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述评 EDITORIAL

诱导多能干细胞–iPSCs在胆管病研究中的应用

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Application of induced pluripotent stem cells in cholangiopathies

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Abstract

Induced pluripotent stem cells (iPSCs) are similar to embryonic stem cells (ESCs) in morphology, gene expression, cell self-renewal and differentiation potential. They avoid the problem of immune rejection and ethical issues associated with the application of ESCs. The application of iPSCs in a variety of diseases provides favorable experiences to the research of liver diseases. Cholangiopathies, such as primary biliary cirrhosis and primary sclerosing cholangitis, refer to a category of uncommon diseases that possess unclear pathogenesis, lack effective treatment and have a poor prognosis. Hence, investigating cholangiopathies-derived, individualized iPSCs and their differentiation into functional cells can mimic the disease phenotype and pathological process *in vitro*. The application of these cells has great significance for pathogenesis exploration, drug screening and therapeutic evaluation.

Key Words: Induced pluripotent stem cells; Cholangiopathies; Biliary epithelial cells

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背景资料
诱导多能干细胞(induced pluripotent stem cells, iPSCs)是在形态、基因表达、细胞自我更新、以及分化潜能等方面与胚胎干细胞相似的一类细胞, 但却避免了后者应用中的免疫排斥和伦理道德等问题, iPSCs技术的创立被认为是生命科学及再生医学研究领域中的革命.

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研发前沿
使用特定患者的iPSCs既可重演单基因遗传性疾病表型也可重演迟发性多基因遗传性疾病的表型. 用这些细胞来分析发病机制和作为药物筛选平台研究新的治疗方法, 已经成为研究的兴奋点及趋势.

摘要

诱导多能干细胞(induced pluripotent stem cells, iPSCs)是在形态、基因表达、细胞自我更新、以及分化潜能等方面与胚胎干细胞(embryonic stem cells, ESCs)相似的一类细胞, 但却避免了ESCs应用中的免疫排斥和伦理道德等问题. iPSCs在多种疾病研究上取得的成就为肝脏疾病提供了良好的借鉴. 以原发性胆汁性肝硬化、原发性硬化性胆管炎等为代表的一组胆管病虽非常见疾病, 但发病机制不清、有效治疗手段匮乏、预后差. 因此发展胆管病患者源性的、个体化的iPSCs及其诱导分化的功能细胞可在体外模拟疾病的表型及病理过程, 应用此细胞模型对研究发病机制、药物筛选和疗效评估等具有重要意义.

关键词: 诱导多能干细胞; 胆管病; 胆管上皮细胞

核心提要: 胆管病是一组以胆管上皮细胞为共同攻击靶标的慢性肝脏疾病的统称, 发病机制不清、有效治疗手段匮乏. 诱导多能干细胞(induced pluripotent stem cells, iPSCs)研究为此提供了新的契机. 诱导iPSCs向胆管细胞分化和应用iPSCs技术构建患者特异性的疾病模型具有较大的临床应用前景.

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0 引言

2006年, Yamanaka等[1]通过将*Oct3/4*、*Sox2*、*c-Myc*和*Klf4*四种基因, 以逆转录病毒为载体导入小鼠成纤维细胞, 诱导体细胞回归到类似于胚胎干细胞(embryonic stem cells, ESCs)的、未分化、具有高度发育潜能的状态, 从而创造了诱导多能干细胞(induced pluripotent stem cells, iPSCs)新技术. iPSCs是在形态、基因表达、细胞自我更新、以及分化潜能等方面与ESCs相似的一类细胞, 但却避免了后者应用中的免疫排斥和伦理道德等问题[2,3]. 因此, 此后有关iPSCs的研究迅速增长并在再生医学领域不断取得进展, 为多种疾病的治疗提供新的契机[4]. 如Jaenisch实验室开创性用镰刀型贫血病小鼠自身皮肤来源的iPSCs治疗该疾病[5], 其他例如治疗帕金森氏病[6]、血小板缺乏症[7]、脊髓损伤[8]、黄斑变性[9]等相继涌现.

