

## 干细胞及类器官在特发性肺纤维化修复和再生中的应用

左 鹤, 刘 哲, 梅倩茹, 杨振华\*, 屈 静\*

(华中科技大学同济基础医学院, 湖北 武汉 430030)

**摘要:** 特发性肺纤维化 (idiopathic pulmonary fibrosis, IPF) 是一种不可逆转的、死亡率较高的间质性疾病, 发病率在全世界范围内呈逐渐增长趋势, 严重危害人类的健康, 给社会带来巨大的经济负担。目前, 传统的治疗方法虽然能够减缓疾病的进展, 但是无法有效控制病情。临床上两种针对 IPF 的药物吡非尼酮和尼达尼布也存在较多的不良反应。因此, 探求让纤维化逆转和肺再生的新方法显得至关重要。干细胞的修复和再生能力对于 IPF 的治疗具有独特的优势。近年来, 由干细胞生成的肺类器官的出现让纤维化肺再生的研究进入了一个全新的阶段, 其结构和功能类似于活体器官, 与亲代细胞具有相似的遗传特性, 在研究肺组织的发育过程以及药物实验方面发挥了巨大作用, 尤其是在再生医学方面, 为研究者提供了一个良好的体外模型。本综述主要对目前干细胞及类器官在 IPF 修复和再生中的作用进行总结, 希望能为临床治疗 IPF 提供参考。

**关键词:** 特发性肺纤维化; 修复; 再生; 类器官; 干细胞

中图分类号: R966 文献标识码: A 文章编号: 0513-4870(2021)11-2900-08

## Application of stem cells and organoids in repair and regeneration of idiopathic pulmonary fibrosis

ZUO He, LIU Zhe, MEI Qian-ru, YANG Zhen-hua\*, QU Jing\*

(School of Basic Medicine, Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430030, China)

**Abstract:** Idiopathic pulmonary fibrosis (IPF) is an irreversible and highly mortal interstitial disease. The incidence of IPF is increasing around the world, which seriously harms human health and brings huge economic burden to the society. While traditional treatments can slow the progression of the disease, they are far to cure this disease. Clinically, pirfenidone and nintedanib are two main drugs that used for the treatment of IPF. However, severe adverse reactions were reported in some patients. Therefore, it is very important to explore novel therapeutic strategies to reverse fibrosis and regenerate lung. The repair and regeneration ability of stem cells has unique advantages in the treatment of pulmonary fibrosis. The structure and function of organoids produced by stem cells have similar characteristics with live organs. Therefore, lung stem cells play an important role in the discovery of novel anti-IPF drugs, and in the formation and development of lung tissue. In addition, organoids produced by stem cells also serve as a perfect model for regenerative medicine. In this review, we mainly summarize the role of stem cells and organoids in the repair and regeneration of pulmonary fibrosis, and hope to provide a reference for the development of clinical treatment of pulmonary fibrosis.

**Key words:** idiopathic pulmonary fibrosis; repair; regeneration; organoid; stem cell

收稿日期: 2021-06-09; 修回日期: 2021-07-05.

基金项目: 国家自然科学基金资助项目 (81900067).

\*通讯作者 Tel: 13545148826, E-mail: jingqu@hust.edu.cn;

Tel: 18008365026, E-mail: zhenhua@hust.edu.cn

DOI: 10.16438/j.0513-4870.2021-0828

特发性肺纤维化 (idiopathic pulmonary fibrosis, IPF) 是一种严重的致命性肺间质纤维化疾病, 造成不可逆转的、渐进性肺部瘢痕, 最终导致呼吸衰竭和死亡。确诊后, 大约一半的患者 3~5 年生存率仅为 20%~30%<sup>[1]</sup>。IPF 疾病进展不可预测, 一部分患者在最初几年内病

情稳定,然而一旦发生急性加重,症状会在数周或数天内恶化,这是IPF患者死亡的首要原因<sup>[2,3]</sup>。目前全世界大约有500多万患者,并呈逐年增长的趋势。该病主要在50岁后确诊,随着人口日益老龄化,预计在不久的将来会有更高的患病率和发病率,给家庭和社会造成巨大的经济负担<sup>[4]</sup>。

IPF的特征是肺泡上皮受损,肺组织的正常结构被破坏,肺间质中细胞外基质(extracellular matrix, ECM)过度沉积以及成纤维细胞的增殖和侵袭能力增强<sup>[5,6]</sup>。ECM过度沉积被认为是IPF的标志。随着ECM的增厚,肺组织将失去促进气体交换并为细胞提供氧气的功能。在病理过程中,功能性肺泡数量减少,肺泡区室逐渐被纤维组织替代,II型肺泡上皮细胞(type II alveolar epithelial cells, AEC2)增殖并缓慢分化为I型肺泡上皮细胞(type I alveolar epithelial cells, AEC1),修复受损的肺泡。如果该过程失调,可能会导致促纤维化因子、细胞因子、趋化因子的释放,多种炎症介质和免疫细胞浸润,促进成纤维细胞和肌成纤维细胞的活性,这些肌成纤维细胞不仅能表达 $\alpha$ -平滑肌动蛋白(alpha smooth muscle actin,  $\alpha$ -SMA),还会造成ECM的过度沉积从而导致IPF<sup>[7-10]</sup>。

