfo-NHS-LC-biotin (CSB).

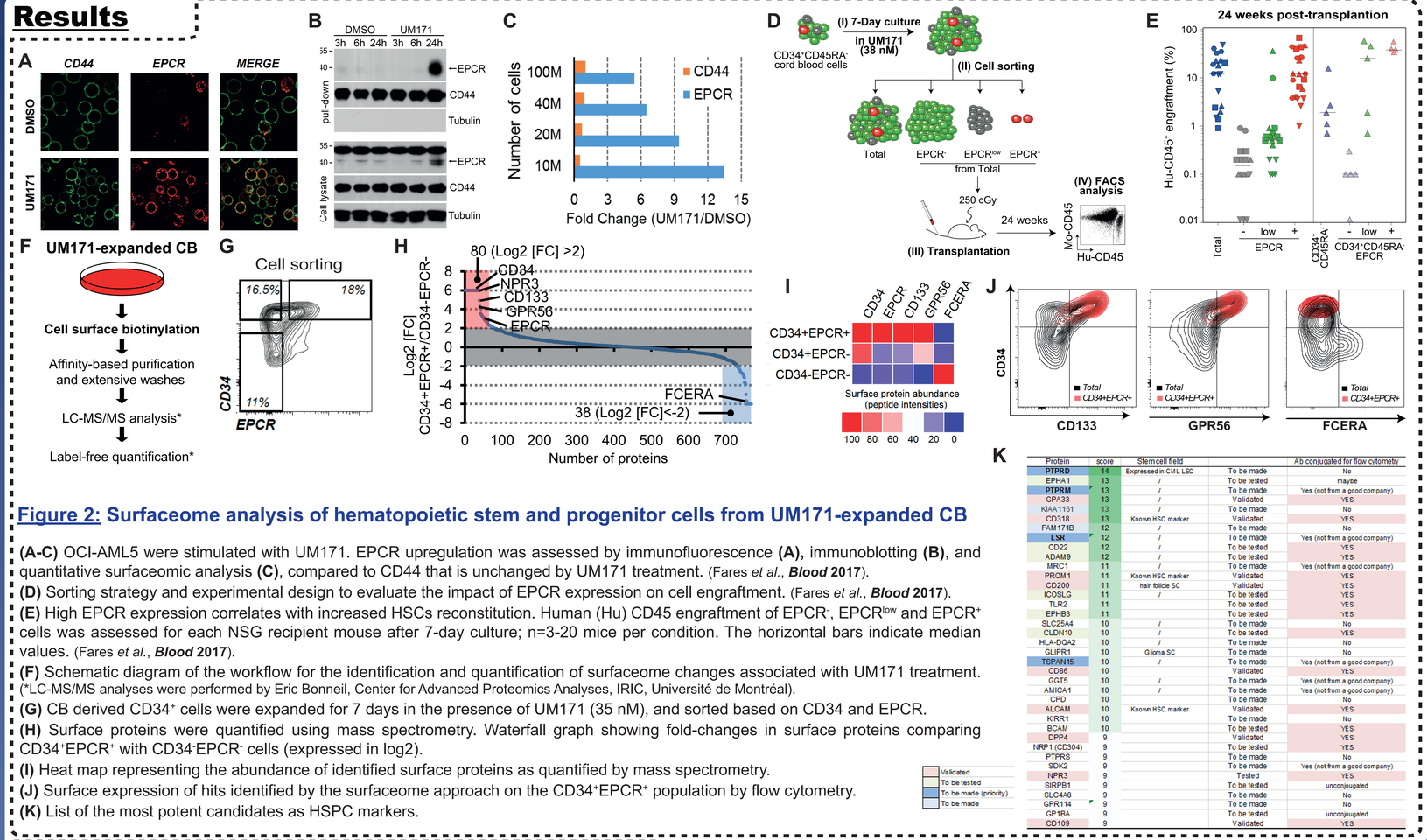
(C) FACS analysis showing the efficiency of cell surface labeling with CSC or CSB method.