iPSCs在多种疾病研究上取得的成就为肝脏疾病提供了很好的借鉴, 多个国内外研究组[10-12]相继应用不同的方法诱导啮齿类和人的iPSCs分化为具有功能性的肝细胞样细胞. 其中作为iPSCs治疗肝脏遗传性疾病的代表, Espejel等[13]将野生型小鼠的iPSCs转染入延胡索酰乙酰乙酸水解酶(aumarylacetoacetate hydrolase, FAH)缺陷鼠(人酪氨酸血症1型模型), 证实iPSCs在体内可发展为正常肝细胞, 并且可以保护FAH缺陷鼠使其免于发展为肝功能衰竭. 但纵观iPSCs在肝脏疾病中的研究大都集中于肝细胞, 对于肝脏中另一重要的细胞类型-胆管上皮细胞(biliary epithelial cells, BECs), 国内外却少有报道, 而BECs对于胆管病研究的重要性毋庸置疑.

1 胆管病的范畴和胆管上皮细胞

胆管病(cholangiopathies)是一组以胆管上皮细胞为共同攻击靶标的慢性肝脏疾病的统称[14,15]. 包括原发性胆汁性肝硬化(primary biliary cirrhosis, PBC)[16]、原发性硬化性胆管炎(primary sclerosing cholangitis, PSC)[17]、胆道闭锁、多囊性肝病、胆管细胞癌等. 此组疾病由于进行性发展的特性和缺乏有效的治疗方法, 通常导致终末期肝病, 肝脏移植常为延长生存的唯一有效方法[18]. 据统计, 美国1988-2014间, 约16%的肝脏移植由胆管病所致. 而费用昂贵、供肝匮乏等限制肝脏移植的因素不易解除. 一种疾病有效治疗方法的缺乏多源于发病机制的不清. 就每种胆管病而言具有自身特异的表现及病程, 但此组胆管病的共性-BECs作为攻击靶标, 是胆管病发病机制中重要的环节.

既往研究已知BECs是被覆于肝内、外胆管的单层柱状上皮细胞, 约占肝细胞总数的3%-5%. 其形态和功能上具有多态性和复杂性, 在胆汁加工及运输过程中起着重要的作用[19-21]. 近年来在胆管病研究[15]中揭示BECs作为疾病所攻击的靶标, 是发病机制中重要的组成部分. 胆管细胞通过与内、外源性刺激的相互作用参与肝损伤和修复[22]. 多种致病因素导致的胆管细胞反应过程, 以促炎症

反应因子表达增加[如白介素-6(interleukin-6, IL-6)、IL-8、肿瘤坏死因子、多种生长因子]和细胞信号通路的激活(如Notch、Hedgehog)等为特征[14]. 所释放的分子以自分泌或旁分泌方式影响胆管细胞的增殖、凋亡和衰老并可导致局部血管新生、纤维化、募集免疫细胞、间质细胞和内皮细胞. 这些与胆管细胞相关的活动所致的胆道反应中出现伴随白细胞和淋巴细胞浸润的小胆管数量的增加、肝祖细胞的激活、基质蛋白含量的增加. 除非得以逆转, 上述活动可导致门脉纤维化、胆管缺失直至最终胆汁性肝硬化[23]. 但同时此类反应也可防护胆管进一步损伤和有助于胆管树损伤的修复. 除此之外, 基因变异、表观遗传学机制和转录后调控也可影响参与反应的胆管细胞进展为胆管病或回归到正常表型[24].

尽管具有生理及病理功能上的重要性, 但由于BECs有限的数量和肝内的解剖位置限制了研究其分子功能的体外细胞模型的发展[25]. 目前, 仅有的少数胆管细胞系多来自胆管癌或被SV40病毒永生化的正常细胞[26], 且大多数用于体外研究的胆管细胞来源于啮齿类[27,28]. 因此, 获得疾病源性的胆管上皮细胞并深入研究其生物学特性, 对揭示胆管病的发病机制及其诊治大有裨益.

