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Human Induced Pluripotent Stem Cell–Derived Macrophages for Unraveling Human Macrophage Biology

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Abstract—Despite a substantial appreciation for the critical role of macrophages in cardiometabolic diseases, understanding of human macrophage biology has been hampered by the lack of reliable and scalable models for cellular and genetic studies. Human induced pluripotent stem cell (iPSC)–derived macrophages (IPSDM), as an unlimited source of subject genotype-specific cells, will undoubtedly play an important role in advancing our understanding of the role of macrophages in human diseases. In this review, we summarize current literature in the differentiation and characterization of IPSDM at phenotypic, functional, and transcriptomic levels. We emphasize the progress in differentiating iPSC to tissue resident macrophages, and in understanding the ontogeny of in vitro differentiated IPSDM that resembles primitive hematopoiesis, rather than adult definitive hematopoiesis. We review the application of IPSDM in modeling both Mendelian genetic disorders and host–pathogen interactions. Finally, we highlighted the potential areas of research using IPSDM in functional validation of coronary artery disease loci in genome-wide association studies, functional genomic analyses, drug testing, and cell therapeutics in cardiovascular diseases.

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Key Words: coronary artery disease ■ genome-wide association study ■ hematopoiesis ■ induced pluripotent stem cells ■ macrophages

Macrophages represent a critical cell type in the pathogenesis of chronic inflammatory cardiometabolic diseases, such as atherosclerosis, central obesity, and insulin resistance.[1] The differentiation of human induced pluripotent stem cells (iPSCs) to macrophages (iPSC-derived macrophages [IPSDM]) offers a powerful platform to study human macrophage biology with enormous potential in disease modeling, functional genomic analyses, drug screening, and cell therapeutics. The goal of this brief review is to provide a concise but comprehensive overview of new advances in the differentiation and application of IPSDM, with a major focus on cardiovascular research, while also highlighting cutting edge applications across the vast landscape of macrophage biology in health and diseases (Figure 1).

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IPSDM Provide an Important Tool to Study Human Macrophage Biology

Primary murine macrophages, despite being widely used as a powerful model in the study of macrophage biology, have fundamental interspecies differences compared with human macrophages.[2,3] Functionally, humans are physiologically more sensitive than mice to lipopolysaccharide stimulation.[2] Human monocyte-derived macrophages (HMDM) have human-specific *TLR4* target genes, for example, *CXCL13*,[4] and relative to murine macrophages slower induction of several negative feedbacks of the toll-like receptor pathway.[3] In HMDM, pattern recognition receptor stimulation drives release of endogenous ATP that triggers inflammasome activation,[5] but murine macrophage inflammasome activation requires cholesterol crystals.[6] Further, compared with mouse, acetylated low-density lipoprotein loading in HMDM induces much greater triglyceride enrichment yet many-fold lower levels of cholesteryl ester (≈30 versus >150 μg/mg protein).[7] Transcriptomically, compared with mRNA, noncoding RNAs are mostly not conserved in mice, highlighting the need to study human macrophage-specific noncoding RNAs in human macrophages.

Experimental human macrophages are derived mostly from 2 sources: tumor-derived cell lines (eg, U937, THP-1 cells) and primary cells, including isolated tissue resident macrophages such as microglia and alveolar macrophages, and

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Nonstandard Abbreviations and Acronyms	
Cas9	CRISPR–associated 9
CRISPR	clustered regularly interspaced short palindromic repeats
EB	embryoid body
GM-CSF	granulocyte macrophage colony-stimulating factor
HMDM	human monocyte-derived macrophages
iPSC	induced pluripotent stem cell
IPSDM	iPSC-derived macrophages
M-CSF	macrophage colony-stimulating factor

HMDM. THP-1 derived macrophages (THP-1Φ) have unlimited replicative potential but are karyotypically abnormal and functionally immature.[8] Tissue macrophages, especially those from healthy subjects, have extremely limited availability. HMDM are relatively easy to obtain, but are difficult to be genetically manipulated, and do not self-renew limiting scalability. IPSDM provides a genotype-specific, scalable, and renewable source of human macrophage[8] that are amenable to genetic manipulation by clustered regularly interspaced short palindromic repeats/clustered regularly interspaced short palindromic repeat–associated 9 (CRISPR/Cas9)–mediated gene editing. With continual improvement in differentiation efficiency and fidelity, IPSDM is emerging as a powerful tool in multiple areas of research for disease modeling and more recently, drug testing and cell therapeutics.

Generation, Functional Feature and Molecular Profiling of IPSDM

Multiple protocols have been described to differentiate iPSC into macrophages. The first 2 IPSDM differentiation protocols published by Choi et al[9] and Senju et al[10] in 2011 used coculture of iPSC with OP9 mouse stromal cell monolayers to induce hematopoietic differentiation, followed by expansion of multipotent myeloid progenitors and differentiation into macrophages with GM-CSF (granulocyte macrophage colony-stimulating factor)[9] or M-CSF (macrophage colony-stimulating factor).[9,10] In 2013, modified from a protocol published by Karlsson et al[11] for human embryonic stem cell to macrophage differentiation, van Wilgenburg et al[12] established an embryoid bodies (EBs)-based protocol for IPSDM differentiation in which hematopoiesis was induced under serum-free condition followed by directed differentiation to macrophages with M-CSF either in serum-free media or in media with serum. Yanagimachi et al[13] developed a monolayer-based protocol by culturing iPSC on a layer of extracellular matrix component under serum-free condition and differentiated floating monocyte-like cells to macrophage in media with serum and M-CSF. In 2015, our group[8] published an EB-based differentiation protocol and for the first time performed transcriptomic characterization using deep RNA-sequencing in HMDM and IPSDM lines derived from the same subjects. The results highlighted the transcriptome similarity between HMDM and IPSDM with a small percentage of genes (≈12%) differentially expressed.[8] Further, more sophisticated functional characteristics, such as cholesterol efflux, cholesteryl ester hydrolysis,[14] and cytokine secretion profile in macrophages with M1 (lipopolysaccharide+interferon-γ) and M2 (interleukin-4) activation, were compared between multiple HMDM and IPSDM lines and have demonstrated