IPF确切的病理生理学机制至今尚未阐明,临床治疗药物极少,常见的糖皮质激素、免疫调节剂等传统药物治疗效果不理想<sup>[11]</sup>。目前,两种针对IPF的药物吡非尼酮和尼达尼布,虽然能减缓疾病进展,但也产生较多的不良反应。IPF唯一可以治愈的方法是肺移植,但是由于供体器官供应不足和异体排斥的限制,肺移植仅适用于极少数患者,并且移植后患者的平均生存期也只有4~5年<sup>[12]</sup>。因此,开发新的治疗方法刻不容缓。近年来,持续的肺泡上皮微损伤被认为是IPF发生的关键因素<sup>[13]</sup>。AEC2是肺泡干细胞,具有增殖和分化的能力,在稳态期间或损伤后更新AEC1,维持肺泡的完整和功能,在肺的修复和再生中发挥重要作用<sup>[14]</sup>。利用AEC2肺泡干细胞修复潜力逆转肺部病理性重塑是治疗IPF的一种潜在策略。因此,深入了解肺泡干细胞功能及调节机制至关重要。目前,干细胞衍生的类器官可以用来模拟器官的发育和疾病,在基础研究、药物开发和再生医学中有广泛的应用。培养肺类器官不仅有助于IPF的病理生理机制研究,也有助于药物筛选和器官移植研究,在具有重要科学意义的同时,还具有重大的临床价值。

## 1 肺泡干细胞在IPF中的功能和机制

**1.1 肺泡干细胞** AEC2是一种异质性群体,在肺泡中具有重要的分泌和再生作用,是肺泡再生的原始干细胞<sup>[15]</sup>。在胚胎发育期间,AEC1和AEC2来自双能祖

细胞,而在出生后AEC1来自成熟的AEC2,当肺组织受到损伤时,AEC2的干细胞能力被充分激活,分化为AEC1<sup>[16]</sup>。小鼠的谱系追踪实验表明,由表面活性蛋白C(surfactant protein C, SPC)表达决定的AEC2能够长期自我更新和多潜能分化产生AEC1,而且SPC阳性(SPC<sup>+</sup>)的AEC2细胞亚群在体内表现出比AEC2细胞更强的克隆潜能<sup>[15]</sup>。当AEC肺泡干细胞不能再生正常的肺泡上皮并产生足够的表面活性蛋白时,肺泡上皮将面临机械张力增加的继发性损伤,这会加剧上皮细胞的枯竭;并在AEC2中触发促纤维化的转化因子 $\beta$ 信号通路,这种激活可以促进纤维化形成<sup>[17,18]</sup>,也可以抑制AEC2向AEC1的转分化,扰乱肺再生过程,从而形成恶性循环<sup>[19]</sup>。

**1.2 肺泡干细胞在IPF中的功能和作用** IPF发生的病理性纤维化是一个涉及上皮细胞、成纤维细胞、免疫细胞(巨噬细胞、T细胞)和内皮细胞之间复杂相互作用的动态过程<sup>[20]</sup>。AEC2作为肺泡上皮的兼性祖细胞,在肺组织受到损伤后,AEC2可以分化为AEC1<sup>[21]</sup>。当AEC2受到持续损伤时,不仅会导致这些兼性祖细胞的耗尽,而且还会导致这些重要细胞执行修复功能的能力发生不可逆转的改变。纤维化肺中功能失调的AEC2也会产生促纤维化因子,进一步导致纤维化<sup>[22]</sup>。将原代AEC2细胞移植到博莱霉素诱导的IPF小鼠模型中,观察到AEC2对于逆转IPF有一定的作用<sup>[23]</sup>。在构建IPF疾病的小鼠模型中,向小鼠气管内滴注博莱霉素会导致AEC2的丢失,此时存活的AEC2会增殖分化为AEC1以补充肺泡上皮<sup>[24]</sup>。如果肺泡上皮细胞不完全修复,则会导致肺泡纤维化闭塞,这与肺泡干细胞的修复能力密切相关<sup>[25,26]</sup>。AEC2对于维持肺内环境稳态及受损肺组织的修复具有重大意义<sup>[27]</sup>。

AEC2的功能障碍在IPF发病过程中起重要作用,在IPF患者中,AEC2在总肺泡上皮细胞中的百分比显著下降<sup>[26]</sup>。AEC2的衰老会引起其修复能力降低,导致纤维增生。基因组分析发现了几个与IPF相关的遗传变异,如端粒酶逆转录酶(telomerase reverse transcriptase, TERT)、端粒酶RNA组分(telomerase RNA component, TERC)和端粒延伸解旋酶调节因子1(regulator telomere extension helicase 1, RTEL1),与AEC2的过早衰老有关<sup>[28]</sup>。有研究表明,内质网(endoplasmic reticulum, ER)应激是促进AEC2衰老凋亡的重要因素<sup>[29]</sup>,可能通过诱导细胞衰老来削弱AEC2的修复能力<sup>[30,31]</sup>。端粒酶的功能障碍是引起细胞衰老的主要原因,吸烟作为IPF的一个危险因素,一方面通过引起剂量依赖性的端粒缩短导致AEC2衰老<sup>[32,33]</sup>,另一方面可诱导DNA损伤反应来损害AEC2的功能<sup>[34]</sup>。衰老上

皮细胞的分泌体,包括白细胞介素(interleukin, IL)-1 $\beta$ 、IL-6和IL-8,都会促进成纤维细胞分化为肌成纤维细胞并抵抗凋亡,从而导致IPF<sup>[35,36]</sup>。如何降低AEC2功能损伤对于AEC2维持自身的数量和向AEC1的分化再生都有重要作用。

**1.3 肺泡干细胞调控IPF修复的分子机制** 在肺损伤过程中,AEC1很容易受到损伤而死亡,此时AEC2会充当祖细胞,增殖产生新的AEC2,然后分化成AEC1。YAP/TAZ是Hippo通路中重要的转录共激活因子,最近有研究表明,YAP/TAZ在肺泡修复过程中发挥重要作用<sup>[37]</sup>。TAZ直接参与了肺泡上皮损伤时AEC2向AEC1分化的过程。AEC2特异性YAP/TAZ基因敲除小鼠肺再生过程中表现出AEC2增殖和AEC2向AEC1分化的异常,甚至出现了纤维化病变。在博莱霉素诱导的肺损伤模型中,AEC2中TAZ的条件性缺失显著降低了AEC2向AEC1分化的能力,导致肺泡损伤和纤维化加剧<sup>[38]</sup>。另一项研究显示,在肺切除代偿性肺生长模型中,YAP信号在AEC2中被激活,AEC2特异性缺失YAP抑制了AEC2的增殖以及向AEC1的分化<sup>[39]</sup>。在动态平衡期间,抑制YAP也会降低AEC2的增殖<sup>[40]</sup>。

