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Mini Review

Membrane Properties of Human Induced Pluripotent Stem Cell-Derived Cultured Red Blood Cells

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Abstract

Cultured red blood cells from human induced pluripotent stem cells (cRBC_iPSCs) are a promising source for future concepts in transfusion medicine. Before cRBC_iPSCs will have entrance into clinical or laboratory use, their functional properties and safety have to be carefully validated. Due to the limitations of established culture systems, such studies are still missing. Improved erythropoiesis in a recently established culture system, closer simulating the physiological niche, enabled us to conduct functional characterization of enucleated cRBC_iPSCs with a focus on membrane properties. Morphology and maturation stage of cRBC_iPSCs were closer to native reticulocytes (nRETs) than to native red blood cells (nRBCs). Whereas osmotic resistance of cRBC_iPSCs was similar to nRETs, their deformability was slightly impaired. Since no obvious alterations in membrane morphology, lipid composition, and major membrane associated protein patterns were observed, reduced deformability might be caused by a more primitive nature of cRBC_iPSCs comparable to human embryonic- or fetal liver erythropoiesis. Blood group phenotyping of cRBC_iPSCs further confirmed the potency of cRBC_iPSCs as a prospective device in pre-transfusional routine diagnostics. Therefore, RBC membrane analyses obtained in this study underscore the overall prospects of cRBC_iPSCs for their future application in the field of transfusion medicine.

Keyboard: Red Blood Cells, Induced Pluripotent Stem Cells, Erythropoiesis, Membrane, Blood Group Antigen, Deformability, Osmotic Resistance, Phospholipids, Cholesterol, Reticulocytes

INTRODUCTION

In current medicine, the transfusion of purple blood cells (RBCs) is vital in treating severe diseases. RBC devices are specifically derived from allogeneic donations of healthful volunteers [1]. Already today, shortages exist in blood deliver for sufferers with uncommon blood institution phenotypes or the ones affected by excessive alloimmunization. Worsening of the general scenario in the subsequent a long time because of demographical adjustments is expected A promising alternative for decreasing shortages in allogeneic RBC deliver is the ex vivo production of cultured RBC (cRBCs) from human stem cells [2]. Due to their self-renewing houses and their capacity to distinguish into all tissues of the human body, triggered pluripotent stem cells (iPSCs) provide a completely appealing stem cellular supply for obligatory big-scale manufacturing of cRBCs [3]. It became expected that iPSC strains of some donors could be ok to cowl the necessities of maximum alloimmunized sufferers and people with uncommon blood institution phenotypes. Reprogramming of affected person-derived cells into iPSCs could even permit for the transfusion of autologous cRBCs in person cases. Besides their use as therapeutics, iPSC-derived cRBCs (cRBC_iPSCs) should provide an appealing diagnostic device in phrases of RBC check panels. Laboratories use RBC panels for obligatory screening and identity of abnormal RBC antibodies earlier than transfusion [4]. The non-stop manufacturing of RBC check panels is hard and relies upon at the accessibility of blood institution O donors, having the applicable blood institution antigens [5].

Since the pioneering discovery that somatic cells may be reprogrammed for pluripotency, numerous subculture structures for the ex vivo technology of cRBC_iPSCs had been hooked up, Recent procedures already consciousness

at the switch of static cellular subculture techniques into scalable bioreactor structures However, the protection and capability of cRBC_iPSCs should be very well proven earlier than those cells may be utilized in medical or laboratory settings. Thus far, the low performance of hooked up subculture structures, in phrases of growth and enucleation, have avoided enough technology of enucleated cRBC_iPSCs for practical analyses. Especially, the absence of a physiological area of interest would possibly impair cellular increase and lineage-unique differentiation. Our institution currently mentioned progressed erythroid differentiation of iPSCs through offering cell interactions in a third-dimensional shape termed "hematopoietic cellular forming complex" (HCFC) [6]. From this HCFC, CD43+ hematopoietic cells (purity > 95%) have been launched into the subculture supernatant over a duration of three–five weeks and can be accrued again and again for in addition differentiation into glycophorin A (GPA) fine erythroid cells. Mean enucleation fees close to 40% (as much as 70%) allowed for the manufacturing of numerous hundreds of thousands of enucleated cRBC_iPSCs from one six-nicely plate [7]. The very last output and standard exceptional of cultured cells became in addition progressed through optimized cholesterol supplementation at some point of culturing. In the prevailing have a look at, we showed the efficacy of our hooked up machine and performed a complete practical characterization of enucleated cRBC_ iPSCs with a focal point on membrane houses. In cRBC_ iPSCs have been analyzed for his or her maturation degree, morphology, deformability, osmotic resistance, lipid profile, and expression of transfusion applicable blood institution antigens and in comparison with peripheral blood (PB)-derived local RBCs (nRBCs) and twine blood (CB)-derived local reticulocytes (nRETs) [8]. These controls fluctuate now no longer simplest of their maturation degree, however additionally they constitute special developmental waves of human erythropoiesis. Whereas CB cells originate from fetal liver erythropoiesis, PB cells originate from grownup bone marrow (BM) erythropoiesis. There is growing proof that RBCs from special developmental waves fluctuate of their cell capabilities like hemoglobin (Hb) composition, Hb content material, and cellular length [9].