2 诱导人iPSCs向胆管细胞分化体系的建立

iPSCs具有与ESCs相似的分化潜能, 啮齿类和人iPSCs经诱导可分化为具有功能性的肝细胞样细胞[29]. 肝细胞与胆管上皮细胞在胚胎发育时期共同来源于肝母细胞[30], 因此在对肝内外胆管发育理解的基础上以及借鉴iPSCs向肝细胞分化的经验[31], 研究者探讨了诱导人iPSCs向胆管细胞分化的策略, 以期建立有效的细胞分化体系.

2014年Dianat等[32]实验组建立了一种向胆管细胞诱导分化的方法, 应用该体系成功诱导人ESCs和iPSCs获得功能性的胆管细胞样细胞(cholangiocyte-like cells, CLCs). 研究者采用无滋养细胞层和特定的培养条件, 首先诱导ESCs和iPSCs分化为肝祖细胞后, 再应用生长激素、表皮生长因子、IL-6和牛磺胆酸钠进一步分化为胆管细胞. 诱导后的CLCs表达胆系细胞标识如细胞角蛋白7(cytokeratin 7, CK7)、骨桥蛋白、转录因子SOX9和肝细胞核因子6, 并且表达承担胆管细胞功能所需的特殊蛋白, 如囊性纤维化跨膜转运调节因子(cystic fibrosis transmembrane conductance regulator, CFTR)、促胰液素受体和核受体, 形成初级纤毛以及可通过增加细胞内Ca^{2+}对激素刺激做出反应. 在三维(three dismensional, 3D)培养基质中, 细胞形成具有表皮/顶面-底侧极性和功能性囊泡及胆管. 以上结果均提示该体系诱导后的CLCs与正常肝脏胆管细胞具有结构和功能上的相似性.

我实验组[33]同期也探讨了诱导人iPSCs向胆管细胞分化的方法. 应用Yamanaka因子和仙台病毒(sendai virus, SeV)载体, 体外重编程人皮肤成纤维细胞为iPSCs, 并模拟胆管上皮细胞发育过程, 采用一些关键的形态发生素(morphogen)诱导iPSCs向成熟的胆管细胞分化. SeV是单负链的RNA病毒. 其作为病毒载体的优势主要在于表达外源基因高效且可调控, 生活周期完全在胞质中进行, 无DNA相, 无整合风险, 安全性高, 故被定义为高效的基因转导工具[34]. 使用SeV载体获取的非整合性iPSCs安全性得以提高. 诱导后获得的iPSCs源性胆管细胞(iPSC-derived cholangiocytes), 称之为iDCs, 在基因和蛋白水平高表达CK7、19等胆系细胞标志, 电镜下观察细胞表面具有纤毛, 3D培养中形成胆管样结构. iDCs具有的胆系细胞的标志揭示了设计的逐步式、无饲养层细胞、无血清诱导分化体系的可行性.

iPSCs向胆管细胞诱导分化体系的建立以及所获得的CLCs可用于体外研究胆管发育的分子机制, 作为种子细胞应用于生物人工肝、组织工程化胆管等领域, 可成为治疗终末期胆病新的细胞来源, 具有潜在的临床应用价值.

3 应用iPSCs技术构建患者特异性的疾病模型

使用特定患者的iPSCs既可重演单基因遗传性疾病表型也可重演迟发性多基因遗传性疾病的表型[35,36], 如帕金森氏病[37]、阿尔茨海默氏病[38]. 用这些细胞来分析发病机制和作为药物筛选平台研究新的治疗方法, 已经成为研究的兴奋点及趋势[39]. 以PSC、PBC等为代表的一组胆管病发病机制不清、有效治疗手段匮乏、预后差. 重编程技术的掌握及建立的可行的胆管细胞诱导分化体系, 可保障应用iPSCs技术构建胆管病患者特异性的疾病模型, 诱导分化后的胆管细胞具有相应疾病表型的属性,

创新盘点

本文系统阐述了胆管病的范畴和胆管上皮细胞在胆管病发病机制中的重要作用, 以及诱导人iPSCs向胆管细胞分化体系的建立和应用iPSCs技术构建患者特异性的疾病模型等研究进展.