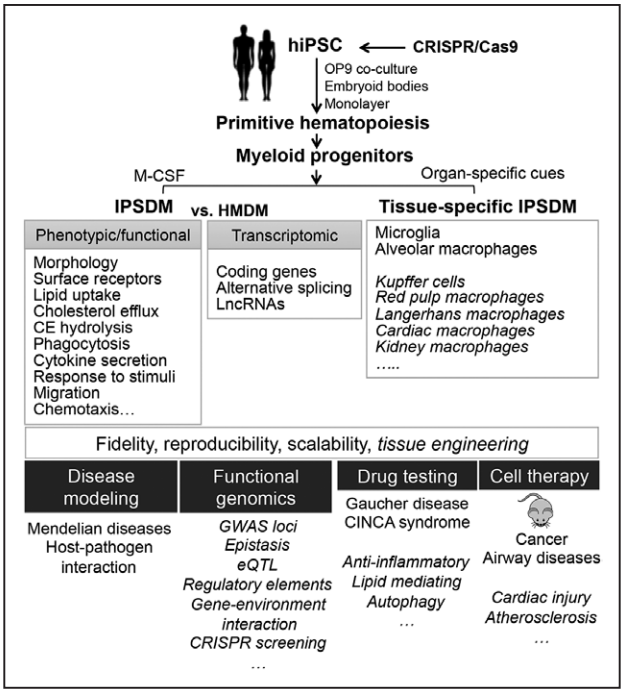

Figure 1. Current status of and future directions for differentiation and application of human induced pluripotent stem cell–derived macrophages (IPSDM). Current IPSDM differentiation protocols use coculture, embryoid body(EB)- or monolayer-based approaches to produce myeloid progenitors and use M-CSF (macrophage colony-stimulating factor) for directed differentiation to mature macrophages. Several macrophage functional features have been characterized, including morphology, surface receptor expression, lipid uptake, phagocytosis, cholesterol efflux, cholesteryl ester (CE) hydrolysis, cytokine secretion, response to stimuli, etc, often in direct comparison with human monocyte-derived macrophages (HMDM). Transcriptome landscape, including alternative splicing events and lncRNA profile, also has been analyzed comprehensively. Emerging evidence supports that in vitro IPSDM differentiation represents primitive hematopoiesis rather than adult definitive hematopoiesis. Protocols for induced pluripotent stem cell (iPSC) differentiation to human and murine microglial-like cells and murine alveolar macrophages have been implemented while other tissue-specific macrophages and organoid culture for tissue engineering remain to be established. IPSDM have been widely used to model Mendelian disorders and host–pathogen interaction. Additional studies to model functional impact of coronary artery diseases(CAD) genome-wide association study (GWAS) loci, epistatic effects, quantitative traits, and gene–environment interaction are being pursued. Use of IPSDM in genome-wide clustered regularly interspaced short palindromic repeats (CRISPR) screening to discover novel genes and noncoding transcripts regulating human macrophage function, and identify functional regulatory and causal variants holds great promise. Use of IPSDM in drug testing and IPSDM-based cell therapy are emerging. Improvement in the fidelity, reproducibility and scalability of IPSDM differentiation in conjunction with CRISPR/clustered regularly interspaced short palindromic repeat–associated 9 (Cas9) gene editing technologies will further reveal the remarkable potential of IPSDM in multiple areas of functional and translational research in human macrophage biology and pathophysiology. CINCA indicates chronic infantile neurological cutaneous and articular; eQTL, expression quantitative trait loci; hiPSC, human induced pluripotent stem cells; and lncRNA, long noncoding RNA. Text in italics indicates potential areas of future research.

remarkable similarity.[8] Two additional studies from our group further elaborated that IPSDM recapitulate important alternative splicing events[15] and long noncoding RNA profiles (article under review) of HMDM during macrophage activation, identifying IPSDM as uniquely suited to study human macrophage-specific transcriptome regulation. These protocols[8,12,13] used sequential exposure of iPSC to different cytokines that resemble hematopoietic specification and myeloid expansion, and myelomonocytic cells were harvested for directed differentiation to macrophages with M-CSF. The differentiation efficiency was evaluated differently across studies, but generally these protocols have demonstrated high efficiency and scalability with >10 million IPSDM generated from 1 6-well plate of cultured iPSCs.[8,12]

