

## 诱导多能干细胞向巨噬细胞分化研究进展

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**摘要:** 诱导多能干细胞 (Induced pluripotent stem cells, iPSCs) 是通过体细胞重编程得到类似胚胎干细胞特性的一种细胞类型。通过 iPSCs 的体外分化, 可以了解巨噬细胞的进化历史和各种特性。iPSCs 来源的巨噬细胞不仅是药物筛选的良好模型, 也是进行免疫治疗的重要手段。本文综述了近年来 iPSCs 及其向巨噬细胞分化的相关研究进展、所面临的问题以及未来的发展方向。

**关键词:** 诱导多能干细胞, 巨噬细胞, 细胞分化, 拟胚体诱导, 单层细胞诱导, 类器官共培养

## Induction and differentiation of induced pluripotent stem cells into macrophages: a review

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**Abstract:** Induced pluripotent stem cells (iPSCs) are a type of cells similar to embryonic stem cells but produced by reprogrammed somatic cells. Through *in vitro* differentiation of iPSCs, we can interrogate the evolution history as well as the various characteristics of macrophages. iPSCs derived macrophages are not only a good model for drug screening, but also an important approach for immunotherapy. This review summarizes the advances, challenges, and future directions in the field of iPSCs-derived macrophages.

**Keywords:** iPSCs, macrophages, cell differentiation, embryoid bodies induction, monolayer cell induction, organoid co-culture

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## 1 诱导多能干细胞

2006年, Yamanaka 等在小鼠成纤维细胞中同时导入 Oct3/4、Sox2、c-Myc 和 Klf4 (简称 OSKM) 后获得了与胚胎干细胞 (Embryonic stem cells, ESCs) 相似特性的干细胞, 并将这些细胞命名为诱导多能干细胞 (Induced pluripotent stem cells, iPSCs)<sup>[1]</sup>。这也为曾经的体细胞核移植时核基因组恢复其分化前的功能状态 (重编程) 这一现象提供了分子基础<sup>[2]</sup>。2007年, Takahashi 等将 OSKM 导入人皮肤成纤维细胞产生了人 iPSCs<sup>[3]</sup>, 同期 James Thomson 等使用不同转录因子组合 (Oct4, Sox2, Nanog, Lin28) 同样将体细胞重编程为 iPSCs<sup>[4]</sup>。随着研究的深入, 越来越多的体细胞都可以被重编程为 iPSCs<sup>[5]</sup>, 重编程

的方法也随之增加。到目前为止, 只有转录因子 Oct3/4 被认为是必不可少的, 而 Sox2、Klf4、c-Myc 则被认为是可以替代的转录因子<sup>[6]</sup>。与原代分离的多能干细胞相比, iPSCs 的细胞来源丰富, 操作简便, 应用范围广, 为许多疾病的研究与治疗提供了全新的思路和技术方法, 目前已有使用 iPSCs 来模拟人体各类组织<sup>[7]</sup>、器官<sup>[8]</sup>和其他系统<sup>[9]</sup>的发生发育发展过程及各类疾病模型 (基于 iPSCs 的疾病建模方法详见图 1)。

### 1.1 iPSCs 的来源及获取方法

自 Yamanaka 等将 OSKM 四种转录因子导入小鼠成纤维细胞中将其重编程为 iPSCs 后, 不同体细胞诱导 iPSCs 的方法相继出现<sup>[10]</sup>。目前, 已被证实可诱导为 iPSCs 的细胞种类不断增多, 成纤维细胞<sup>[11]</sup>、角蛋白细胞<sup>[12]</sup>、大鼠眼细胞<sup>[13]</sup>、