MATERIALS AND METHODS

Human Material

Five human iPSC strains derived from erythroblasts or CB CD34+ cells have been used Native RBCs have been acquired from RBC devices inside 24 h after donation, and nRETs have been remoted from CB with magnetic beads inside 12 h postpartum (CD71 Microbead Kit; Miltenyi Biotec, Bergisch Gladbach, Germany). Written knowledgeable consent became given previous to sampling. The have a look at became accredited through the nearby ethics committee in step with the Declaration of Helsinki (EK 27 165ex 14/15) [10].

Cultivation of cRBC_iPSCs

Hematopoietic and erythroid differentiation of iPSCs have been triggered as currently defined and illustrated For embryoid body (EB) formation, colonies (>23 passages) have been indifferent with collagenase IV (Sigma-Aldrich, St. Louis, MO, USA). Cell clumps have been seeded on low-binding suspension plates (Nunclon Sphera, Thermo Fisher Scientific, Waltham, MA, USA) and incubated for five days in hESC medium without bFGF Afterward, round EBs have been transferred into six-nicely tissue subculture plates (Sarstedt, Nümbrecht, Germany) with STEMdiff APEL2 Medium (STEMCELL Technologies, Vancouver, BC, Canada), five% Protein-Free Hybridoma Medium (Thermo Fisher Scientific, Waltham, MA, USA), five ng/mL interleukin-three (IL-three; PeproTech), a hundred ng/mL SCF (PeproTech, London, UK), and three U/mL erythropoietin (EPO) (Erypo, Janssen Biologics BV, Leiden, Netherlands). The medium became changed weekly [11]. For erythroid differentiation, unmarried cells launched into the supernatant have been accrued and cultured for an extra 18 days in a longtime three-section erythropoiesis assay. Cells have been cultured in Iscove's medium (Biochrom, Berlin, Germany) with five% human plasma (Octapharma, Vienna, Austria), 10 µg/ mL insulin (Sigma-Aldrich, St. Louis, MO, USA), 330 µg/mL human holotransferrin (BBI Solutions, Salisbury, UK), and from day eight onward four mg/dl ldl cholesterol-wealthy lipids (Sigma-Aldrich, St. Louis, MO, USA). Cells have been inspired with a hundred ng/mL SCF, five ng/mL IL-three, three U/mL EPO, and 10−6 M hydrocortisone (Sigma-Aldrich, St. Louis, MO, USA) from days zero to eight, with a hundred mg/mL SCF and three U/mL EPO from day eight today eleven, and with three U/mL EPO from days eleven to 18. Cells have been filtered thru a syringe filter (Acrodisc WBC Pall, Port Washington, NY, USA), to achieve the natural enucleated portion [12].

Phenotype and Maturation Stage of cRBC_iPSCs

Phenotypic characterization became performed thru microscopic evaluation of cytospin arrangements and thru float cytometry. For float cytometry analyses, cells have been incubated with respective antibodies towards CD45, CD71 (Becton Dickinson, Franklin Lakes, NJ, USA), CD36, CD49d, GPA (Beckman Coulter, Krefeld, Germany), and band three (Bric 6, IBGRL, Bristol, UK). Measurements have been performed the use of a CytoFLEX float cytometer (Beckman Coulter, Krefeld, Germany). The maturation degree of filtered cells became evaluated on the idea of thiazole orange staining (Retic Count, Becton Dickinson) and the expression of CD71 [13]. Cells have been in addition characterized after staining with new methylene blue (Reticulocyte Stain, Sigma-Aldrich), and as a minimum three hundred cells have been enumerated microscopically (Axioscope, Zeiss, Oberkochen, Germany). Cell length became assessed thru float cytometry the use of scatter traits and thru microscopy (EVOS M5000, Thermo Fisher Scientific, and Waltham, MA, USA) after staining with May–Grunewald–Giemsa (Hemafix, Biomed,

and Oberschleißheim, Germany). The hemoglobin (Hb) attention became measured the use of Drabkin's Reagent (Sigma-Aldrich, St. Louis, MO, USA). An overall of 7 × 106 cells have been incubated with 500 µL Drabkin's answer and study at a wavelength of 540 nm on a Shimadzu-1800 spectrophotometer (Shimadzu, Korneuburg, Austria) [14-15].