成纤维细胞生长因子(fibroblast growth factor, FGF)信号转导参与了IPF的发病机制。FGF家族有18个分泌配体组成,它们与4个不同的信号受体(fibroblast growth factor receptor, FGFR)结合<sup>[41]</sup>。FGFR对肺的发育至关重要<sup>[42]</sup>,AEC2中FGFR2b的失活导致AEC2几乎全部死亡<sup>[43]</sup>。在气道上皮修复的过程中,FGF信号对于分化再生有重要作用。FGF2在博莱霉素诱导的急性肺损伤后SPC<sup>+</sup>的AEC2恢复中起关键作用,FGF2过表达或气管内滴注FGF2可以减轻博莱霉素诱导的纤维化<sup>[44]</sup>。在博莱霉素引起的肺损伤中,FGF10促进分泌细胞直接分化为AEC2或去分化为基底细胞,并进一步分化为AEC2<sup>[43]</sup>。最近的研究表明,FGF10可预防多发性应激所致的肺损伤,并促进肺上皮细胞再生<sup>[45-49]</sup>。

**1.4 干细胞在IPF治疗中的应用** 组织特异性干细胞在正常情况下处于静止状态,当组织受到损伤时开始增殖分化<sup>[50,51]</sup>。当肺组织受到损伤时,少数表达基底细胞限制性转录因子P63和角蛋白5(keratin 5, KRT5)的远端气道干细胞(distal airway stem cells, DASC)被激活,肺组织表现出再生能力<sup>[52-55]</sup>。有研究发现,在IPF肺远端可见KRT5<sup>+</sup>细胞,而在健康的肺中则难以发现这些细胞<sup>[56]</sup>。将小鼠气道远端干细胞(mouse distal airway stem cells, MDASC)移植到纤维化小鼠肺中,在21天左右分化为肺泡样结构,在40天

左右发现表达AEC1和AEC2的标志物,50天左右分化为AEC2<sup>[53,54]</sup>。可以发现,MDASC可以增殖分化为AEC1和AEC2,参与肺损伤后的修复和再生。羟脯氨酸是小鼠肺纤维化病变中胶原蛋白合成的关键氨基酸,与正常小鼠相比,纤维化病变肺组织随着时间的推移表现出羟脯氨酸水平明显升高,用MDASC治疗后显著降低了肺组织中羟脯氨酸的含量,说明MDASC对于纤维化肺的修复再生有重要作用<sup>[57,58]</sup>。

肺球细胞来源的肺干细胞(lung spheroid cell, LSC)是包含肺上皮细胞和间充质细胞的异质细胞群体。研究发现,LSC分泌蛋白可通过减少胶原蛋白的积累和成纤维细胞的增殖来恢复肺泡结构,减轻博莱霉素引起的纤维化,对于肺修复具有治疗潜力<sup>[59]</sup>。此外,将人脐带间充质干细胞(human umbilical cord mesenchymal stem cells, HUMSC)移植入博莱霉素诱导肺纤维化大鼠中,发现HUMSC增强了巨噬细胞基质金属蛋白酶9(macrophage matrix metalloproteinase-9, MMP-9)表达以降解胶原蛋白,促进肺部Toll样受体4(Toll-like receptor-4, TLR-4)表达以促进肺泡再生,有效逆转纤维化肺<sup>[60]</sup>。

## 2 类器官在纤维化肺修复和再生中的意义

**2.1 类器官** 类器官是一种复杂的三维结构,是细胞在体外增殖分化形成的克隆性产物,其结构和功能类似于活体器官,与亲代细胞具有相似的生物学特性,由干细胞或器官特异性前体细胞通过自组织过程发展而来<sup>[61,62]</sup>。类器官可以用来模拟器官的发育和疾病,在基础研究、药物开发和再生医学中有广泛的应用。与单一细胞类型的传统细胞培养相比,类器官培养能够在器官水平上模拟病理过程。此外类器官还可以用来创建类器官库,从而建立强大的筛查平台。如建立胃肠道癌类器官库来比较患者对药物的临床反应和体外反应;建立膀胱癌类器官库来比较各自亲代肿瘤的组织病理学和分子特征<sup>[63,64]</sup>。

类器官体外培养与传统的细胞体外培养有很大不同,不仅要考虑培养环境的物理特征,还要考虑对内源性信号和外源性信号的要求以及起始细胞的类型。在某些情况下,外源信号只对初始细胞类型的诱导是必要的,然后这些细胞依赖于系统的自主信号来完成自组织步骤。如人多能干细胞(human pluripotent stem cells, HPSC)必须在特定生长因子的刺激下才能形成输尿管上皮和肾间充质细胞的混合体,随后这些细胞自组织成肾类器官而不需要加入更多的生长因子<sup>[65,66]</sup>。肺类器官可以通过自组织过程从干细胞或器官特异性前体细胞中衍生,既可以模拟肺的发育过程,也可以在体外模拟肺的三维组织结构和功能<sup>[61,67-69]</sup>。基质胶含

有复杂的ECM,为类器官体外培养提供支持框架,促进细胞的分化和生长,肠、大脑、胃和乳腺已经在基质胶条件下成功产生类器官<sup>[70-73]</sup>。基质胶已经成为用于3D培养肺类器官广泛应用的产品<sup>[15,67,74]</sup>。

类器官作为移植组织和功能细胞的来源,在再生医学细胞治疗中具有重要作用。用小鼠的胚胎干细胞培养成类器官,从中提取视网膜片移植到视网膜退化的小鼠模型中,移植的组织长出了成熟的光感受器,甚至可以建立突触连接,恢复光响应<sup>[75,76]</sup>。用小鼠结肠上皮干细胞培养成的类器官移植到小鼠体内,能够不同程度地再生受损的结肠黏膜<sup>[77]</sup>。有关肝脏和肾脏类器官在动物模型上移植也有相关的报道<sup>[78-80]</sup>。肺类器官为基底细胞的黏液纤毛上皮再生提供了体外模型,可以用来研究其在体内的再生机制,筛选可以调节细胞可塑性和谱系的药物、小分子途径以及关键上皮细胞的功能<sup>[81]</sup>。