Although IPSDM have demonstrated similar phenotypic, functional, and transcriptomic characteristics to HMDM, their ontogeny and identity have not been fully defined and this recently has received further study. It has been recognized that in vitro hematopoietic differentiation of PSCs resembles in vivo primitive hematopoiesis rather than definitive hematopoiesis.[16] Vanhee et al[16] used a reporter human embryonic stem cell line with *MYB-eGFP*, a marker for hematopoietic stem cell-dependent hematopoiesis, and confirmed the absence of in vitro generation of *MYB*$^+$ hematopoietic stem cell during EB-based IPSDM differentiation. The results were further validated in human iPSCs,[17] showing that knockout of *MYB* in iPSC by CRISPR/Cas9 did not impact IPSDM differentiation.[12] In contrast, when *SPI1* and *RUNX1*, important transcription factors in yolk sac hematopoiesis, were knocked out, iPSCs were unable to produce CD14$^+$ macrophages. Thus, IPSDM may developmentally relate to and be a good model for *MYB*-independent tissue resident macrophages. Indeed, several iPSC to microglia-like cell differentiation protocols were published this year using cytokines representing the main driver of microglia lineage,[18,19] coculture with iPSC-derived neurons[20,21] and astrocytes,[22] or incubating with cytokines derived from those cell types[23] to recapitulate organ-specific cues. Takata et al[21] has established a protocol that specifically resembles primitive hematopoiesis and yolk sac macrophages for further differentiation into tissue macrophage-like cells by coculture or engraftment. The iPSC-derived microglia-like cells showed transcriptome profile highly similar to that of human adult and fetal microglia,[23] and were clustered together with cultured human microglia and away from HMDM and cultured human hepatic macrophages,[18] supporting their role as a more suitable cellular source of microglia than adult hematopoiesis-derived myeloid cells.

It is also noteworthy that the myelomonocytic cells that emerge from EB culture and are collected for subsequent differentiation to macrophages,[8,12] indeed originally termed monocytes,[12] are now recognized as MYB-independent primitive myeloid cells that clustered away from peripheral blood monocytes based on their transcriptome profile.[20] We also have performed RNA-sequencing of myeloid progenitors (CD34$^+$CD43$^+$CD45$^+$CD18$^+$) harvested during progressive commitment and maturation to IPSDM[8] and showed that the expression profile of those progenitors is highly correlated to that of IPSDM but not CD14$^+$ monocytes (Figure 2). Thus, successful differentiation of circulating monocytes will rely on a more complete understanding of mechanisms regulating definitive hematopoietic specification[24] and identification of factors governing the generation of hematopoietic stem cells from iPSC,[25] knowledge gaps that remain major goals of regenerative medicine.

Future studies to further improve efficiency, reproducibility and scalability of IPSDM differentiation with potential for automated differentiation will facilitate the application of IPSDM in multiple areas of research. It has been suggested that the differentiation efficiency and yield are higher when using serum-free media to differentiate EBs into myeloid precursors as opposed to serum-containing media.[26] Monolayer-directed differentiation as opposed to an EB-based differentiation that generates cells of all 3 germ layers may have advantage for differentiation in defined conditions,[21] but direct comparison has not been performed. Novel protocols to differentiate iPSCs to tissue resident macrophages, for example, cardiac macrophages, will further raise the possibilities to delve into their specialized properties and tissue-specific functions. It is

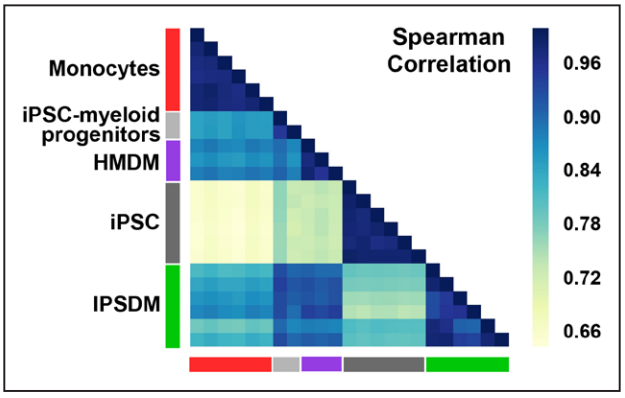

Figure 2. Heat map of Spearman correlation coefficient (*r*) for pairwise comparisons of CD14$^+$ monocytes, human monocyte-derived macrophages (HMDM), induced pluripotent stem cell (iPSC), induced pluripotent stem cell-derived macrophages (IPSDM), and iPSC-derived myeloid progenitor during an intermediate stage of IPSDM differentiation. RNA-sequencing (RNA-seq) data for iPSC, IPSDM, and HMDM derived from 3 subjects were downloaded from GSE55536[8] with 2 lines per subject for iPSC and IPSDM. RNA-seq data for CD14$^+$ monocytes from age/race matched subjects were downloaded from GSE87290.[48] We have generated iPSC-derived myeloid progenitors using our published IPSDM differentiation protocol[8] and performed RNA-seq. Those cells were derived from 2 subjects with 1 line per subject, who are among the 3 subjects described in GSE55536.[8] The iPSC-derived myeloid progenitors are CD43$^+$CD34$^+$CD45$^+$CD18$^+$ and are harvested at day 15 of the differentiation from suspension culture of embryoid bodies (EBs) and then differentiated to mature macrophages using serum and M-CSF (macrophage colony-stimulating factor) in adherent culture for 7 d.[8] Depth coverage among samples were normalized as described by Anders and Huber.[49] Genes expressed at FPKM (fragments per kilobase of transcript per million mapped reads) > 1% expression of all genes were included for each sample. FPKM values were then normalized across samples. Spearman correlation coefficient was calculated for each pair of samples and visualized by heat map. The transcriptome of iPSC-myeloid progenitors are highly correlated with that of IPSDM, but to a lesser extent with that of CD14$^+$ monocytes, supporting their identity as macrophage precursors during a progressive commitment and maturation of macrophages, rather than monocytes as suggested also by Haenseler et al[20] using a different differentiation protocol to obtain IPSDM.[12]

also important to consider whether IPSDM may resemble the heterogeneity of human tissue resident macrophages at single cell levels and the mechanisms driving the heterogeneity and downstream functional consequences.