DISCUSSION

Our have a look at investigated the membrane houses of cRBC_iPSCs because the prerequisite for his or her potential diagnostical or medical application. The RBC membrane contains a lipid bilayer with embedded proteins and is anchored to an elastic community of skeletal proteins. The membrane structure determines the antigenic houses in addition to the mechanical capabilities of RBCs. Altered balance or deformability of cells consequences in declined half-lifestyles due to splenic sequestration or maybe lysis and could consequently decide the survival of cRBC_iPSCs after transfusion. Since cRBCs from human hematopoietic stem or progenitor cells (HSPCs) or iPSCs do now no longer attain whole terminal maturation into biconcave formed RBCs ex vivo, we to begin with decided the maturation degree and morphology of cRBC_iPSCs through their assessment with nRETs and nRBCs. Contrary to the delicate and much less deformable multilobular reticulocyte, the biconcave RBC demonstrates excessive shear resistance and membrane flexibility withinside the blood float. This allows the RBC to go through big passive and reversible deformations at some point of non-stop capillary passages. In vivo, the terminal transition of RETs to biconcave RBCs is related to RNA degradation and organelle clearance. Concurrently, giant membrane reworking takes place, decreasing the extent and cellular floor place through 20–30%. The mechanisms worried in terminal maturation steps are poorly defined. According to current reports, lack of membrane floor takes place through an endosome–exosome pathway, while degradation of residual organelles consists of autophagic mechanisms. Both pathways would possibly interfere. In our have a look at, enucleated cRBC_iPSCs exhibited a few residual chromatin spots and sporadic organelles, maximum of them already encapsulated in vacuoles for in addition degradation or exocytosis, and consequently a maturation degree among nRETs and nRBCs at the cytosolic level. In line with terminal membrane maturation, cellular floor expression of CD36 became low, and CD49d became nearly absent. Nevertheless, expression of the transferrin receptor CD71 became continual and did now no longer correlate with different warning signs of terminal membrane reorganization. This is probably resulting from inadequate iron deliver (330 µg/mL holo-transferrin) at some point of big ex vivo amplification, compensated through extended expression of the transferrin receptor. Further research with more desirable iron deliver at some point of culturing might be important to verify this hypothesis. Cell length and Hb content material of cRBC _iPSCs have been more desirable and extra similar with the ones of nRETs than nRBCs. As mentioned in extra element below, cellular length and Hb content material may not simplest be motivated through the maturation degree of erythroid cells, however additionally through the developmental wave of human erythropoiesis. Besides special increase kinetics and changed expression of transcription elements at some point of erythropoiesis, fetal liver-derived RBCs like CB cells are defined to have a better extent and Hb content material in assessment to BM-derived grownup RBCs. Interestingly, the Hb attention in grownup nRBCs became decrease than in cRBC_iPSCs and CB-derived nRETs, however akin to cRBCs derived from grownup HSPCs (cRBC_adult). This would possibly suggest a developmental effect at the Hb content material as opposed to a subculture-associated phenomenon. Although the ex vivo growth of cRBC_adult is out of the point of interest of the prevailing paper, for assessment reasons, information acquired from cRBC_adult improved with inside the identical subculture machine defined right here for cRBC_iPSCs, are summarized in Supplementary. Finally, SEM demonstrated the morphology of cRBC_iPSCs similar with that of nRETs or maybe pro-biconcave established RBCs. Most importantly, no outstanding traits indicating a faulty membrane business enterprise like echinocyte formation have been noticeable.

The principal biophysical membrane traits of RBCs are deformability and OR. Our consequences demonstrated better OR in nRETs than in nRBCs, as formerly mentioned. OR of cRBC_iPSCs became similar with that of nRETs. OR is stricken by the membrane floor place-to-extent ratio (S/V ratio) and the membrane integrity. The consequences could consequently argue for membrane S/V ratio and membrane integrity of cRBC_iPSCs similar with that of nRETs. It is nicely hooked up that younger reticulocytes showcase declined membrane deformability, as additionally visible on this have a look at However, the deformability of cRBC_iPSCs became fairly decrease than that of nRETs and sundry notably from that of nRBCs. To the exceptional of our knowledge, to this point simplest. tested the deformability of cRBC_ iPSCs acquired from a affected person with sickle cellular anemia and mentioned similarity to healthful controls. Nevertheless, in that have a look at, deformability became triggered through osmotic changes as opposed to shear strain and a huge variety for healthful controls became given (EImax zero.38–zero.58) in assessment with the prevailing .Deformation capability of human RBCs is a characteristic of (1) geometry of the cells, mainly the membrane S/V ratio; (2) intracellular viscosity decided through the Hb content material and the hydration state; and (three) membrane composition. In cRBC_iPSCs, those capabilities might also additionally range from local cells due to the culturing method or the extra primitive nature of iPSC-derived cells. Larger cellular length and a better Hb content material that would have an effect on the S/V ratio have additionally been defined for cRBC_adult and have been ascribed to strain erythropoiesis and more desirable fetal hemoglobin (HbF) expression. However, the deformability of cRBC_