**2.2 类器官在IPF中的应用** 尽管IPF的治疗方法在不断地涌现,但是由于缺乏一个理想的IPF模型,确定其病理生理学机制非常困难。类器官的出现让研究人员看到了希望。到目前为止,从成体组织、胚胎干细胞(embryonic stem cells, ESC)、诱导多能干细胞(induced pluripotent stem cells, iPSC)和肺芽尖中提取培养的肺类器官已有不同程度的报道。

目前,已经有许多方法能够将ESC分化为功能性肺泡细胞<sup>[82]</sup>。有研究人员把内胚层细胞扩增为FOXA2<sup>+</sup>NKX2.1<sup>+</sup>祖细胞,这种祖细胞在体内外均可分化为AEC1和AEC2<sup>[83]</sup>。通过刺激iPSC形成NKX2.1<sup>+</sup>的前内胚层细胞,从中分离出表达羧肽酶M(carboxypeptidase M, CPM)的细胞,辅以肺泡相关生长因子和人类肺成纤维细胞,可以产生肺泡球体。这些球体中含有表达NKX2.1和CPM的细胞,以及表达水通道蛋白5(aquaporin 5, AQP5)和SPC的分化细胞,它们分别是AEC1和AEC2的标志物<sup>[84-86]</sup>。在球体生长过程中,刺激HH(Hedgehog)通路,可以增强NKX2.1的表达,在含有FGF10的培养基中扩大球体生长成更复杂的结构,持续培养可以形成有组织的近端气道样上皮管,含有许多在天然气道上皮中发现的细胞类型,包括基底细胞、纤毛细胞和杆状细胞,这些细胞被SMA表达的间质组织包围,维持早期的双能肺泡祖细胞<sup>[86,87]</sup>。

2015年,首次报道了由HPSC产生的肺类器官,它的结构特征与天然肺类似,由近端气道上皮、远端肺泡上皮和间充质谱系组成的细胞类型组成<sup>[74]</sup>。有研究用成人原代支气管上皮细胞、肺成纤维细胞和肺微血管内皮细胞结合在一起,在3D培养条件下产生气道类器官<sup>[88]</sup>。此外,从HPSC生成3D前肠前部球体,将前肠

球体植入基质胶中,并在高水平FGF10和1%的胎牛血清(fetal bovine serum, FBS)中培养,就可以产生人肺类器官,形成被间充质细胞群包围的类气道结构和一些表达AEC1和AEC2标志物的细胞,这些类器官的转录图谱与胎肺高度相似<sup>[74,89,90]</sup>。

人胚肺上皮尖是多能祖细胞,可以在体外扩增为长期自我更新的有机体<sup>[91]</sup>。但成人来源的肺祖细胞还没有类似的进展,有待于进一步的研究<sup>[88]</sup>。有研究用原代小鼠肺基质细胞和肺泡干细胞成功培养出类器官,而中国仓鼠肺成纤维细胞系(Chinese hamster lung fibroblast line, CCL39)和肺泡干细胞则没有成功培养出类器官<sup>[92]</sup>。之所以产生这种差异是因为细胞的分泌特性对类器官的培养至关重要,CCL39不能培养成功可能是关键性生长因子分泌不足所致,当把肺基质细胞换成高浓度FGF10,肺泡干细胞可以形成能力较低的一类器官,HPSC来源的AEC2不需要成纤维细胞就可以形成3D肺泡球,这说明肺泡类器官的发育需要特定的生长因子<sup>[68,93]</sup>。取自小鼠和人的肺的原代基底细胞自组织成的类器官称为气管球或支气管球<sup>[85]</sup>。用实验试剂(如萘和SO<sub>2</sub>气体)处理小鼠肺或用病毒感染后,基底细胞的行为会发生变化,迅速再生上皮并恢复屏障功能。到现在为止,肺泡球的形成一般需要某种支持细胞,且要求上皮细胞与间充质细胞紧密结合<sup>[92]</sup>。常见支持细胞有PDGFRA<sup>+</sup>成纤维细胞、脂成纤维细胞、小鼠肺成纤维细胞系和肺内皮细胞等<sup>[15,94,95]</sup>。

有研究用小鼠AEC2培养出了类器官<sup>[96]</sup>,关于人类的AEC2已经有报道用人类诱导多能干细胞(human induced pluripotent stem cells, hiPSC)来源的AEC2进行类器官培养<sup>[68,90,97]</sup>。内源性人AEC2可以与糖原合成酶激酶-3(glycogen synthase kinase-3 beta, GSK-3 $\beta$ )、TGF- $\beta$ 和骨形态发生蛋白4(bone morphogenetic protein 4, BMP4)抑制剂以及Notch和FGF7配体一起培养和传代,并产生两个AEC2细胞系。利用该培养体系,将突变的表面活性蛋白C(SPC<sup>exon4</sup>)导入AEC2中,表现出与IPF患者上皮细胞相似的转录特征<sup>[98]</sup>。SPC突变常见于家族性肺纤维化,了解SPC突变是如何导致肺纤维化对于抗纤维化治疗是必要的<sup>[99]</sup>。有研究用重组工程的方法,选择性将表达NH<sub>2</sub>末端HA标记的小鼠SPC<sup>173T</sup>的编码序列转入内源性小鼠SPC基因座,培育了一只突变的SPC(SPC<sup>173T</sup>)小鼠。在成年小鼠中SPC<sup>173T</sup>的表达增加,导致弥漫性实质肺损伤的增加,并进一步导致IPF的发展<sup>[100]</sup>。突变SPC的AEC2显示出与表面活性物质动态平衡相关的基因表达下调。当基因谱系标记的小鼠AEC2用于肺泡球的培养时,所形成的3D结构内部包含高级糖基化终产物特异