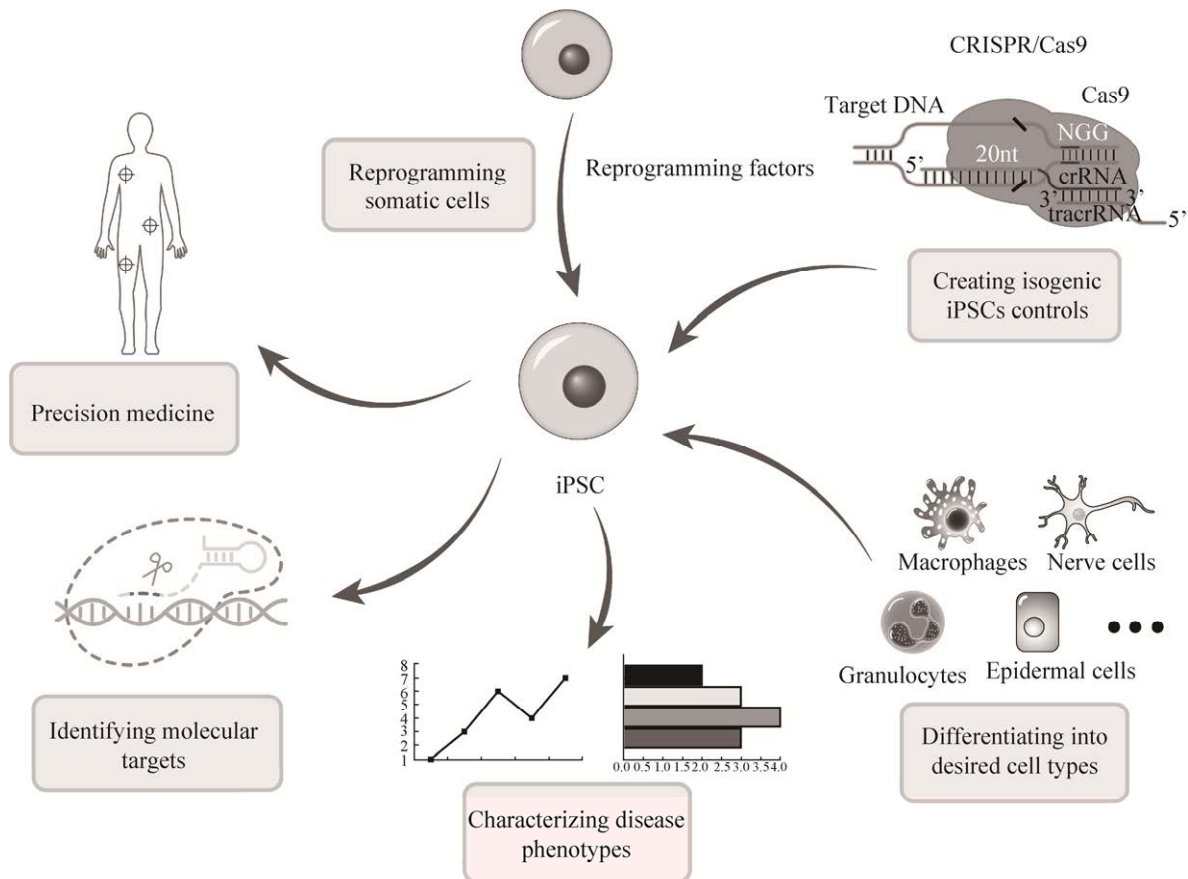


图 1 基于 iPSCs 的疾病建模示意图

Fig. 1 Schematic representation for human iPSCs-based disease modeling.

小鼠<sup>[10]</sup>和兔的肝脏与胃细胞<sup>[14]</sup>、胰岛 $\beta$ 细胞<sup>[15]</sup>、神经祖细胞<sup>[13]</sup>等均可以重编程为 iPSCs。但因为在诱导过程中引入了肿瘤基因 c-Myc, 高致瘤性仍然是一个关键问题<sup>[16]</sup>。2008年, 通过优化重编程方法, 获得了不需要 c-Myc 逆转录病毒的 iPSCs<sup>[11]</sup>。同期, Huangfu 等仅使用 Oct4 和 Sox2 成功重编程得到 iPSCs<sup>[17]</sup>。人们进一步发现只有 Oct4 是重编程过程中所必需的转录因子<sup>[18]</sup>, Oct4 在 ESCs 的多能维持和成熟细胞重新编程至 iPSCs 中起着突出的作用<sup>[19]</sup>。2009年 James A Thomson 等使用非整合的表观载体完成了对人体细胞的重编程<sup>[20]</sup>, 相比于整合型重编程方法, 使用非整合重编程不需要基因组整合或外源重编程因子的持续表达, 消除了人类 iPSCs 临床应用的一个障碍<sup>[21]</sup>。随后越来越多可用于重编程的方法被研究出来, 如使用仙台病毒<sup>[22]</sup>、附加体<sup>[23]</sup>、mRNA 转染<sup>[24]</sup>和转座子<sup>[25]</sup>等。之后, 很多学者利用小分子进行体细胞的重编程<sup>[26]</sup>, 这种化学重编程避免了基因组的改变, 降低了致瘤性, 为 iPSCs 临床应用提供了更多的可能性<sup>[27]</sup>, 为人类获取 iPSCs 提供了新方法、新思路。

## 1.2 iPSCs 应用现状

iPSCs 广泛应用于构建人类疾病模型、发育模型与再生医学中<sup>[28]</sup>, 对分析病因、探究疾病治疗靶点和药物筛选具有重要意义。iPSCs 在功能和发育方面更加类似于胚胎干细胞<sup>[29]</sup>, 理论上可以诱导成机体的各类细胞<sup>[30]</sup>, 且具有无限增殖的能力<sup>[31]</sup>。因此, 可以使用 iPSCs 及其衍生细胞来模拟身体组织、器官。如今, 已经可以将 iPSCs 诱导为内皮细胞<sup>[32]</sup>、神经元<sup>[9]</sup>、自然杀伤细胞<sup>[33]</sup>、生殖细胞<sup>[34]</sup>、巨噬细胞<sup>[35]</sup>等, 为疾病模型的建立和分化机制的研究提供了平台。患者与供体 ESCs 之间的组织不兼容性以及人类 ESCs 相关的伦理问题是目前限制发展的严重阻碍, 而 iPSCs 技术有望解决这些问题<sup>[36]</sup>。巨噬细胞作为人体的第一道防线, 利用 iPSCs 诱导分化为巨噬

