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博士学位论文

诱导多能干细胞来源的移植物的免疫原性  
及其功能的研究

The immunogenicity and function of iPSC- derived graft

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## 纪念移植免疫理论提出 55 周年

*By Peter Brian Medawar 1960*



### **Nobel Prize in Physiology or Medicine 1960**

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## 摘要

**目的:** 诱导多能干细胞 (induced pluripotent stem cells, iPSCs) 因其来源于供者体细胞, 易获得, 易制备, 且具有与胚胎干细胞(embryonic stem cells, ESCs) 相似的多能性, 经分化发育, 可为供者提供无限的量身定做的移植物而为再生医学的发展带来极大的希望。然而, iPSCs 来源组织或器官的免疫原性、功能性及安全性仍备受质疑。这些疑问不仅限制其临床转化, 甚至动摇了该研究方向的可行性。本研究的目的是要全面的评价 iPSCs 经体内正常分化发育而形成的移植物的免疫原性, 功能性和安全性。

**方法:** 该研究以四倍体补偿的方法构建 iPSCs 来源小鼠(iPSC-derived mouse, iPSm) 作为移植物的来源, 并与 ESCs 来源小鼠 (ESCs-derived mouse, ESm) 和同系小鼠相应的移植物进行比较。首先通过流式细胞术和 q-PCR 检测组织相容性抗原, 并通过混合淋巴细胞反应 (Mixed lymphocyte reactivity, MLR) 确定组织配型成功。然后, 进行 iPSm 到同基因受体鼠的皮肤, 胰岛以及心脏移植, 通过生存期观察、组织学分析、T 细胞应答水平和抗体水平检测, 来全面评价 iPSCs 经体内正常分化发育而形成的各胚层来源的组织和器官的免疫原性。同时, 移植后跟踪监测各组移植物的功能。iPS 移植物的安全性是通过 iPSCs 本身的遗传稳定性检测, 受体鼠体重和状态, 以及大体解剖、组织学分析和受体淋巴细胞分型等检测, 观察肿瘤或感染等出现的可能性。

**结果:** iPSm 体细胞均来源于 iPSCs 并具有正常的主要组织相容性抗原(Major histocompatibility complex antigen, MHC) 表型。供体细胞在 MLR 中未刺激同基因受体 T 细胞增殖。iPSm 的皮肤移植后能成功存活并修复组织创伤; iPSm 的胰岛能逆转同基因受体小鼠糖尿病进程, 有效的分泌胰岛素并降低血糖; iPSm 的心脏移植物, 在同基因受体小鼠体内长期存活并维持正常跳动。移植 3 个月后, 对移植物的病理检测均未发现炎性细胞浸润。体外二次刺激受体 T 细胞和血清抗供体抗体检测均未发现受体对 iPSm 移植物产生明显的免疫应答。同时, iPSC 本身无明显遗传变异。移植后受体鼠状态良好, 无肿瘤感染等发生。受体鼠 T 细胞、B 细胞及 NK 细胞比例正常。

**结论:** 本研究首次在组织器官水平, 通过移植手段证明 iPSCs 与 ESCs 经过正常的分化发育而获得的各胚层的移植物在免疫原性上无明显差别, 均可以在无

需应用免疫抑制剂治疗的情况下，在同基因受体体内长期存活并维持正常的功能。在 3 个月的观察期内，均无免疫排斥反应发生，也没有明显的副作用产生。该研究为以 iPSCs 来源的细胞，组织和器官作为临床移植物来源的安全性和有效性提供了直接的实验证据。

**展望：**本研究结果前瞻性的证明了 iPS 技术临床转化的可行性，从根本上打消了对其最终是否能形成安全有效且无免疫原性的移植物的质疑。为 iPSCs 在再生医学中的临床应用指明了方向。然而，要真正实现该方法的广泛应用，还要深入研究诱导体细胞重编程的分子机制；进一步明确其他成体细胞诱导的 iPSC 以及人 iPSC 是否安全，有效且无免疫原性，同时还要不断优化 iPSC 的制备、筛选、体外定向分化发育和治疗方法。iPSCs 终将实现临床转化，为人类提供丰富的移植物，从而解决人类面临的各种疾患。

**关键词：**诱导多能干细胞、免疫原性、皮肤移植、胰岛移植、心脏移植

## Abstract

**Objective:** Induced pluripotent stem cells (iPSCs) hold great hopes for regeneration medicine by providing unlimited donor specific grafts because it can be easily obtained from donor somatic cells and induced to be pluripotent stem cells. However, whether physiologically iPSCs derived organs are immunogenic and functional which can be used for transplantation is unclear. This query not only hinders clinical conversion of iPSC, but also vacillates the feasibility of iPSC application. The aim of this project is to roundly evaluate the immunogenicity, function and safety of the in-vivo normally developed graft from iPSC.