性受体 (advanced glycosylation end product-specific receptor, Ager<sup>+</sup>)、足蛋白 (podoplanin, Pdpn<sup>+</sup>)、Hopx<sup>+</sup>的 AEC1 细胞, 外部有 SPC<sup>+</sup>的 AEC2 细胞<sup>[14]</sup>。还有研究发现, 小鼠基底细胞和人的基底细胞已经可以在体外无基质的条件下培养出克隆球, 并且具备自我更新能力, 基底细胞被鉴定为小鼠和人的干细胞, 有整合素  $\alpha 6$  和神经因子生长受体两个细胞表面标记<sup>[101]</sup>。

目前, 对 IPF 新疗法的临床评估仍依赖于动物模型, 由于其和人类肺部的生物学差异, 无法概括人类 IPF 的进行性和顽固性, 阻碍了药物的研发<sup>[102]</sup>。采用患者来源的肺泡干细胞, 通过培养产生 AEC2, 再将其培养成类器官, 有可能克服这些障碍。类器官培养的成功让研究者不仅能够更清楚地研究肺泡干细胞与肺内其他结构细胞之间的相互作用, 而且为了解人类远端肺疾病的发病机制提供了独特的平台, 为纤维化肺的修复和再生过程的研究提供了良好的模型。

### 3 讨论

临床上对于 IPF 的治疗仍缺乏有效的方法, 现有药物治疗只能延缓其进展, 有关纤维化肺修复和再生的研究还停留在实验室阶段, 基于器官再生的治疗方法还有很大的改进空间。AEC2 作为肺泡干细胞, 在纤维化肺修复和再生中具有重要作用, 但其中涉及的具体的分子机制还有待于进一步的研究。此外, 通过干细胞移植、抑制体内的相关信号通路等其他方式, 可以抑制成纤维细胞的活化和增殖、促进细胞外基质降解以及促进肺泡再生, 这些方法都为临床 IPF 的治疗提供了一种思路。

类器官能够模拟真实器官的 3D 结构、细胞类型和某些特定的功能, 提供有重建器官能力的细胞以及保护移植器官免受恶劣的病理环境影响, 可作为研究器官发育和再生的模型。与动物模型相比, 类器官更有利于深入地了解器官发生过程。类器官在多种疾病治疗中有一定的优势, 并且在未来再生医学中的应用也具有较好的前景。目前, 虽然不能完整地复制肺泡细胞功能和体内细胞的相互作用, 但类器官却是研究肺泡细胞行为的特定属性和分子特征的有力工具。此外, 类器官模型能够创建患者特定的类器官, 对于家族性肺纤维化和个性化用药有很大的价值。然而, 类器官也存在许多局限性, 一是分化为成熟组织受限, 不能发育为成熟的器官; 二是由于营养供应的限制导致的非血管化; 三是基质成分的限制, 如免疫成分阻碍了类器官在模拟炎症和药物渗透研究中的使用<sup>[103]</sup>。但是, 通过更好地了解 ECM 成分和活细胞成像技术, 可能突破这些限制, 期望不久的将来类器官技术能广泛地应用于临床。

随着人们对肺类器官的研究深入, 为探索 IPF 具体的病理生理机制提供了良好的模型, 类器官在纤维化肺再生研究中将会发挥更大的作用。尽管在理解纤维化肺再生机制上有很大困难, 但随着生物技术和生物信息学的快速发展有望改变对这些机制的理解, 探索纤维化肺修复和再生的有效方法, 对临床治疗产生更加深远的影响。

**作者贡献:** 左鹤负责初期文献检索与整理以及论文初稿的撰写; 刘哲与梅倩茹对本文后期文献补充提供帮助; 屈静和杨振华负责本文的思路设计、修改以及提出指导性意见; 全体作者都阅读并同意最终的版本。

**利益冲突:** 本论文所有作者声明无任何利益冲突。

### References

- [1] Nalysnyk L, Cid-Ruzafa J, Rotella P, et al. Incidence and prevalence of idiopathic pulmonary fibrosis: review of the literature [J]. *Eur Respir Rev*, 2012, 21: 355-361.
- [2] Weng D, Chen XQ, Qiu H, et al. The role of infection in acute exacerbation of idiopathic pulmonary fibrosis [J]. *Eur Respir J*, 2019, 2019: 5160694.
- [3] Gui X, Qiu X, Xie M, et al. Prognostic value of serum osteopontin in acute exacerbation of idiopathic pulmonary fibrosis [J]. *Biomed Res Int*, 2020, 2020: 3424208.
- [4] Richeldi L, Rubin AS, Avdeev S, et al. Idiopathic pulmonary fibrosis in BRIC countries: the cases of Brazil, Russia, India, and China [J]. *BMC Med*, 2015, 13: 237.
- [5] Qu J, Yang SZ, Zhu Y, et al. Targeting mechanosensitive MDM4 promotes lung fibrosis resolution in aged mice [J]. *J Exp Med*, 2021, 218: e20202033.
- [6] Chen H, Qu J, Huang X, et al. Mechanosensing by the alpha6-integrin confers an invasive fibroblast phenotype and mediates lung fibrosis [J]. *Nat Commun*, 2016, 7: 12564.
- [7] Wynn TA. Integrating mechanisms of pulmonary fibrosis [J]. *J Exp Med*, 2011, 208: 1339-1350.
- [8] Wolters PJ, Collard HR, Jones KD. Pathogenesis of idiopathic pulmonary fibrosis [J]. *Annu Rev Pathol*, 2014, 9: 157-179.
- [9] Noble PW, Barkauskas CE, Jiang D. Pulmonary fibrosis: patterns and perpetrators [J]. *J Clin Invest*, 2012, 122: 2756-2762.
- [10] Kasper M, Barth K. Potential contribution of alveolar epithelial type I cells to pulmonary fibrosis [J]. *Biosci Rep*, 2017, 37: BSR20171301.
- [11] Behr J. The diagnosis and treatment of idiopathic pulmonary fibrosis [J]. *Dtsch Arztebl Int*, 2013, 110: 875-881.
- [12] Raghu G, Collard HR, Egan JJ, et al. An official ATS/ERS/JRS/ALAT statement: idiopathic pulmonary fibrosis: evidence-based guidelines for diagnosis and management [J]. *Am J Respir Crit Care Med*, 2011, 183: 788-824.
- [13] Joannes A, Brayer S, Besnard V, et al. FGF9 and FGF18 in