细胞为相关疾病建模及巨噬细胞相关机理研究提供了良好的基础。

## 2 巨噬细胞

### 2.1 巨噬细胞起源

1893年 Élie Metchnikoff 等在观察组织炎症和吞噬病原体时, 首次发现了嗜中性粒细胞和巨噬细胞吞噬病原体的现象<sup>[37]</sup>。因为其吞噬病原体的功能, 后来的科学家将其归类于组织免疫细胞<sup>[38]</sup>。1968年, Ralf van Furth 和 Zanvil Cohn 共同定义了单核吞噬细胞系统, 认为巨噬细胞是来源于骨髓干细胞起源的单核细胞 (Monocytes), 具有很强的吞噬能力, 且细胞核不分叶。单核吞噬细胞系统是当时巨噬细胞起源的主要模型<sup>[39]</sup>。但是, 最近的研究表明, 巨噬细胞存在着不依赖造血干细胞 (Hematopoietic stem cells, HSCs) 的起源方式, 其中一类是在早期卵黄囊中经原位分化直接产生, 并迁移到胚胎各处; 另一类则由卵黄囊来源的红系髓系祖细胞 (Yolk sac-derived myeloid-biased progenitors, YSMPs) 先形成单核细胞后分化为巨噬细胞, 这类巨噬细胞具有自我更新能力, 并且不依赖于单核细胞的分化<sup>[40]</sup>, 如肝脏 (库佛氏细胞)、大脑 (小胶质细胞)、表皮 (朗格汉斯细胞) 和肺 (肺泡巨噬细胞) 中的绝大多数组织驻留巨噬细胞 (Tissue-resident macrophage, TRM), 是来自与 HSCs 不同的 Tek 细胞通路<sup>[41]</sup> (巨噬细胞起源相关模型见图 2)。然而, 形成 TRM 的 YSMPs 的确切性质仍然存在争议, 使巨噬细胞群体维持的机制也不明确<sup>[42]</sup>。但这也为巨噬细胞起源阐明了新的途径, 也为 iPSCs 诱导巨噬细胞提供了新的思路与方法。

### 2.2 巨噬细胞表型与功能

巨噬细胞具有极高的可塑性, 可以从一种表型转换到另一种表型<sup>[43]</sup>。巨噬细胞极化是一种特定的表型, 并对每个特定组织中遇到的微环境刺激和信号作出功能性反应的过程<sup>[44]</sup>。根据巨

噬细胞的表型与功能, 目前普遍认为巨噬细胞存在一系列连续的功能状态, 而经典激活或促炎 (M1) 型和交替激活或抗炎 (M2) 型巨噬细胞是这一连续状态的两个极端<sup>[45]</sup>。M1 型巨噬细胞通常由 Th1 细胞因子(如 IFN- $\gamma$  和 TNF- $\alpha$ )或细菌脂多糖(Lipopolysaccharide, LPS)诱导, 并分泌较高水平的促炎细胞因子 TNF- $\alpha$ 、IL-1 $\alpha$ 、IL-1 $\beta$ 、IL-6、IL-12、IL-23 和环氧合酶-2 (COX-2), 并专职提呈抗原, 参与正向免疫应答, 发挥免疫监视的功能; M2 型巨噬细胞通常通过 IL-4 受体  $\alpha$  (IL-4R $\alpha$ ) 激活 STAT6, 或通过 IL-10 受体 (IL-10R) 激活 STAT 3

来促进其极化, 有较弱抗原提呈能力, 并通过分泌抑制性细胞因子 IL-10 或 TGF- $\beta$  等下调免疫应答, 在免疫调节中发挥重要作用<sup>[46]</sup>。因此, M1 型巨噬细胞有较强的抗微生物和抗肿瘤活性, 介导活性氧 (Reactive oxygen species, ROS) 所致的组织损伤, 限制组织再生和伤口愈合。为了防止这种组织损伤, M2 型巨噬细胞通过较强的吞噬能力, 清除碎片和凋亡的细胞, 促进组织修复和伤口愈合, 从而抑制慢性炎症反应<sup>[47]</sup>。然而, M1、M2 连续体的界限并不清晰, 目前公认的 M1 型和 M2 型巨噬细胞表面标记及分泌蛋白见图 2<sup>[48]</sup>。