**Materials and methods:** Here, we generated iPSC-derived mouse (iPSm) through 4n complementation as the origin of the grafts to compare with ESC-derived grafts and autogenic mouse's grafts. Flow cytometry and RT-PCR were used to detect the tissue-compatible antigen before transplantation. MLR was also used for in vitro tissue matching. Then, iPSC-derived skin, islet, and heart representing three germ layers of the body through 4n complementation were transplanted to syngeneic C57BL/6 mouse to evaluate their therapeutic efficacy. The immunogenicity of these grafts was evaluated by tissue survival time, histology H&E staining, and T-cell infiltration of each graft type. Recipient's T cell activation and function were also evaluated by T cell proliferation and IFN- $\gamma$  secretion during second T cell stimulation. What's more, the anti-donor antibody level was assessed after transplantation. The function of the graft was also followed-up. The ability of iPSC-derived skin graft to heal skin lesions was examined using a wound healing mouse model, while the effect of iPSC-derived islet on blood glucose depression was assessed in diabetic mice. Vascularized heterotopic transplantation of iPSC-derived heart was also performed to observe its beating ability. The recipient's weight and state was followed. Tumor formation and infection was detected by gross anatomy, histology analysis and lymphocyte subtype rate to evaluate the safety of the graft.

**Results:** The results showed that cells in iPSm origin from iPSC, with normal level of MHC and tissue specific antigen which do not stimulate recipient's T cells

in vitro. Upon transplantation into recipient mice, the skin grafts from iPSm grow healthily with regenerated hairs and repair local tissue wounds; the islet grafts from iPSm could rescue diabetic mice and lower blood glucose to basal levels; the heart grafts from iPSm maintained normal beating for more than 100 days. These transplanted tissues could survive and work normally in the receptor for a long term without T cell infiltration and secondary immune response. The immunogenicity of these iPSC-derived tissues were indistinguishable from that of the ESC-derived tissues and the syngeneic tissues. Importantly, no gene variation was observed in those iPSCs clone. After transplantation, the recipients are in good condition. Physiologically iPSC-derived skin, heart, and islet transplants function well in recipients without tumor formation or other defects. The rate of each class of lymphocyte is normal.

**Conclusion:** Our study first demonstrated there is no difference between iPSCs and ESCs derivatives after normal differentiation by organ transplantation. The iPSC-derived skin, heart, and islet showed limited immunogenicity, leading to acceptance of these organs by syngeneic recipients without the need for immunosuppression upon transplantation. No rejection and other side effect happened within 3 months' observation. The result not only demonstrates the fundamental immunogenicity and function of iPSC derivatives, but also provides preclinical evidence to support the feasibility of using iPSC-derived skin, islet, and heart for therapeutic use.

**Prospect:** Our data prospect the feasibility of the iPS clinical translation which eliminate the suspicion about the ultimate function, safety and immunogenicity of iPSCs derivative. However, to realize the clinical widely application of iPSCs, it should be clear about the molecular mechanism of somatic cell reprogramming and regulation, the characteristics of other source and human iPSCs, what's more, several conditions should be optimized about iPSCs induction, selection and differentiation. It is foreseeable that the successful clinical translation of iPSC will offer unlimited graft to healing different kinds of human diseases.

**Keywords:** induced pluripotent stem cell, immunogenicity, skin transplantation, islets transplantation, heart transplantation

厦门大学博硕士学位论文摘要库

## 第一章 前言

随着人类寿命的延长和生活方式的改变，糖尿病、慢性肾病、肝硬化、冠心病等器官功能损伤或退化的病患人数逐年攀升。而这些疾病往往会发展到不可逆的器官衰竭导致死亡。这些终末期器官衰竭患者生存下去的唯一希望就是器官移植。随着近几年临床器官移植的发展，胰岛移植术后 1 年生存率以及肾移植术后 5 年生存率均达到 90% 以上，器官移植已经取得了极大成功，有望从根本上解决终末期器官衰竭的治疗问题。然而，器官匮乏和免疫排斥严重制约了器官移植的发展<sup>[1]</sup>。一方面，全世界需要器官移植手术的患者数量与所捐献人体器官的数量比为 20:1<sup>[2]</sup>。UNOS 网站上公布 (<http://www.unos.org/>)，截止到 2015 年 3 月 20 日美国有 12 万人在等待器官移植，而 2014 年全年只有 1.4 万器官捐献者。同时，我国从今年起全面停止使用死囚器官，公民自愿捐献成为器官移植供体的唯一来源。中国器官移植网数据显示，2015 年初两个月内我国公民器官捐献已达 381 例，共 1200 个器官，其中大器官 937 例。但我国目前每年约 30 万患者等待器官移植，器官供体缺口仍旧很大 ([www.transplant-china.com](http://www.transplant-china.com))。人类对于器官移植的巨大需求与供体器官的匮乏之间的矛盾还滋生出了器官买卖等一系列恶劣的重大社会问题。另一方面，受移植配型的限制，患者找到配型成功的供体只有万分之一的可能。尽管免疫抑制剂的开发和治疗水平的提高正逐渐打破 MHC 配型和血型障碍，但患者仍需要终生服用大量免疫抑制剂。这不仅给社会和家庭都带来沉重的负担，同时，感染、慢性排斥和免疫抑制剂的毒副作用等严重影响了患者的生存率和生活质量。而 iPSC 有无限增殖和多向分化的潜能，同时其来源于患者自身，理论上可以为患者源源不断的提供无免疫原性的各种移植物，有望从根本上解决以上两个问题。它将再生医学的发展推向了新的阶段。

前言部分将从介绍 iPSC 的发现及研究现状入手，探讨其临床转化的方向和可能存在的问题。从而明确本研究的目的、意义和研究内容。



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