- idiopathic pulmonary fibrosis promote survival and migration and inhibit myofibroblast differentiation of human lung fibroblasts *in vitro* [J]. *Am J Physiol Lung Cell Mol Physiol*, 2016, 310: L615-L629.
- [14] Jain R, Barkauskas CE, Takeda N, et al. Plasticity of Hopx(+) type I alveolar cells to regenerate type II cells in the lung [J]. *Nat Commun*, 2015, 6: 6727.
- [15] Barkauskas CE, Crouse MJ, Rackley CR, et al. Type 2 alveolar cells are stem cells in adult lung [J]. *J Clin Invest*, 2013, 123: 3025-3036.
- [16] Desai TJ, Brownfield DG, Krasnow MA. Alveolar progenitor and stem cells in lung development, renewal and cancer [J]. *Nature*, 2014, 507: 190-194.
- [17] Cabrera-Benitez NE, Parotto M, Post M, et al. Mechanical stress induces lung fibrosis by epithelial-mesenchymal transition [J]. *Crit Care Med*, 2012, 40: 510-517.
- [18] Wu H, Yu Y, Huang H, et al. Progressive pulmonary fibrosis is caused by elevated mechanical tension on alveolar stem cells [J]. *Cell*, 2020, 180: 107-121.e17.
- [19] Riemondy KA, Jansing NL, Jiang P, et al. Single cell RNA sequencing identifies TGF-beta as a key regenerative cue following LPS-induced lung injury [J]. *JCI Insight*, 2019, 5: e123637.
- [20] Barkauskas CE, Noble PW. Cellular mechanisms of tissue fibrosis. 7. New insights into the cellular mechanisms of pulmonary fibrosis [J]. *Am J Physiol Cell Physiol*, 2014, 306: C987-C996.
- [21] Johansson HM, Newman DR, Sannes PL. Whole-genome analysis of temporal gene expression during early transdifferentiation of human lung alveolar epithelial type 2 cells *in vitro* [J]. *PLoS One*, 2014, 9: e93413.
- [22] Selman M, Pardo A. The leading role of epithelial cells in the pathogenesis of idiopathic pulmonary fibrosis [J]. *Cell Signal*, 2020, 66: 109482.
- [23] Serrano-Mollar A, Nacher M, Gay-Jordi G, et al. Intratracheal transplantation of alveolar type II cells reverses bleomycin-induced lung fibrosis [J]. *Am J Respir Crit Care Med*, 2007, 176: 1261-1268.
- [24] Zhang Z, Newton K, Kummerfeld SK, et al. Transcription factor Etv5 is essential for the maintenance of alveolar type II cells [J]. *Proc Natl Acad Sci U S A*, 2017, 114: 3903-3908.
- [25] Lederer DJ, Martinez FJ. Idiopathic pulmonary fibrosis [J]. *N Engl J Med*, 2018, 378: 1811-1823.
- [26] Xu Y, Mizuno T, Sridharan A, et al. Single-cell RNA sequencing identifies diverse roles of epithelial cells in idiopathic pulmonary fibrosis [J]. *JCI Insight*, 2016, 1: e90558.
- [27] Qu J, Zhu L, Zhou Z, et al. Reversing mechanoinductive DSP expression by CRISPR/dCas9-mediated epigenome editing [J]. *Am J Respir Crit Care Med*, 2018, 198: 599-609.
- [28] Moore C, Blumhagen RZ, Yang IV, et al. Resequencing study confirms that host defense and cell senescence gene variants contribute to the risk of idiopathic pulmonary fibrosis [J]. *Am J Respir Crit Care Med*, 2019, 200: 199-208.
- [29] Kropski JA, Blackwell TS. Endoplasmic reticulum stress in the pathogenesis of fibrotic disease [J]. *J Clin Invest*, 2018, 128: 64-73.
- [30] Borok Z, Horie M, Flodby P, et al. Grp78 loss in epithelial progenitors reveals an age-linked role for endoplasmic reticulum stress in pulmonary fibrosis [J]. *Am J Respir Crit Care Med*, 2020, 201: 198-211.
- [31] Chilosi M, Carloni A, Rossi A, et al. Premature lung aging and cellular senescence in the pathogenesis of idiopathic pulmonary fibrosis and COPD/emphysema [J]. *Transl Res*, 2013, 162: 156-173.
- [32] Arish N, Petukhov D, Wallach-Dayana SB. The role of telomerase and telomeres in interstitial lung diseases: from molecules to clinical implications [J]. *Int J Mol Sci*, 2019, 20: 2996.
- [33] Morla M, Busquets X, Pons J, et al. Telomere shortening in smokers with and without COPD [J]. *Eur Respir J*, 2006, 27: 525-528.
- [34] Sheng G, Chen P, Wei Y, et al. Viral infection increases the risk of idiopathic pulmonary fibrosis: a meta-analysis [J]. *Chest*, 2020, 157: 1175-1187.
- [35] Tian Y, Li H, Qiu T, et al. Loss of PTEN induces lung fibrosis *via* alveolar epithelial cell senescence depending on NF-kappaB activation [J]. *Aging Cell*, 2019, 18: e12858.
- [36] Chen X, Xu H, Hou J, et al. Epithelial cell senescence induces pulmonary fibrosis through nanomediated fibroblast activation [J]. *Aging Cell*, 2019, 12: 242-259.
- [37] Lacanna R, Liccardo D, Zhang P, et al. Yap/Taz regulate alveolar regeneration and resolution of lung inflammation [J]. *J Clin Invest*, 2019, 129: 2107-2122.
- [38] Sun T, Huang Z, Zhang H, et al. TAZ is required for lung alveolar epithelial cell differentiation after injury [J]. *JCI Insight*, 2019, 5: e128674.
- [39] Liu Z, Wu H, Jiang K, et al. MAPK-mediated YAP activation controls mechanical-tension-induced pulmonary alveolar regeneration [J]. *Cell Rep*, 2016, 16: 1810-1819.
- [40] Zhou B, Flodby P, Luo J, et al. Claudin-18-mediated YAP activity regulates lung stem and progenitor cell homeostasis and tumorigenesis [J]. *J Clin Invest*, 2018, 128: 970-984.
- [41] Itoh N, Ornitz DM. Fibroblast growth factors: from molecular evolution to roles in development, metabolism and disease [J]. *J Biochem*, 2011, 149: 121-130.
- [42] Moura RS, Coutinho-Borges JP, Pacheco AP, et al. FGF signaling pathway in the developing chick lung: expression and inhibition studies [J]. *PLoS One*, 2011, 6: e17660.
- [43] Yuan T, Volckaert T, Redente EF, et al. FGF10-FGFR2B signaling generates basal cells and drives alveolar epithelial regeneration by bronchial epithelial stem cells after lung injury [J]. *Stem Cell*