Disease Modeling and Functional Genomic Analyses With IPSDM

IPSDM overcome the limitations of the lack of an adequate supply of disease-specific primary macrophages in studying rare diseases, patients-derived IPSDM are increasingly used to model Mendelian diseases, for example, Tangier disease (mutations in *ABCA1*), lysosomal storage diseases including neuronal ceroid lipofuscinoses[27] and Gaucher disease (mutations in *GBA*),[28,29] chronic granulomatous disease (defective NADPH [nicotinamide adenine dinucleotide phosphate] oxidase),[30] Blau syndrome (mutation in *NOD2*),[31] and chronic infantile neurological cutaneous and articular syndrome (mutation in *NLRP3*).[32] Some of these studies compared the phenotypes of IPSDM and HMDM derived from the same subjects[8,29] to further validate the fidelity of IPSDM in assessing the functionality of genetic mutations. CRISPR/Cas9 gene editing technologies further expanded the potential by introducing disease mutation in iPSC lines derived from healthy subjects and to correct mutations in the iPSC lines of affected patients to demonstrate causality and the functional impact of the disease mutations on a specific isogenic background.[14,33] IPSDM also serves as a powerful tool to study macrophage host–pathogen interaction. IPSDM can be infected with various strains of HIV-1,[34] and support *Salmonella*[26] and *Chlamydia* infection.[35] Knockout of *IRF5/IL10RA* by CRIPSR/Cas9 led to increased susceptibility to *Chlamydia trachomatis* infection in IPSDM. This approach can potentially be extended to study other pathogens to advance our understanding of mechanisms of host–pathogen interactions in macrophages and the role of human genetics in influencing the susceptibility to specific pathogens and outcome of infections.

IPSDM provides a unique platform for functional genomic analyses. To date, genome-wide association studies have identified 73 coronary artery diseases–associated regions.[36] Genome editing in human iPSCs to introduce separately each risk allele, or a combination of alleles, and to correct risk alleles and then differentiate to macrophages allows the interrogation of the macrophage-specific function of the causal variants at genome-wide association study coronary artery disease loci. IPSDM are also powerful tools for modeling genetic interactions, alternative splicing, and macrophage-specific functional noncoding transcripts and regulatory elements. By establishing a biobank of iPSC lines derived from a large number of healthy subjects,[37,38] one can study gene–environment interaction and map quantitative trait locus under various stimuli to determine how genetic differences between individuals may alter immune cells' response to environmental stimuli, and whether variation in the response may predict susceptibility or resilience to macrophage-related disorders in humans including atherosclerosis and coronary artery disease. The coculture or 3-dimensional systems, involving IPSDM and other iPSC-derived cell types relevant to atherosclerosis, including endothelial cells and smooth muscle cells, to form 3D tissue-engineered blood vessel organoids, will be crucial to advance in vitro disease modeling, for example, organs on a chip,[39] and better recapitulate the complexity of the tissue-level in vivo environment. The scalability of the IPSDM platform can allow genome-wide CRISPR screening of human macrophage-specific regulatory element or knockout screening to identify novel regulators of functional phenotypes, which to date have only been performed in immortalized cell lines or PSC lines.[40]

Drug Testing and Cell Therapeutics

Patient-derived IPSDM-based in vitro assays are starting to impact the drug discovery process. IPSDM derived from Gaucher disease patients because of mutations in *GBA*, a gene encoding glucocerebrosidase, were used to test the efficacy of a noninhibitory small-molecule chaperone drug previously identified from high-throughput screening of compound libraries to enhance glucocerebrosidase activity and reverse the disease phenotype.[29] IPSDM derived from chronic infantile neurological cutaneous and articular syndrome patients carrying mutations in *NLRP3* were used to test existing anti-inflammatory compounds in inhibiting the abnormal IL-1β secretion,[32] and can be potentially used to seek drug candidates that directly modulate NLRP3-inflammasome activation for chronic inflammatory conditions such as atherosclerosis.[32] Beyond the use of patient-derived IPSDM, CRISPR/Cas9-edited IPSDM for key regulators in inflammation and lipid metabolism pathways can be exploited for the development of novel therapeutics and delivery systems.[41] The development of organoid systems derived from isogenic iPSC lines will allow drug effects to be tested within the context of the symbiotic interactions between macrophages and their host tissue with the potential for development of individualized treatment strategies.