应用要点
应用iPSCs技术构建胆管病患者特异性疾病模型和诱导分化后的胆管细胞具有相应疾病表型的属性, 可用于研究疾病的病理及发病机制、新药筛选和疗效评估等.

可用于研究疾病的病理及发病机制、新药筛选和疗效评估等领域.

近来, Sampaziotis等[40]探讨了上述研究的可行性. 该实验组首先针对既往诱导iPSCs向胆管细胞分化中存在的低效、功能不全等问题[41,42], 模拟更接近生理胆管发育的诱导体系以提高效率. 此体系强调FGF10、activin-TGF-β对早期胆管特异性形成的重要性以及维甲酸的作用[43,44]. 研究结果显示经此体系诱导获得的CLCs在转录和功能水平均更接近于真正的胆管细胞, 可用于发育、疾病模型、疗效验证和药物筛选等方面的研究.

之后研究者应用多囊性肝病、囊性纤维化相关胆管病患者来源iPSCs建立疾病模型, 并利用此细胞模型评估药物疗效. 如研究所述, 常染色体隐性遗传性疾病CF是由于*CFTR*基因突变所致[45], 为体外模拟该疾病, 研究者将从野生型和具有最常见纯和突变CF患者皮肤成纤维细胞获得的hiPSCs诱导分化为CLCs, CF-hiPSC来源的CLCs表达胆管上皮细胞标志和具有功能特征. 结果显示野生型来源的CLCs可根据培养基相应调整胞内氯化物的浓度, 而CF-CLCs无此变化, 从而证实该细胞CFTR功能的缺失. CF实验性药物VX809具有稳定CFTR和提高功能的作用[46]. 将该药物与CF-CLCs孵育48 h后应用氯离子荧光探针检测观察到CFTR功能的改善. 上述研究结果表明疾病源性iPSCs获得的CLCs体系适于评估药效, 尤其是对于缺乏高通量药物筛选平台的胆管病.

4 问题及展望

iPSCs技术的创立和由此衍生的研究方法为多种疾病诊疗提供了新的契机, 具有广阔的临床应用前景, 但仍有较多亟待解决的问题. 例如安全性, iPSCs诱导过程中, 重编程因子的种类、剂量以及培养条件, 对于产生的iPSCs表观遗传状态和多能性潜力的差异有着重要影响. 早期使用的病毒转染体系易引发致癌基因的激活, 具有致瘤风险. 在近年来对如何提高重编程安全性及效率问题的研究中, 重编程因子更安全的传递方法有附着体载体[47], mRNA转染[48], 以及重组蛋白传导[49]等. iPSCs技术另一种研究趋势是将一种体细胞“直接重编程”为另一种细胞, 直接重编程简单、快速. 此方面我国研究者[50]将鼠成纤维细胞直接重编程为肝细胞取得的突破, 可为其他种类细胞提供借鉴. 再有, 如何提高定向诱导分化效率等问题. 现有的分化体系所产生的CLCs纯度、质量和功能差别较大, 尤其在功能上和真正的BECs还存在一定的差距. 对此, 深入对胆管发育的认识将会更好的体外动态模拟分化体系.

5 结论

iPSCs技术的创立被认为是生命科学及再生医学研究领域中的革命, 应用此技术探索解决影响国计民生的重大疾病具有广阔的需求. 以PSC、PBC等为代表的一组胆管病虽非常见疾病, 但危害严重, 大多数患者最终发展为终末期肝病. 因此发展胆管病患者源性的、个体化的iPSCs及其诱导分化的功能细胞可在体外模拟胆管疾病的表型及病理过程, 应用此细胞模型对研究发病机制、药物筛选和疗效评估等具有重要意义.

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同行评价

本文对iPSCs在胆管病研究中的应用作了较为全面的综合分析与论述，提炼和阐述了该领域的问题和研究方向，并提出了未来的研究方向与临床应用，具有较好的学术价值.

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