- Reports, 2019, 12: 1041-1055.
- [44] Guzy RD, Stoilov I, Elton TJ, et al. Fibroblast growth factor 2 is required for epithelial recovery, but not for pulmonary fibrosis, in response to bleomycin [J]. *Am J Respir Cell Mol Biol*, 2015, 52: 116-128.
- [45] Quantius J, Schmoldt C, Vazquez-Armendariz AI, et al. Influenza virus infects epithelial stem/progenitor cells of the distal lung: impact on fgfr2b-driven epithelial repair [J]. *PLoS Pathog*, 2016, 12: e1005544.
- [46] Tong L, Bi J, Zhu X, et al. Keratinocyte growth factor-2 is protective in lipopolysaccharide-induced acute lung injury in rats [J]. *Respir Physiol Neurobiol*, 2014, 201: 7-14.
- [47] Bi J, Tong L, Zhu X, et al. Keratinocyte growth factor-2 intratracheal instillation significantly attenuates ventilator-induced lung injury in rats [J]. *J Cell Mol Med*, 2014, 18: 1226-1235.
- [48] Fang X, Wang L, Shi L, et al. Protective effects of keratinocyte growth factor-2 on ischemia-reperfusion-induced lung injury in rats [J]. *Am J Respir Cell Mol Biol*, 2014, 50: 1156-1165.
- [49] Ringelhan M, O'connor T, Protzer U, et al. The direct and indirect roles of HBV in liver cancer: prospective markers for HCC screening and potential therapeutic targets [J]. *J Pathol*, 2015, 235: 355-367.
- [50] Kolios G, Moodley Y. Introduction to stem cells and regenerative medicine [J]. *Respiration*, 2013, 85: 3-10.
- [51] Leeman KT, Fillmore CM, Kim CF. Lung stem and progenitor cells in tissue homeostasis and disease [J]. *Curr Top Dev Biol*, 2014, 107: 207-233.
- [52] Kumar PA, Hu Y, Yamamoto Y, et al. Distal airway stem cells yield alveoli *in vitro* and during lung regeneration following H1N1 influenza infection [J]. *Cell*, 2011, 147: 525-538.
- [53] Vaughan AE, Brumwell AN, Xi Y, et al. Lineage-negative progenitors mobilize to regenerate lung epithelium after major injury [J]. *Nature*, 2015, 517: 621-625.
- [54] Zuo W, Zhang T, Wu DZ, et al. p63(+)/Krt5(+) distal airway stem cells are essential for lung regeneration [J]. *Nature*, 2015, 517: 616-620.
- [55] Yang Y, Riccio P, Schotsaert M, et al. Spatial-temporal lineage restrictions of embryonic p63(+) progenitors establish distinct stem cell pools in adult airways [J]. *Dev Cell*, 2018, 44: 752-761.e4.
- [56] Shi Y, Dong M, Zhou Y, et al. Distal airway stem cells ameliorate bleomycin-induced pulmonary fibrosis in mice [J]. *Stem Cell Res Ther*, 2019, 10: 161.
- [57] Zhao L, Wang X, Chang Q, et al. Neferine, a bisbenzylisoquinoline alkaloid attenuates bleomycin-induced pulmonary fibrosis [J]. *Eur J Pharmacol*, 2010, 627: 304-312.
- [58] Arizmendi N, Puttagunta L, Chung KL, et al. Rac2 is involved in bleomycin-induced lung inflammation leading to pulmonary fibrosis [J]. *BioMed Central*, 2014, 15: 71.
- [59] Dinh PC, Paudel D, Brochu H, et al. Inhalation of lung spheroid cell secretome and exosomes promotes lung repair in pulmonary fibrosis [J]. *Nat Commun*, 2020, 11: 1064.
- [60] Chu KA, Wang SY, Yeh CC, et al. Reversal of bleomycin-induced rat pulmonary fibrosis by a xenograft of human umbilical mesenchymal stem cells from Wharton's jelly [J]. *Theranostics*, 2019, 9: 6646-6664.
- [61] Clevers H. Modeling development and disease with organoids [J]. *Cell*, 2016, 165: 1586-1597.
- [62] Lancaster MA, Knoblich JA. Organogenesis in a dish: modeling development and disease using organoid technologies [J]. *Science*, 2014, 345: 1247125.
- [63] Vlachogiannis G, Hedayat S, Vatsiou A, et al. Patient-derived organoids model treatment response of metastatic gastrointestinal cancers [J]. *Science*, 2018, 359: 920-926.
- [64] Lee SH, Hu W, Matulay JT, et al. Tumor evolution and drug response in patient-derived organoid models of bladder cancer [J]. *Cell*, 2018, 173: 515-528.e17.
- [65] Takasato M, Little MH. A strategy for generating kidney organoids: recapitulating the development in human pluripotent stem cells [J]. *Dev Biol*, 2016, 420: 210-220.
- [66] Takasato M, Er PX, Becroft M, et al. Directing human embryonic stem cell differentiation towards a renal lineage generates a self-organizing kidney [J]. *Nat Cell Biol*, 2014, 16: 118-126.
- [67] Mccauley KB, Hawkins F, Serra M, et al. Efficient derivation of functional human airway epithelium from pluripotent stem cells *via* temporal regulation of wnt signaling [J]. *Cell Stem Cell*, 2017, 20: 844-857.e6.
- [68] Jacob A, Morley M, Hawkins F, et al. Differentiation of human pluripotent stem cells into functional lung alveolar epithelial cells [J]. *Cell Stem Cell*, 2017, 21: 472-488.e10.
- [69] Chen YW, Huang SX, De Carvalho A, et al. A three-dimensional model of human lung development and disease from pluripotent stem cells [J]. *Nat Cell Biol*, 2017, 19: 542-549.
- [70] Sato T, Vries RG, Snippert HJ, et al. Single Lgr5 stem cells build crypt-villus structures *in vitro* without a mesenchymal niche [J]. *Nature*, 2009, 459: 262-265.
- [71] Simian M, Hirai Y, Navre M. The interplay of matrix metalloproteinases, morphogens and growth factors is necessary for branching of mammary epithelial cells [J]. *Development*, 2001, 128: 3117-3131.
- [72] Lancaster MA, Renner M, Martin CA, et al. Cerebral organoids model human brain development and microcephaly [J]. *Nature*, 2013, 501: 373-379.
- [73] Mccracken KW, Cata EM, Crawford CM, et al. Modelling human development and disease in pluripotent stem-cell-derived gastric organoids [J]. *Nature*, 2014, 516: 400-404.
- [74] Dye BR, Hill DR, Ferguson MA, et al. *In vitro* generation of human pluripotent stem cell derived lung organoids [J]. *Elife*, 2015, 4: e05098.
- [75] Assawachananont J, Mandai M, Okamoto S, et al. Transplanta-