IPSDM-based cell therapeutics have been studied mainly in cancer[42,43] and airway diseases[44] using murine models particularly relating to the protective benefits of macrophage-mediated phagocytosis. The intraperitoneal injection of iPSC-derived myeloid cells[10] genetically engineered to express interferon-β significantly inhibited the growth of human gastric and pancreatic cancers implanted in the peritoneal cavity,[42] as well as inhibiting the growth of disseminated human melanoma cells in immunocompromised mice.[43] iPSC-derived primitive macrophages intranasally transferred to the lung matured into alveolar macrophages and eliminated the surfactant protein accumulated as a result of *Csf2ra* deficiency in mice.[44] Indeed, evidence is emerging that iPSC-derived myeloid cells may have specific advantages as in cell therapeutics. In mice, iPSC-derived primitive macrophages exhibit greater capacity to be differentiated into microglia-like cells by coculturing with neurons than monocytes and bone marrow-derived macrophages.[21] Similarly, murine iPSC-derived alveolar macrophages engraft in lung better than bone marrow-derived macrophages.[44]

Adult mammalian myocardium contains diverse macrophage populations with distinct origins and function.[45] At steady state, the majority of the resident macrophages is derived from yolk sac progenitors and primarily maintained

through local proliferation. However, during injury, circulating monocytes are recruited, differentiated to macrophages and reside, whereas resident macrophages expand through proliferation. Transcriptional analyses reveal that monocyte-derived macrophages coordinate cardiac inflammation, while playing lesser roles in antigen sampling and efferocytosis.[46] Similarly, embryonic and neonatal macrophages regulate tissue remodeling during development with a lower capacity to generate inflammatory response compared with monocyte-derived macrophages.[47] Therefore, it has been proposed that preserving resident cardiac macrophages expansion, while targeting peripheral monocyte recruitment may lead to improved myocardial recovery after injury.[46] Our RNA-sequencing data suggested that IPSDM may be less inflammatory, as genes expressed at lower levels in IPSDM versus HMDM were enriched in gene ontology terms immune response, antigen processing and presentation, and response to stimulus,[8] but whether IPSDM could exert therapeutic benefits in cardiac injury remains an unexplored question. Deeper understanding of the functions of cardiac-resident macrophages in the steady state and injured heart and macrophage populations in the diseased vasculature, will reveal the potential of IPSDM as a cell therapeutic strategy in cardiovascular diseases. Yet significant challenges and barriers for clinical application of IPSDM and any PSC-based products remain to be addressed.

Limitations and Cautions

Application of iPSC-derived cells for functional genomics and translational studies has many strengths, but current limitations should be considered carefully. These include (1) clonal variability can occur during iPSC reprogramming and has been well recognized to cause variations in differentiation efficiency and the functional properties of differentiated cells. Somatic cell source and reprogramming methods also may affect differentiation efficiency, but the impact on IPSDM has not been systematically evaluated. It is therefore essential to use well-characterized iPSC lines, highly consistent differentiation protocols with quality assessment at multiple stages of the differentiation process, and multiple iPSC lines/clones per subject with independent replications to minimize variability and render statistically significant and meaningful experimental outcomes. (2) The gene of interest and functional phenotypes to be tested in IPSDM need to be carefully chosen and evaluated, for example, whether the target genes or noncoding transcripts show similar expression profile between IPSDM and HMDM, or whether the alternative splicing events or the regulatory features of HMDM are also represented by IPSDM. For initial screening, our studies characterizing the coding genes[8] and lincRNA (long intergenic noncoding RNA) transcriptome (article under review), and alternative splicing events[15] in isogenic IPSDM and HMDM lines provide a powerful resource for planning IPSDM studies to model such events, but specific validation for each chosen target is essential. (3) Some target genes may affect iPSC reprogramming or IPSDM differentiation efficiency, and cannot be reliably studied in IPSDM. Also IPSDM modeling of aging-associated cellular phenotypes has not been established. (4) Carefully designed controls and a combination of experimental strategies are essential for faithful interpretation of the functional consequences of target genes when using IPSDM, for example, patient-derived iPSC lines with correction of disease causing mutations in conjunction with wild-type lines with gene knockout or knockin of the mutations, and CRISPRi- and CRISPRa-mediated gene inhibition and activation add powerful complementary layers of information. (5) Use of IPSDM does not eliminate the need for traditional cellular models or distinct genetic manipulations, for example, siRNA or antisense oligonucleotides mediated knockdown, which are complementary and alternative approaches to further ensure validity and reproducibility.

Conclusions

IPSDM possess enormous value in advancing our understanding of diseases that involve human macrophages and already have demonstrated proof of principle utility in the development of novel therapies. Future studies will focus on automation of IPSDM differentiation, via both primitive and definitive hematopoiesis, with high scalability and reproducibility to produce the full range of myeloid cells that have tissue-specific properties and are applicable for tissue engineering. In such contexts, IPSDM will be instrumental in validating human genetic discoveries, advancing functional genomics, and facilitating clinical and therapeutic translation for diseases such as atherosclerosis and coronary artery disease in which macrophages play a modulatory role in the clinical outcome.

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Disclosures

None.