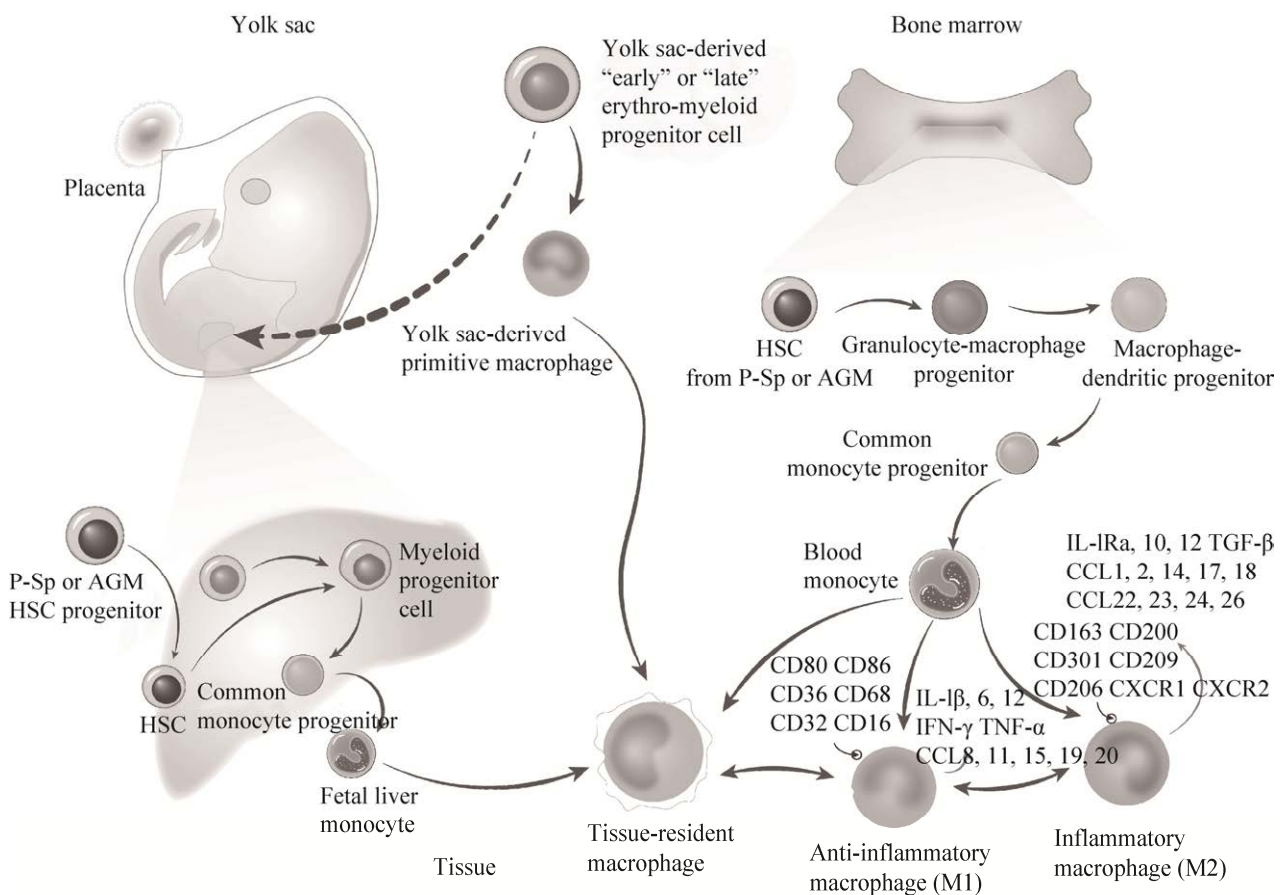


图 2 巨噬细胞起源及相关标记模式图 (P-Sp: 主动脉旁内脏胸膜; AGM: 主动脉-性腺-中肾)

Fig. 2 Model of macrophage origin and related markers. P-Sp: para-aortic splanchnopleure, AGM: orta-gonad-mesonephros.



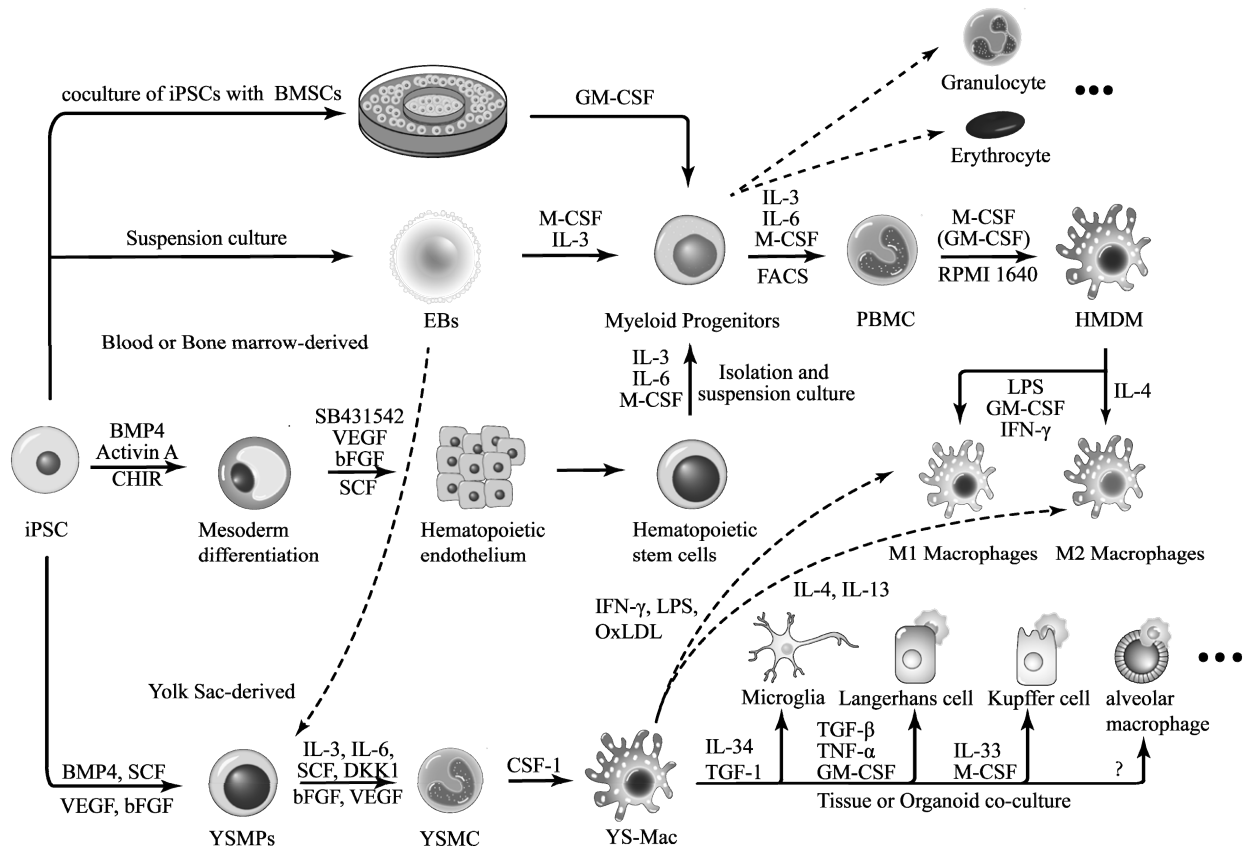


图3 iPSCs 诱导分化巨噬细胞体外模型 (GM-CSF: 粒细胞-巨噬细胞集落刺激因子; M-CSF: 巨噬细胞集落刺激因子; IL: 白介素; BMP4: 骨形态发生蛋白4; Activin A: 激活素A; VEGF: 血管内皮生长因子; bFGF: 成纤维细胞生长因子; SCF: 干细胞因子; TPO: 血小板生成素; IFN: 干扰素; TGF: 转化生长因子; TNF: 肿瘤坏死因子)

Fig. 3 Model of macrophage differentiation induced by iPSCs *in vitro*. GM-CSF: granulocyte-macrophage colony stimulating factor; M-CSF: macrophage colony stimulating factor; IL: interleukin; BMP4: bone morphogenetic protein 4; VEGF: vascular endothelial growth factor; bFGF: basic fibroblast growth factor; SCF: stem cell factor; TPO: thermoplastic polyolefin; IFN: interferon; TGF: transforming growth factor; TNF: tumor necrosis factor.

