

COMMENTARY

iPSC diversity: A key for better use and improved targeting

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Induced pluripotent stem cells (iPSCs) are potentially an inexhaustible source of megakaryocytes, which can produce human platelets resembling the ones from peripheral blood in many aspects, including ultrastructure, surface antigen expression, and function. iPSCs can be used for studying either cell physiology or pathology by starting from disease cells. Their use is wide ranging, from basic science and pathophysiology to drug and toxicology screening. The need for cells that could overcome donor-derived platelet scarcity for transfusions has prompted several research groups to develop new culture systems for the massive production of megakaryocytes and platelets from iPSCs.¹

Considering the importance of iPSC applications, we believe it would be useful to agree on new protocols and guidelines including a broader characterization of iPSC-derived megakaryocytes and platelets.

Evidence has shown that megakaryocytes from different sources, such as cord or peripheral blood, bone marrow, embryonic stem cells, and iPSCs can present different phenotypes and variable function in terms of proplatelet formation and platelet production.²⁻⁵ iPSC-derived megakaryocytes are smaller and do not extend long and branched proplatelets. However, it is still unclear whether this is an intrinsic defect or whether the right trigger to promote proper platelet production from iPSCs is still missing. Although iPSCs are considered a model for studying human cell processes, they have distinct characteristics that need to be taken into consideration. The first step to identify the origin of iPSC phenotype is to analyze their transcriptome and proteome.

The article by Kammer et al.,⁶ published in this issue of *Journal of Thrombosis and Haemostasis*, provides an integrated analysis of the

transcriptome and proteome of megakaryocytes differentiated from iPSCs derived from a large group of healthy subjects of different ages, race, and sex (Figure 1).

Linear regression and gene set enrichment analysis identified functional groups of genes whose expression was related positively or negatively to megakaryocyte maturation, the former including genes important in regulating platelet function, cell-cell adhesion, and cytoskeleton remodeling, while the latter a variety of cellular regulatory genes, including organelle-related genes and gene sets related to immune and inflammatory responses and leukocyte function.

None of these genes had differential expression in iPSC-derived megakaryocytes when applying a linear regression model to samples categorized by ages, while a significant differential gene expression was found after categorization by race, namely European American (EA) and African American (AA), though gene set enrichment analysis showed no significant differences. In contrast, differences were observed when dividing samples by sex. More than 400 genes showed increased expression in iPSC-derived megakaryocytes from women compared to men. Of these, the most significant expressed genes were those coding for five different types of collagen, the bone morphogenic protein (BMP1), and β -tropomyosin (TPM2). By applying gene set enrichment analysis, 265 gene sets were found significantly upregulated in females compared to males, including genes related to cell motility and adhesion, angiogenesis, and collagen fibril organization, which is an interesting finding in light of the known higher susceptibility of platelets from women to aggregate.⁷⁻¹⁰ Some genes were also significantly upregulated in males compared to females, these included a telomere protection gene (POT1), a secretory gene expressed in CD34⁺ hematopoietic cells (CYTL1), a tyrosine kinase involved in cell adhesion (SYK), and a cell adhesion receptor (TMIGD2), though no gene sets were found for those genes.

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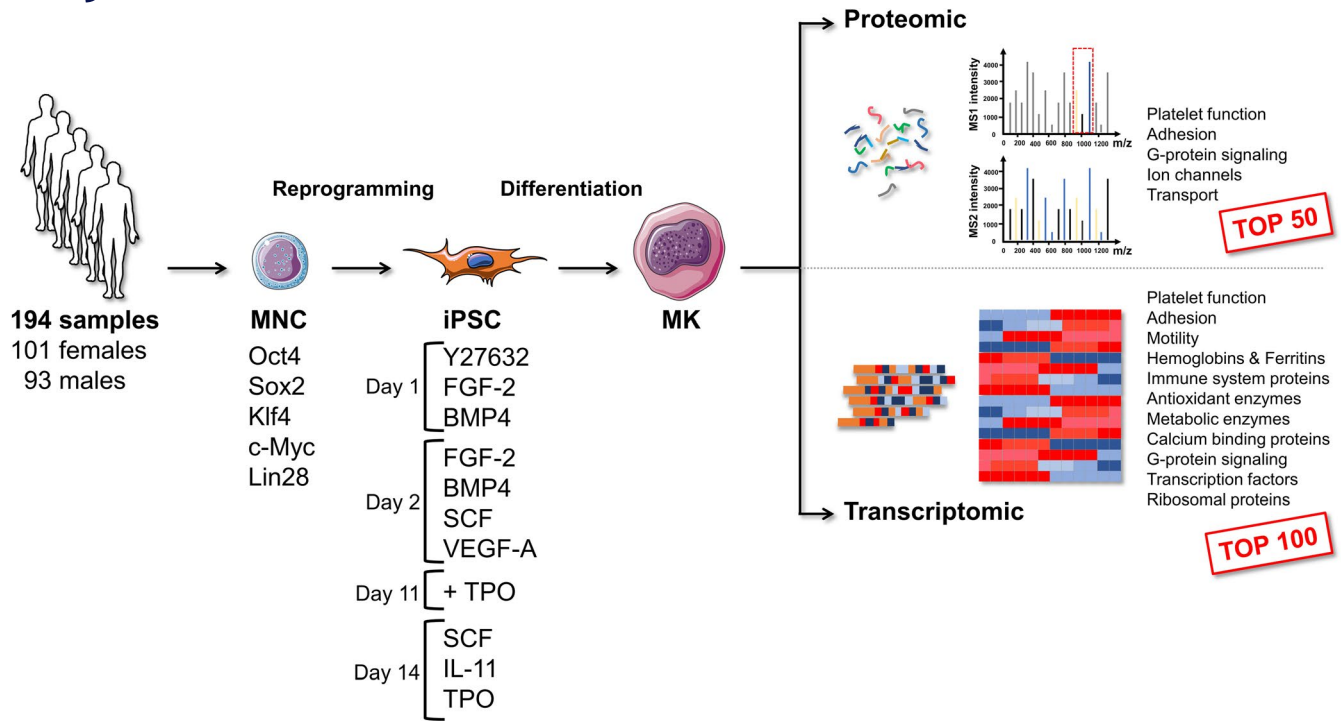