References

1. McNelis JC, Olefsky JM. Macrophages, immunity, and metabolic disease. *Immunity*. 2014;41:36–48. doi: 10.1016/j.immuni.2014.05.010.
2. Copeland S, Warren HS, Lowry SF, Calvano SE, Remick D; Inflammation and the Host Response to Injury Investigators. Acute inflammatory response to endotoxin in mice and humans. *Clin Diagn Lab Immunol*. 2005;12:60–67. doi: 10.1128/CDLI.12.1.60-67.2005.
3. Schroder K, Irvine KM, Taylor MS, et al. Conservation and divergence in toll-like receptor 4-regulated gene expression in primary human versus mouse macrophages. *Proc Natl Acad Sci U S A*. 2012;109:E944–E953.
4. Carlsen HS, Baekkevold ES, Morton HC, Haraldsen G, Brandtzaeg P. Monocyte-like and mature macrophages produce CXCL13 (B cell-attracting chemokine 1) in inflammatory lesions with lymphoid neogenesis. *Blood*. 2004;104:3021–3027. doi: 10.1182/blood-2004-02-0701.
5. Piccini A, Carta S, Tassi S, Lasiglie D, Fossati G, Rubartelli A. ATP is released by monocytes stimulated with pathogen-sensing receptor ligands and induces IL-1beta and IL-18 secretion in an autocrine way. *Proc Natl Acad Sci U S A*. 2008;105:8067–8072.
6. Duewell P, Kono H, Rayner KJ, et al. NLRP3 inflammasomes are required for atherogenesis and activated by cholesterol crystals. *Nature*. 2010;464:1357–1361. doi: 10.1038/nature08938.
7. Kritharides L, Christian A, Stoudt G, Morel D, Rothblat GH. Cholesterol metabolism and efflux in human THP-1 macrophages. *Arterioscler Thromb Vasc Biol*. 1998;18:1589–1599.
8. Zhang H, Xue C, Shah R, et al. Functional analysis and transcriptomic profiling of iPSC-derived macrophages and their application in