巨噬细胞是先天免疫系统的关键组成部分，在发育过程中分布在不同的组织和器官中，并通过局部增殖和稳态募集而终生维持<sup>[49]</sup>。这些组织驻留巨噬细胞群体在各种组织特异性的生理和病理过程中起着核心作用<sup>[50]</sup>。巨噬细胞还提供了抵御入侵病原体的一道防线，协调先天免疫反应，并激活适应性免疫应答<sup>[51]</sup>。而且在大多数癌症中，巨噬细胞与肿瘤浸润和预后不良相关，并会导致化疗耐药性，这使得肿瘤相关巨噬细胞无论是通过肿瘤消融还是从亲肿瘤状态向抗肿瘤

状态的再分化，都是人类抗癌治疗的良好靶点<sup>[52]</sup>。因此，在生物学以及人类疾病的背景下，巨噬细胞的功能及表征是许多研究者的主要重点。而实验性人类巨噬细胞主要有两个来源：肿瘤衍生细胞系，如U937、THP-1细胞等，或原代细胞，如外周血单核细胞 (Peripheral blood mononuclear cell, PBMC)、单核细胞源性巨噬细胞 (Human-monocyte derived macrophages, HMDM)。前者虽具有无限增殖的能力，但核型异常、表型不成熟，不能进行特定基因型的研究<sup>[53]</sup>。后者是一

种应用广泛的巨噬细胞功能实验模型<sup>[54]</sup>，虽然相对容易获得，但 HMDM 是终末分化的，不能进行自我更新，缺乏增殖能力<sup>[55]</sup>，且较难对其进行基因编辑<sup>[42]</sup>。利用 iPSCs 诱导产生的巨噬细胞 (Induced pluripotent stem cell-derived macrophages, IPSDM) 可以解决巨噬细胞的获取问题，并可利用先前构建的基因型特异性的 iPSCs 来衍生出核型正常和基因型稳定的巨噬细胞<sup>[56]</sup>。这种方法为研究巨噬细胞特定功能、免疫应答机制、活化和极化分子及巨噬细胞相关疾病或肿瘤治疗提供了良好的工具。

### 3 诱导多能干细胞向巨噬细胞分化的方法

目前巨噬细胞研究主要通过使用血液单核细胞衍生的巨噬细胞<sup>[57]</sup>，这些不仅需要来自献血者大量的血液，并且相对难以进行基因编辑。因此，IPSMD 为人类巨噬细胞研究提供了一个有力的解决方法。通过对小鼠和人 iPSCs 产生的畸胎瘤进行分析，证实了 iPSCs 具有分化为三胚层不同细胞的潜力<sup>[58]</sup>。此外，iPSCs 来源的细胞能够嵌合到多种组织、器官甚至生殖系<sup>[59]</sup>。小鼠和人类 iPSCs 已被广泛用于研究造血和免疫系统的发展<sup>[60]</sup>。虽然并非所有细胞都可以以相同的效率获得，但免疫细胞，如巨噬细胞和自然杀伤细胞等，可以较便捷地利用 iPSCs 获得，并利用其研发新的细胞疗法<sup>[61]</sup>。iPSCs 衍生的巨噬细胞也可以作为癌症免疫治疗的新方法以及修复或再生患病/受损组织和器官的新策略。图 3 展示了 iPSCs 诱导分化巨噬细胞的主要体外模型。

#### 3.1 拟胚体生成法

拟胚体生成法 (Embryoid bodies, EBs) 是目前最常用的使 iPSCs 诱导分化为巨噬细胞的方法。EBs 生成法是使未分化的克隆状 iPSCs 在悬液中生长，形成一种被称为 EBs 的结构<sup>[62]</sup>。EBs 可以分化为内胚层、中胚层、外胚层的三胚层结构<sup>[63]</sup>。一旦形成，EBs 就可以使用特定的酶分

离，获得一个细胞群体，该细胞群体利用特定诱导条件可以分化为三胚层中所有类型的细胞<sup>[64]</sup>。常见的 EBs 生产方法主要有静态悬浮培养、旋转悬浮培养<sup>[65]</sup>、悬挂滴片法<sup>[66]</sup>、微孔和微模型芯片法<sup>[67]</sup>等。将由 iPSCs 产生的 EBs 在无血清状态下添加特定的细胞因子，如 GM-CSF、M-CSF、IL-3、IL-4 等将 EBs 诱导分化为巨噬细胞<sup>[68]</sup>。Choi 等将 EBs 诱导为髓样前体细胞，再加入 M-CSF 和 IL-1 诱导分化为巨噬细胞<sup>[69]</sup>。Mukherjee 等则利用由 iPSCs 衍生出的 EBs，并加入 M-CSF、IL-3 产生髓样前体细胞，最后用高浓度的 M-CSF 将髓样前体细胞发育成熟为功能性巨噬细胞，并可使用 IFN- $\gamma$  和 IL-4 刺激后分别极化为 M1 或 M2 型巨噬细胞，且在功能上，这些巨噬细胞能对微生物或病原体刺激作出吞噬反应<sup>[70]</sup>。Gao 等使用 M-CSF、IL-3 和 IL-6 先使 EBs 向中胚层分化产生单核细胞 (通过诱导得到的 CD14<sup>+</sup>单核细胞起初只占细胞总量的 10%–25%，在诱导 25–40 d 后可达 90%–95%)，经过流式细胞荧光分选技术 (Fluorescence activated cell sorting, FACS) 分选，再使用 M-CSF 诱导至 M0 型巨噬细胞，在有 M-CSF 的条件下可使用 LPS、GM-CSF 或 IFN- $\gamma$  诱导 M0 型巨噬细胞向 M1 型巨噬细胞转变，使用 IL-4 向 M2 型巨噬细胞转变<sup>[71]</sup>。仅从一个 6 孔板培养的 iPSCs 细胞中便可以生成  $4 \times 10^6$  个 IPSMD<sup>[72]</sup>，证明了现阶段诱导方案的可行性与高效性。