FIGURE 1 Graphical summary of the article. Induced pluripotent stem cells (iPSCs) were derived by reprogramming peripheral blood mononuclear cells (MNCs) of 194 healthy subjects of different ages, ethnicity, and sex. After reprogramming, iPSCs were differentiated in a cell population enriched for CD41⁺/CD42a⁺ megakaryocytes (MKs). Transcriptomic profiles were obtained by RNA sequencing. The expressed proteome was analyzed in 62 of the subjects by using independent acquisition mass spectrometry. This figure was created using some templates from Servier Medical Art licensed under a Creative Commons Attribution 3.0 Unported License (<https://smart.servier.com>)

The comparison between gene expression and protein synthesis highlighted a correspondence of transcript/protein pairs. Specifically, the proteomic analysis identified more than 1000 distinct proteins detected in >50% of the samples analyzed. Among these, proteins involved in platelet function, cell adhesion, actin-binding proteins, G-protein signaling, and ion channels were positively associated with increasing CD41⁺/CD42a⁺ expression, while proteins involved in basic cellular functions, immune and inflammatory responses, and leukocyte function were associated with immature stages. Age, sex, and race were not associated with differential protein expression, and this was probably due to the relatively small sample size available. It is not known whether the differences found in autosomal gene expression in iPSC-derived megakaryocytes by sex and race derive from incomplete reprogramming of the parent cells, which thus continue to carry the epigenetic signature of the donor mononuclear cells, as already reported in other studies focusing on iPSCs.¹¹⁻¹³ In the case of gender differences, sex chromosomes could contribute to the differences through both cis and trans mechanisms. The validation of the differential gene expression by sex and race in primary bone marrow megakaryocytes or circulating platelets would certainly add insight into these aspects.

This study was the first to identify, in a large sample size, a set of genes and proteins expressed in iPSC-derived megakaryocytes that is significantly different depending on sex and race. iPSC-derived megakaryocytes appeared to maintain a transcriptomic and proteomic profile similar to human megakaryocytes derived from hematopoietic

stem and progenitor cells.¹⁴⁻¹⁶ Gene expression varies between individuals with sex and race accounting for some differences for a small number of genes. Consistently, variations in race, genetic mutations, and diseases have an impact on the type of transcripts transferred from megakaryocytes to platelets. Several exon-skipping events were identified in platelets, such as exon 14 of SELP (P-selectin), which is differently retained in platelet transcripts among individuals.¹⁷ This platelet splice site quantitative trait locus was shown to account for differences between Black, AA, and White individuals. In support of this, it has been demonstrated that platelet RNA profiles can be associated with demographic variables explaining, for example, the increased platelet reactivity upon protease-activated receptor 4 thrombin receptor-mediated activation observed in Black individuals.¹⁸ These are important analyses as most of the genome-wide association study analysis to date has been performed on populations of European ancestry and few data are available on individuals of other origins. The same applies to the identification of variables between individuals of different sex and age.

The results of the paper by Kammers et al., together with data from the literature, raise the question of how we should address the use of iPSCs to model human physiological or pathological processes or to derive mature blood cells for research and clinical applications.^{4,5,19} In the era of precision and personalized medicine, should we always consider the use of iPSCs from different origins to generate more reliable data? Or vice versa, could we exploit the origin of iPSCs to understand the mechanisms of predisposition to certain

diseases in association with the demographic variables? Is this a feasible approach in a research project? We now have the possibility to expand and at the same time tailor our studies to a specific population.

The same considerations should be applied to the use of iPSCs to generate mature blood cells for transfusions, clinical study, or drug testing. In these cases, due to the unique characteristics of human platelets, it would be fundamental to promote a better understanding of the exact nature of iPSC-derived megakaryocytes. Extended studies are still necessary to clarify their intrinsic characteristics, including the best course of management *in vitro*.²⁰ Because these cells differ from ones derived from other sources, we need to discover how to trigger platelet production specifically from iPSC-derived megakaryocytes. Moving forward, all analysis should take into account variables such as sex, race, or age for a better definition of our *in vitro* models and systems for platelet production.

In conclusion, iPSC-derived megakaryocytes may function differently depending on the origin of the iPSCs. This added complexity should be viewed as an advantage and source of new research opportunities. Similar, but different.

CONFLICTS OF INTEREST

The authors have no conflicts of interest to declare.

AUTHOR CONTRIBUTIONS

V.A. and C.A.D.B. contributed to drafting and prepared the figure; A.B. wrote the manuscript.

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