modeling Mendelian disease. *Circ Res*. 2015;117:17–28. doi: 10.1161/CIRCRESAHA.117.305860.
9. Choi KD, Vodyanik M, Slukvin II. Hematopoietic differentiation and production of mature myeloid cells from human pluripotent stem cells. *Nat Protoc*. 2011;6:296–313. doi: 10.1038/nprot.2010.184.
10. Senju S, Haruta M, Matsumura K, Matsunaga Y, Fukushima S, Ikeda T, Takamatsu K, Irie A, Nishimura Y. Generation of dendritic cells and macrophages from human induced pluripotent stem cells aiming at cell therapy. *Gene Ther*. 2011;18:874–883. doi: 10.1038/gt.2011.22.
11. Karlsson KR, Cowley S, Martinez FO, Shaw M, Minger SL, James W. Homogeneous monocytes and macrophages from human embryonic stem cells following coculture-free differentiation in M-CSF and IL-3. *Exp Hematol*. 2008;36:1167–1175. doi: 10.1016/j.exphem.2008.04.009.
12. van Wilgenburg B, Browne C, Vowles J, Cowley SA. Efficient, long term production of monocyte-derived macrophages from human pluripotent stem cells under partly-defined and fully-defined conditions. *PLoS One*. 2013;8:e71098. doi: 10.1371/journal.pone.0071098.
13. Yanagimachi MD, Niwa A, Tanaka T, Honda-Ozaki F, Nishimoto S, Murata Y, Yasumi T, Ito J, Tomida S, Oshima K, Asaka I, Goto H, Heike T, Nakahata T, Saito MK. Robust and highly-efficient differentiation of functional monocytic cells from human pluripotent stem cells under serum- and feeder cell-free conditions. *PLoS One*. 2013;8:e59243. doi: 10.1371/journal.pone.0059243.
14. Zhang H, Shi J, Hachet MA, Xue C, Bauer RC, Jiang H, Li W, Tohyama J, Millar J, Billheimer J, Phillips MC, Razani B, Rader DJ, Reilly MP. CRISPR/Cas9-mediated gene editing in human iPSC-derived macrophage reveals lysosomal acid lipase function in human macrophages—brief report. *Arterioscler Thromb Vasc Biol*. 2017;37:2156–2160. doi: 10.1161/ATVBAHA.117.310023.
15. Lin J, Hu Y, Nunez S, Foulkes AS, Cieply B, Xue C, Gerelus M, Li W, Zhang H, Rader DJ, Musunuru K, Li M, Reilly MP. Transcriptome-wide analysis reveals modulation of human macrophage inflammatory phenotype through alternative splicing. *Arterioscler Thromb Vasc Biol*. 2016;36:1434–1447. doi: 10.1161/ATVBAHA.116.307573.
16. Vanhee S, De Mulder K, Van Caeneghem Y, Verstichel G, Van Roy N, Menten B, Velghe I, Philippé J, De Bleser D, Lambrecht BN, Taghon T, Leclercq G, Kerre T, Vandekerckhove B. In vitro human embryonic stem cell hematopoiesis mimics MYB-independent yolk sac hematopoiesis. *Haematologica*. 2015;100:157–166. doi: 10.3324/haematol.2014.112144.
17. Buchrieser J, James W, Moore MD. Human induced pluripotent stem cell-derived macrophages share ontogeny with MYB-independent tissue-resident macrophages. *Stem Cell Reports*. 2017;8:334–345. doi: 10.1016/j.stemcr.2016.12.020.
18. Douvaras P, Sun B, Wang M, Kruglikov I, Lallos G, Zimmer M, Terrenoire C, Zhang B, Gandy S, Schadt E, Freytes DO, Noggle S, Fossati V. Directed differentiation of human pluripotent stem cells to microglia. *Stem Cell Reports*. 2017;8:1516–1524. doi: 10.1016/j.stemcr.2017.04.023.
19. Muffat J, Li Y, Yuan B, Mitalipova M, Omer A, Corcoran S, Bakiasi G, Tsai LH, Aubourg P, Ransohoff RM, Jaenisch R. Efficient derivation of microglia-like cells from human pluripotent stem cells. *Nat Med*. 2016;22:1358–1367. doi: 10.1038/nm.4189.
20. Haenseler W, Sansom SN, Buchrieser J, Newey SE, Moore CS, Nicholls FJ, Chintawar S, Schnell C, Antel JP, Allen ND, Cader MZ, Wade-Martins R, James WS, Cowley SA. A highly efficient human pluripotent stem cell microglia model displays a neuronal-co-culture-specific expression profile and inflammatory response. *Stem Cell Reports*. 2017;8:1727–1742. doi: 10.1016/j.stemcr.2017.05.017.
21. Takata K, Kozaki T, Lee CZW, et al. Induced-pluripotent-stem-cell-derived primitive macrophages provide a platform for modeling tissue-resident macrophage differentiation and function. *Immunity*. 2017;47:183–198.e6. doi: 10.1016/j.immuni.2017.06.017.
22. Pandya H, Shen MJ, Ichikawa DM, Sedlock AB, Choi Y, Johnson KR, Kim G, Brown MA, Elkahloun AG, Maric D, Sweeney CL, Gossa S, Malech HL, McGavern DB, Park JK. Differentiation of human and murine induced pluripotent stem cells to microglia-like cells. *Nat Neurosci*. 2017;20:753–759. doi: 10.1038/nn.4534.
23. Abud EM, Ramirez RN, Martinez ES, et al. iPSC-derived human microglia-like cells to study neurological diseases. *Neuron*. 2017;94:278–293.e9. doi: 10.1016/j.neuron.2017.03.042.
24. Creamer JP, Dege C, Ren Q, Ho JTK, Valentine MC, Druley TE, Sturgeon CM. Human definitive hematopoietic specification from pluripotent stem cells is regulated by mesodermal expression of CDX4. *Blood*. 2017;129:2988–2992. doi: 10.1182/blood-2016-11-749382.
25. Sugimura R, Jha DK, Han A, et al. Haematopoietic stem and progenitor cells from human pluripotent stem cells. *Nature*. 2017;545:432–438. doi: 10.1038/nature22370.
26. Hale C, Yeung A, Goulding D, Pickard D, Alasoo K, Powrie F, Dougan G, Mukhopadhyay S. Induced pluripotent stem cell derived macrophages as a cellular system to study salmonella and other pathogens. *PLoS One*. 2015;10:e0124307. doi: 10.1371/journal.pone.0124307.
27. Uusi-Rauva K, Blom T, von Schantz-Fant C, Blom T, Jalanko A, Kyttala A. Induced pluripotent stem cells derived from a cln5 patient manifest phenotypic characteristics of neuronal ceroid lipofuscinoses. *Int J Mol Sci*. 2017;18:955. doi: 10.3390/ijms18050955.
28. Panicker LM, Miller D, Park TS, et al. Induced pluripotent stem cell model recapitulates pathologic hallmarks of gaucher disease. *Proc Natl Acad Sci U S A*. 2012;109:18054–18059.
29. Aflaki E, Stubblefield BK, Maniwang E, Lopez G, Moaven N, Goldin E, Marugan J, Patnaik S, Dutra A, Southall N, Zheng W, Tayebi N, Sidransky E. Macrophage models of Gaucher disease for evaluating disease pathogenesis and candidate drugs. *Sci Transl Med*. 2014;6:240ra73. doi: 10.1126/scitranslmed.3008659.
30. Jiang Y, Cowley SA, Siler U, Melguizo D, Tilgner K, Browne C, Dewilton A, Przyborski S, Saretzki G, James WS, Seger RA, Reichenbach J, Lako M, Armstrong L. Derivation and functional analysis of patient-specific induced pluripotent stem cells as an in vitro model of chronic granulomatous disease. *Stem Cells*. 2012;30:599–611. doi: 10.1002/stem.1053.
31. Takada S, Kambe N, Kawasaki Y, et al. Pluripotent stem cell models of Blau syndrome reveal an IFN-gamma-dependent inflammatory response in macrophages [published online ahead of print June 3, 2017]. *J Allergy Clin Immunol*. doi: 10.1016/j.jaci.2017.04.013. http://dx.doi.org/10.1016/j.jaci.2017.04.013.
32. Tanaka T, Takahashi K, Yamane M, et al. Induced pluripotent stem cells from CINCA syndrome patients as a model for dissecting somatic mosaicism and drug discovery. *Blood*. 2012;120:1299–1308. doi: 10.1182/blood-2012-03-417881.
33. Flynn R, Grundmann A, Renz P, Hänseler W, James WS, Cowley SA, Moore MD. CRISPR-mediated genotypic and phenotypic correction of a chronic granulomatous disease mutation in human iPS cells. *Exp Hematol*. 2015;43:838–848.e3. doi: 10.1016/j.exphem.2015.06.002.
34. van Wilgenburg B, Moore MD, James WS, Cowley SA. The productive entry pathway of HIV-1 in macrophages is dependent on endocytosis through lipid rafts containing CD4. *PLoS One*. 2014;9:e86071. doi: 10.1371/journal.pone.0086071.
35. Yeung ATY, Hale C, Lee AH, Gill EE, Bushell W, Parry-Smith D, Goulding D, Pickard D, Roumeliotis T, Choudhary J, Thomson N, Skarnes WC, Dougan G, Hancock REW. Exploiting induced pluripotent stem cell-derived macrophages to unravel host factors influencing Chlamydia trachomatis pathogenesis. *Nat Commun*. 2017;8:15013. doi: 10.1038/ncomms15013.
36. Howson JMM, Zhao W, Barnes DR, et al; CARDIoGRAMplusC4D; EPIC-CVD. Fifteen new risk loci for coronary artery disease highlight arterial-wall-specific mechanisms. *Nat Genet*. 2017;49:1113–1119. doi: 10.1038/ng.3874.
37. Kilpinen H, Goncalves A, Leha A, et al. Common genetic variation drives molecular heterogeneity in human iPSCs. *Nature*. 2017;546:370–375. doi: 10.1038/nature22403.
38. Carcamo-Orive I, Hoffman GE, Cundiff P, et al. Analysis of transcriptional variability in a large human iPSC library reveals genetic and non-genetic determinants of heterogeneity. *Cell Stem Cell*. 2017;20:518–532.e9. doi: 10.1016/j.stem.2016.11.005.
39. Jung Y, Ji H, Chen Z, Fai Chan H, Atchison L, Klitzman B, Truskey G, Leong KW. Scaffold-free, human mesenchymal stem cell-based tissue engineered blood vessels. *Sci Rep*. 2015;5:15116. doi: 10.1038/srep15116.
40. Shalem O, Sanjana NE, Hartenian E, Shi X, Scott DA, Mikkelson T, Heckl D, Ebert BL, Root DE, Doench JG, Zhang F. Genome-scale CRISPR-Cas9 knockout screening in human cells. *Science*. 2014;343:84–87. doi: 10.1126/science.1247005.
41. Brault J, Vaganay G, Le Roy A, Lenormand JL, Cortes S, Stasia MJ. Therapeutic effects of proteoliposomes on X-linked chronic granulomatous disease: proof of concept using macrophages differentiated from patient-specific induced pluripotent stem cells. *Int J Nanomedicine*. 2017;12:2161–2177. doi: 10.2147/IJN.S128611.
42. Koba C, Haruta M, Matsunaga Y, Matsumura K, Haga E, Sasaki Y, Ikeda T, Takamatsu K, Nishimura Y, Senju S. Therapeutic effect of human iPS-cell-derived myeloid cells expressing IFN-β against peritoneally disseminated cancer in xenograft models. *PLoS One*. 2013;8:e67567. doi: 10.1371/journal.pone.0067567.