#### 3.2 单层细胞诱导法

单层细胞诱导法是以 2D 培养的方法将 iPSCs 在多种细胞因子的作用下直接诱导至髓系祖细胞而产生巨噬细胞，并可将其 M0 型巨噬细胞分化为 M1 或 M2 型巨噬细胞<sup>[73]</sup>。此种方法将 iPSCs 通过中胚层分化至 HSCs 形成与 PBMC 具有相同基因表型的单核细胞<sup>[74]</sup>；也能通过将 iPSCs 诱导至 YSMPs 进而产生巨噬细胞<sup>[75]</sup>，相比于 HSCs 来源的巨噬细胞，YSMPs 来源的巨噬细胞缺乏 HOXA 基因，故其更类似于 TRM<sup>[76]</sup>，并能够在低温下长

期保存。Hansen 等使用该体系无需饲养层,以新的单细胞来源的 iPSCs 集落分化为基础模拟体内造血步骤,通过多种细胞因子在 8–10 d 内产生多潜能的 HSCs,经过谱系特异性生长因子的补充,这些细胞可以诱导为红系细胞、巨噬细胞和髓系细胞<sup>[77]</sup>。Vordenbäumen 等发现人  $\alpha$ 1 酪蛋白可以促进 iPSCs 诱导的 PBMC 向巨噬细胞分化,具体表现在能刺激 PBMC 促炎细胞因子 GM-CSF 的表达<sup>[78]</sup>。Amanda 等使用 iPSCs 模拟 YSMPs 产生组织驻留巨噬细胞,并使用 IL-34、GM-CSF 向小胶质细胞诱导,并能对  $Ca^{2+}$  响应,展现出与 HMDM 的区别<sup>[79]</sup>。单层细胞诱导法很好地避免了 EBs 的形成,无需特定的 EBs 进行诱导,并可以经历 YSMPs 或 HSCs 分别诱导至 TRM 或 HMDM。且分化步骤清晰,杂细胞比例小,对认清巨噬细胞起源与分化机理具有重大意义,也为巨噬细胞相关疾病提供了一个良好的研究平台,但每一步需要的细胞因子多、成本高且诱导周期长,不利于用于大规模的诱导策略,无法利用巨噬细胞作为“工厂细胞”生产各类细胞制品。

### 3.3 类器官或组织细胞共培养

类器官或组织细胞共培养是使用 iPSCs 与骨髓基质细胞 (Bone marrow stromal cells, BMSCs) 共培养使其向造血方向诱导分化得到巨噬细胞或将 M0 型巨噬细胞与其他原代组织、iPSCs 来源的类器官共培养得到类似 TRM 的巨噬细胞<sup>[80]</sup>。目前,已被证实其可以产生小胶质细胞<sup>[81]</sup>、肺巨噬细胞,并有发展为其他 TRM 的潜力<sup>[35]</sup>。此方法的主要优点是可以在短时间内(8–9 d)实现 iPSCs 的高效定向分化,诱导效率可达 85% 以上,而且不需要添加细胞因子<sup>[82]</sup>,但诱导步骤及促进其分化的细胞因子种类不明确也对后续科学研究造成了一定的困扰。Choi 等于 2011 年使用小鼠骨髓基质细胞与 iPSCs 共培养得到巨噬细胞<sup>[70]</sup>,BMSCs 共培养可用于获取造血干细胞和成熟细胞,包括 T、B 淋巴细胞和巨核细胞,虽然

BMSCs 共培养最初是为 ESCs 的分化而开发的,但这种方法也可以有效地从 iPSCs 生成造血细胞,进而分化为巨噬细胞、粒细胞等,并稳定表达其特异性表面标记,通过 FACS 可以特异性地分离出目的细胞<sup>[83]</sup>,此外该方法通过抑制  $lnk$ <sup>[84]</sup>、强制激活 STAT5A<sup>[85]</sup>、SOX17<sup>[86]</sup> 等均可促进 iPSCs 向造血分化,提高 ESCs 的造血潜力及 IPSDM 的诱导效率。IPSDM 共培养系统可能成为研究组织驻留性巨噬细胞的一种非常有价值的工具。

### 3.4 大规模诱导策略

目前,iPSCs 向巨噬细胞的大规模分化策略主要仍为 EBs 生成法,基于生物反应器可以一次性收获大量 IPSDM,且收获期较长,细胞因子需求种类较少,通过 BMP4、VEGF、SCF 得到 EB 后再添加 M-CSF 和 IL-3 便可得到巨噬细胞,较适合商业化生产<sup>[35]</sup>。Ackermann 等开发了一种基于悬浮 (3D)、连续 (4D) 的造血分化方案,将 EBs 诱导为髓系细胞形成复合体,其可以连续生产 IPSDM 并进行肺内移植,可以有效抑制细菌感染<sup>[87]</sup>。Gutbier 等基于生物反应器利用 iPSCs 建立了一种可大规模生产类似 TRM 的方法,并克服了巨噬细胞前体及巨噬细胞冷冻保存导致的低存活率,且单次收获产量高达  $6 \times 10^8$  个细胞<sup>[88]</sup>。几种诱导分化的特点和优缺点见表 1<sup>[89]</sup>。