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

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

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

Results



Protein	score	Stem cell field		Ab conjugated for flow cytometry
PTPRD	14	Expressed in CML LSC	To be made	No
EPHA1	13	/	To be tested	maybe
PTPRM	13	/	To be made	Yes (not from a good company)
GPA33	13	/	Validated	YES
KIAA1161	13	/	To be made	No
CD318	13	Known HSC marker	Validated	YES
FAM171B	12	/	To be made	No
LSR	12	/	To be made	Yes (not from a good company)
CD22	12	/	To be tested	YES
ADAM9	12	/	To be tested	YES
MRC1	11	/	To be made	Yes (not from a good company)
PROM1	11	Known HSC marker	Validated	YES
CD200	11	hair follicle SC	Validated	YES
ICOSLG	11	/	To be tested	YES
TLR2	11		To be tested	YES
EPHB3	11		To be tested	YES
SLC25A4	10	/	To be made	No
CLDN10	10	/	To be tested	YES
HLA-DQA2	10	/	To be made	No
GLIPR1	10	Glioma SC	To be made	No
TSPAN15	10	/	To be made	Yes (not from a good company)
CD86	10		Validated	YES
GGT5	10	/	To be made	Yes (not from a good company)
AMICA1	10	/	To be made	Yes (not from a good company)
CPD	10		To be made	No
ALCAM	10	Known HSC marker	Validated	YES
KIRR1	10		To be made	No
BCAM	10		To be tested	YES
DPP4	9		Validated	YES
NRP1 (CD304)	9		To be tested	YES
PTPRS	9		To be made	No
SDK2	9		To be made	Yes (not from a good company)
NPR3	9		Tested	YES
SIRPB1	9		To be tested	unconjugated
SLC4A8	9		To be made	No
GPR114	9		To be made	No
GP1BA	9		To be tested	unconjugated
CD109	9		Validated	YES

Validated
To be tested
To be made (priority)
To be made

Figure 2: Surfaceome analysis of hematopoietic stem and progenitor cells from UM171-expanded CB

(A-C) OCI-AML5 were stimulated with UM171. EPCR upregulation was assessed by immunofluorescence **(A),** immunoblotting **(B)**, and quantitative surfaceomic analysis **(C)**, compared to CD44 that is unchanged by UM171 treatment. (Fares *et al.*, ***Blood* 2017**).

(D) Sorting strategy and experimental design to evaluate the impact of EPCR expression on cell engraftment. (Fares *et al.*, ***Blood* 2017**).

(E) 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. (Fares *et al.*, ***Blood* 2017**).

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

(G) CB derived CD34$^+$ cells were expanded for 7 days in the presence of UM171 (35 nM), and sorted based on CD34 and EPCR.

(H) Surface proteins were quantified using mass spectrometry. Waterfall graph showing fold-changes in surface proteins comparing CD34$^+$EPCR$^+$ with CD34$^-$EPCR$^-$ cells (expressed in log2).

(I) Heat map representing the abundance of identified surface proteins as quantified by mass spectrometry.

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

(K) List of the most potent candidates as HSPC markers.

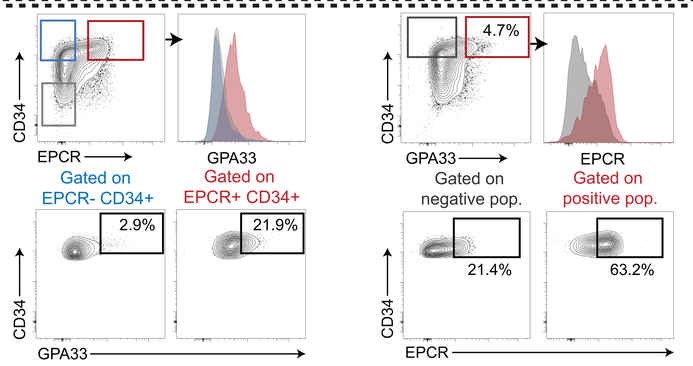


Figure 3: GPA33 further subdivides the CD34$^+$EPCR$^+$ population

FACS analyses in cord blood cells.

Conclusion

- Development of a cutting-edge surface proteomics approach for the characterization and quantification of surfaceome changes in UM171-expanded CB cells.

Work in progress

- FACS analyses + transplantation experiments in NSG mice on a reduced list of several candidates, to evaluate if these surface proteins are specific to HSCs.
- Characterization of the surfaceome in pediatric acute megakaryoblastic leukemia (AMKL), using synthetic human models (PDXs), to identify AMKL-specific antigens (collaboration with **Dr. Sonia Cellot**, see **PO26 - Mathieu Roussy**)

Perspectives

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