adult became mentioned to be similar with reticulocytes. These observations might also additionally contend for a developmental affect on cRBC_iPSCs deformability as opposed to subculture-associated artefacts.

REFERENCES

1. Kurebayashi J, Kanomata N, Yamashita T, Shimo T, Moriya T (2016). Antitumor and anticancer stem cell activities of eribulin mesylate and antiestrogens in breast cancer cells. Breast Cancer. 23: 425–436.
2. Cortes J, O'Shaughnessy J, Loesch D, Blum JL, Vahdat LT, et al. (2011). Eribulin monotherapy versus treatment of physician's choice in patients with metastatic breast cancer (EMBRACE): A phase 3 open-label randomised study. Lancet 377: 914–923.
3. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, et al. (2021).Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. CA Cancer J Clin. 71: 209–249.
4. Dillekas H, Rogers MS, Straume O (2019). Are 90% of deaths from cancer caused by metastases? Cancer Med. 8: 5574–5576.
5. Castro-Giner, F.; Aceto, N. Tracking cancer progression: From circulating tumor cells to metastasis. Genome Med. 12: 31.
6. Cristofanilli M, Pierga JY, Reuben J, Rademaker A, Davis AA, et al. (2019). The clinical use of circulating tumor cells (CTCs) enumeration for staging of metastatic breast cancer (MBC): International expert consensus paper. Crit Rev Oncol Hematol. 134: 39–45.
7. Larsson AM, Jansson S, Bendahl PO, Levin Tykjaer Jorgensen C, Loman N et al. (2018). Longitudinal enumeration and cluster evaluation of circulating tumor cells improve prognostication for patients with newly diagnosed metastatic breast cancer in a prospective observational trial. Breast Cancer Res. 20: 48.
8. Bidard FC, Jacot W, Kiavue N, Dureau S, Kadi A, et al. (2021). Efficacy of Circulating Tumor Cell Count-Driven vs Clinician-Driven First-line Therapy Choice in Hormone Receptor-Positive, ERBB2-Negative Metastatic Breast Cancer: The STIC CTC Randomized Clinical Trial. JAMA Oncol. 7: 34–41.
9. Baccelli I, Schneeweiss A, Riethdorf S, Stenzinger A, Schillert A, et al. (2013). Identification of a population of blood circulating tumor cells from breast cancer patients that initiates metastasis in a xenograft assay .Nat Biotechnol. 31: 539–544.
10. Shibue T, Weinberg RA (2017). EMT, CSCs, and drug resistance: The mechanistic link and clinical implications. Nat Re Clin Oncol. 14: 611–629.
11. Yu M, Bardia A, Wittner BS, Stott SL, Smas ME, et al. (2013). Circulating breast tumor cells exhibit dynamic changes in epithelial and mesenchymal composition. Science. 339: 580–584.
12. Theodoropoulos PA, Polioudaki H, Agelaki S, Kallergi G, Saridaki Z, et al (2010). Circulating tumor cells with a putative stem cell phenotype in peripheral blood of patients with breast cancer. Cancer Lett. 288: 99–106.
13. Papadaki MA, Kallergi G, Zafeiriou Z, Manouras L, Theodoropoulos PA, et al. (2014). Co-expression of putative stemness and epithelial-to-mesenchymal transition markers on single circulating tumour cells from patients with early and metastatic breast cancer. BMC Cancer. 14: 651.
14. Papadaki MA, Aggouraki D, Vetsika EK, Xenidis N, Kallergi G, et al (2021). Epithelial-to-mesenchymal Transition Heterogeneity of Circulating Tumor Cells and Their Correlation with MDSCs and Tregs in HER2-negative Metastatic Breast Cancer Patients. Anticancer Res. 41: 661–670.
15. Kallergi G, Papadaki MA, Politaki E, Mavroudis D, Georgoulias V, et al (2011). Epithelial to mesenchymal transition markers expressed in circulating tumour cells of early and metastatic breast cancer patients. Breast Cancer Res. 13: 59.