### 3.5 IPSDM 特征与鉴定方法

巨噬细胞分布广泛,因其功能状态不同而变化,一般为圆形或椭圆形,并有短小突起,功能活跃者常伸出较长伪足而呈不规则形<sup>[90]</sup>。M1 型巨噬细胞形态常为具有多个突起的星形,而 M2 型巨噬细胞形态较圆,体外培养时可附着在玻璃和塑料表面<sup>[44]</sup>。利用吞噬及释放活性氧和一氧化氮的特性同样可以确定其为巨噬细胞,巨噬细胞利用吞噬作用吞噬带荧光标记的细菌或蛋白可以定位巨噬细胞,如 DiI-乙酰化低密度脂蛋白或大肠杆菌等,并可实时示踪其活动轨迹<sup>[91]</sup>。巨

噬细胞表达 CD11b、CD14、CD18、CD45 和 CD64 标记,但相较于外周血来源的巨噬细胞,CD18 和 CD45 的表达量偏低,M1 型巨噬细胞表达 CD80 和 CD86,而 M2 型巨噬细胞表达 CD163 和 CD206,成熟/激活的巨噬细胞表达 CD195 和 HLA-DR<sup>[92]</sup>。巨噬细胞的另一种免疫调节功能是刺激后释放亲炎或抗炎细胞因子,这使得其释放的各种细胞因子也能成为验证靶点,亲炎细胞因子(称为 M1 相关)包括 IL-6、IL-8、TNF- $\alpha$ 、CXCL8、CXCL10、CCL2 和 CCL4 等,抗炎因子

(称为 M2 相关)包括 IL-1RA、IL-10、VEGF 和 CCL22 等,均可作为验证模型<sup>[93]</sup>。单核-巨噬细胞形态学特征及主要表面标记见表 2 和图 2<sup>[49]</sup>。

## 4 IPSDM 应用现状

巨噬细胞是代谢、免疫、心血管疾病及肿瘤等领域中的关键细胞类型,而 IPSDM 可以无限增殖并表现特定基因型,且易于通过 CRISPR/Cas9 进行基因操作。与 HMDM 相比,IPS DM 不仅有与其相媲美的基因表达特征<sup>[75]</sup>,且

表 1 iPSCs 诱导分化至巨噬细胞的方法及特点

Table 1 Methods and characteristics of differentiation into macrophages induced by iPSCs

Induction strategy	Induced way	Induced content	The advantages and disadvantages
Small scale strategy	Embryoid body formation	iPSCs was generated into EBs, and then induced to differentiate into macrophages by corresponding cytokines	Advantages: it is beneficial to the control of cell differentiation or the study and optimization of the differentiation conditions of a single variable Disadvantages: low differentiation efficiency and high cost
	Monolayer culture	iPSCs was induced into myeloid progenitor cells to produce macrophages step by step under the action of many factors, and the induction efficiency could be increased by using CSN1S1, and the steps were clear	
	Co-culture induction method	iPSCs was co-cultured with bone marrow stromal cells to induce hematopoiesis, and the induction efficiency could be improved by inhibiting Ink or enhancing STAT5	
Large scale strategy	2D monolayer culture method	No concentration gradient of cytokines is directly allowed to enter the cells, reducing the differentiation operation	Advantages: it can be used for large-scale production of target cells with low induction cost and high efficiency Disadvantages: need to rely on expensive production equipment, the investment is large, and is not conducive to research
	3D suspension culture method	IPSCs can be differentiated into macrophages on a large scale, but the environment is not conducive to the formation of dynamic balance	
	Bioreactor culture method	It can improve the use efficiency of raw materials, the product is easy to collect, and the differentiation efficiency is high	

表 2 单核-巨噬细胞形态学特征及主要表面标记

Table 2 Morphological characteristics and main surface markers of monocytes and macrophages

Types	Morphological features	Main surface marks
Monocytes	It is large in size, 15–20 $\mu\text{m}$ . The nuclei are often eccentric, chromatin is loose and reticular, more chromatin than shallow cytoplasm, and basophilic	CD14, CD45
Macrophages	It is generally round or elliptical, and has short protuberance, and the functional active person often stretches out longer pseudopod and shows irregular shape. The nucleus is small, round or elliptical, and darker in color	CD11b, CD14, CD18, CD45, CD64, CD195, HLA-DR
M1 macrophages	It is usually a star with multiple prominences	CD80, CD86
M2 macrophages	Cell morphology is round, basically no protuberance	CD163, CD206



在免疫反应、抗原处理和呈递及对刺激的反应中, IPSDM 均表现出更强的能力<sup>[57]</sup>, 并能分化为 TRM, 为研究疾病建模、药物筛选和细胞治疗提供了一个良好的平台, 为研究巨噬细胞生物学提供了重要工具<sup>[35]</sup>。