43. Miyashita A, Fukushima S, Nakahara S, Kubo Y, Tokuzumi A, Yamashita J, Aoi J, Haruta M, Senju S, Nishimura Y, Jinnin M, Ihn H. Immunotherapy against metastatic melanoma with human iPS cell-derived myeloid cell lines producing type I interferons. *Cancer Immunol Res*. 2016;4:248–258. doi: 10.1158/2326-6066.CIR-15-0096.
44. Litvack ML, Wigle TJ, Lee J, Wang J, Ackerley C, Grunebaum E, Post M. Alveolar-like stem cell-derived Myb(-) macrophages promote recovery and survival in airway disease. *Am J Respir Crit Care Med*. 2016;193:1219–1229. doi: 10.1164/rccm.201509-1838OC.
45. Heidt T, Courties G, Dutta P, Sager HB, Sebas M, Iwamoto Y, Sun Y, Da Silva N, Panizzi P, van der Laan AM, van der Lahn AM, Swirski FK, Weissleder R, Nahrendorf M. Differential contribution of monocytes to heart macrophages in steady-state and after myocardial infarction. *Circ Res*. 2014;115:284–295. doi: 10.1161/CIRCRESAHA.115.303567.
46. Epelman S, Lavine KJ, Beaudin AE, et al. Embryonic and adult-derived resident cardiac macrophages are maintained through distinct mechanisms at steady state and during inflammation. *Immunity*. 2014;40:91–104. doi: 10.1016/j.immuni.2013.11.019.
47. Epelman S, Lavine KJ, Randolph GJ. Origin and functions of tissue macrophages. *Immunity*. 2014;41:21–35. doi: 10.1016/j.immuni.2014.06.013.
48. Xue C, Zhang X, Zhang H, Ferguson JF, Wang Y, Hinkle C, Li M, Reilly MP. De novo RNA sequence assembly during in vivo inflammatory stress reveals hundreds of unannotated lincrnas in human blood CD14+ monocytes and in adipose tissue. *Physiol Genomics*. 2017;49:287–305. doi: 10.1152/physiolgenomics.00001.02017.
49. Anders S, Huber W. Differential expression analysis for sequence count data. *Genome Biol*. 2010;11:R106. doi: 10.1186/gb-2010-11-10-r106.

Highlights

- The differentiation of human induced pluripotent stem cells to macrophages (induced pluripotent stem cell–derived macrophages) offers a powerful platform to study human macrophage biology, understand human disease, and develop novel therapies.
- Induced pluripotent stem cell–derived macrophages show similar phenotypic, functional and transcriptomic characteristics as human monocyte-derived macrophages.
- In vitro hematopoietic differentiation of pluripotent stem cells resembles in vivo primitive hematopoiesis rather than adult definitive hematopoiesis. Protocols that specifically differentiate induced pluripotent stem cells to microglia-like cells have been reported.
- Future studies will focus on automation of induced pluripotent stem cell–derived macrophages differentiation, via both primitive and definitive hematopoiesis, with high scalability and reproducibility to produce the full range of myeloid cells that have tissue-specific properties for use in tissue engineering, functional genomic analyses, drug testing, and cell therapeutics.

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