利用功能基因组学分析和转录组学分析, IPSDM 在疾病模型建立方面拥有着无限的潜力<sup>[94]</sup>。通过体外刺激 IPSDM 以确定个体遗传差异如何改变免疫细胞对环境刺激的影响, 建立模型预测人类巨噬细胞相关疾病的易感性与恢复力<sup>[57]</sup>, 在孟德尔病的建模与研究中起到了关键的作用, 如遗传性肺泡蛋白沉积症<sup>[95]</sup>、丹吉尔病<sup>[54]</sup>等。对于各类传染病, 巨噬细胞吞噬作用是抵抗病原体侵袭的重要防御机制, 利用 IPSDM 建立免疫疾病模型也展现了其极大的优势, Bernard 等利用 IPSDM 研究其对结核杆菌的感染反应及 ESX-1 VII 型分泌系统的作用, 揭示了细菌逃逸时的膜动力学<sup>[96]</sup>。

IPSDM 在细胞治疗, 特别是肿瘤相关治疗方面也展现出其独有的优势。CAR-IPSDM 利用肿瘤特异性靶向作用为巨噬细胞清除癌细胞提供了新方法<sup>[97]</sup>。并利用其表达的干扰素  $\beta$  显著抑制了植入免疫功能低下小鼠腹腔的人胃癌和胰腺癌的生长, 展现出了抗肿瘤能力<sup>[98]</sup>。Ackermann 等将 IPSDM 作为针对细菌性呼吸道感染的细胞治疗方案, 有效治疗下呼吸道的急性感染<sup>[88]</sup>。IPSDM 相比 HMDM 表现出 HLA-DR<sup>dim</sup>, 是低活性低极化细胞<sup>[94]</sup>, 略微偏向于抗炎活性, 具有多种功能活性, 是一种研究病原体和宿主之间的相互作用及研究其抗菌能力的宝贵模型, 用于评估多种遗传背景下的宿主反应<sup>[99]</sup>。在移植或输血过程中使用 IPSDM 能够有效避免免疫排斥和疾病传播风险, 并能增强其功能或赋予其新的治疗特性<sup>[100]</sup>。

利用 IPSDM 可以分化为类似组织驻留巨噬细胞的特性, 通过单细胞测序或 CRISPR/Cas9, 更有利于验证 TRM 所涉及的关键转录因子在机体中的作用及其如何调节组织免疫功能<sup>[69]</sup>, 例如

小胶质细胞的 SALL1<sup>[101]</sup>、肺泡巨噬细胞的 PPAR $\gamma$ <sup>[102]</sup>、朗格汉斯细胞的 RUNX3<sup>[103]</sup>、库弗氏细胞的 Id3<sup>[104]</sup>, 利用 IPSDM 进行此类研究很可能为巨噬细胞相关疾病(如肺泡蛋白沉积症<sup>[105]</sup>、阿尔茨海默病<sup>[106]</sup>等)带来新的治疗靶点。且当移植进体内时, IPSDM 能够适应并获得与相应组织驻留巨噬细胞相同的表型和功能特征, 这为研究组织驻留巨噬细胞提供了极佳的模型<sup>[35]</sup>。IPSDM 的发展为 YSMPs 来源的巨噬细胞提供了一个良好的替代品, 为区分哪些属性是所有巨噬细胞固有的、哪些是组织驻留巨噬细胞特有的提供了一个强大的工具。

因此, IPSDM 为巨噬细胞相关疾病建模、新疗法的开发以及作为治疗特定疾病患者的细胞来源提供了新的机遇。再加上易于通过基因组编辑方法(如 CRISPR/Cas9 和 TALEN)进行操作, IPSDM 代表了一种潜在的、优质的巨噬细胞来源, 可用于大规模生成特定研究材料<sup>[107]</sup>。

## 5 总结与展望

iPSCs 的相关问题已经经过了 10 余年的研究, 取得了一定的研究进展, 与胚胎干细胞相比, iPSCs 解决了免疫排斥问题和伦理道德问题, 为相关领域的研究开辟了新方向、新思路, 但仍有很多问题亟需解决, 重编程效率、致癌风险、分化多样性与多能性不足的问题仍困扰着当代研究者。笔者研究组建立了猪人工诱导多能干细胞(piPSCs), 解析了决定多能性干细胞发育分化的一系列关键因子的调控机理<sup>[108-113]</sup>。然而, 猪诱导多能干细胞虽然具有三胚层分化能力并在体内能够产生畸胎瘤, 但不能得到生殖系嵌合体后代, 说明 piPSCs 的多能性可能存在缺陷<sup>[114-115]</sup>。虽然人和小鼠的多能干细胞能够被定性诱导至巨噬细胞, 但猪诱导多能干细胞能否定向分化成巨噬细胞, 仍是一个未知的问题。而且, 在多能信号通路中, 猪也表现出与人和小鼠的不同<sup>[116-117]</sup>, 人和小鼠的诱导体系是否也适用于猪, 也是值得探索的。

巨噬细胞作为人体内重要的免疫细胞, iPSCs 向巨噬细胞分化为研究者获取巨噬细胞并在体外培养提供了一个便捷、可靠的来源, 为研究巨噬细胞发育规律建立了良好的模型, 且对 IPSDM 进行基因修饰可以针对一些免疫相关疾病提供基因分析工具, 但目前对其相关分化机制的研究还十分有限, 探究其分化机制为人类免疫系统的研究提供了很好的借鉴意义, 且过程可视化。笔者利用猪诱导性多能干细胞作为种子细胞, 利用小分子结合 CRISPR/Cas9 基因编辑技术尝试建立高效的 iPSCs 诱导分化为肺泡巨噬细胞, 挖掘 iPSCs 向免疫细胞的高效体系和决定因子, 进一步筛选用于抗病育种的靶标基因和抗病药物筛选或用作大批量制作疫苗的载体细胞。目前采用多种方法优化提高其诱导分化效率, 其中改进其诱导分化途径、基因敲除技术的联合使用、分化机制的探究都将是今后研究的热点问题。

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