- tion of embryonic and induced pluripotent stem cell-derived 3D retinal sheets into retinal degenerative mice [J]. *Stem Cell Reports*, 2014, 2: 662-674.
- [76] Mandai M, Fujii M, Hashiguchi T, et al. iPSC-derived retina transplants improve vision in rd1 end-stage retinal-degeneration mice [J]. *Stem Cell Reports*, 2017, 8: 69-83.
- [77] Yui S, Nakamura T, Sato T, et al. Functional engraftment of colon epithelium expanded *in vitro* from a single adult Lgr5<sup>+</sup> stem cell [J]. *Nat Med*, 2012, 18: 618-623.
- [78] Huch M, Dorrell C, Boj SF, et al. *In vitro* expansion of single Lgr5<sup>+</sup> liver stem cells induced by Wnt-driven regeneration [J]. *Nature*, 2013, 494: 247-250.
- [79] Taguchi A, Kaku Y, Ohmori T, et al. Redefining the *in vivo* origin of metanephric nephron progenitors enables generation of complex kidney structures from pluripotent stem cells [J]. *Cell Stem Cell*, 2014, 14: 53-67.
- [80] Huch M, Gehart H, Van Boxtel R, et al. Long-term culture of genome-stable bipotent stem cells from adult human liver [J]. *Cell*, 2015, 160: 299-312.
- [81] Barkauskas CE, Chung MI, Fioret B, et al. Lung organoids: current uses and future promise [J]. *Development*, 2017, 144: 986-997.
- [82] Longmire TA, Ikonomidou L, Hawkins F, et al. Efficient derivation of purified lung and thyroid progenitors from embryonic stem cells [J]. *Cell Stem Cell*, 2012, 10: 398-411.
- [83] Huang SX, Islam MN, O'neil J, et al. Efficient generation of lung and airway epithelial cells from human pluripotent stem cells [J]. *Nat Biotechnol*, 2014, 32: 84-91.
- [84] Vazin T, Schaffer DV. Engineering strategies to emulate the stem cell niche [J]. *Trends Biotechnol*, 2010, 28: 117-124.
- [85] Vaughan MB, Ramirez RD, Wright WE, et al. A three-dimensional model of differentiation of immortalized human bronchial epithelial cells [J]. *Differentiation*, 2006, 74: 141-148.
- [86] Green MD, Chen A, Nostro MC, et al. Generation of anterior foregut endoderm from human embryonic and induced pluripotent stem cells [J]. *Nat Biotechnol*, 2011, 29: 267-272.
- [87] Van Haute L, De Block G, Liebaers I, et al. Generation of lung epithelial-like tissue from human embryonic stem cells [J]. *Respir Res*, 2009, 10: 105.
- [88] Tan Q, Choi KM, Sicard D, et al. Human airway organoid engineering as a step toward lung regeneration and disease modeling [J]. *Biomaterials*, 2017, 113: 118-132.
- [89] Dye BR, Dedhia PH, Miller AJ, et al. A bioengineered niche promotes *in vivo* engraftment and maturation of pluripotent stem cell derived human lung organoids [J]. *Elife*, 2016, 5: e19732.
- [90] Miller AJ, Dye BR, Ferrer-Torres D, et al. Generation of lung organoids from human pluripotent stem cells *in vitro* [J]. *Nat Protoc*, 2019, 14: 518-540.
- [91] Nikolic MZ, Caritg O, Jeng Q, et al. Human embryonic lung epithelial tips are multipotent progenitors that can be expanded *in vitro* as long-term self-renewing organoids [J]. *Elife*, 2017, 6: e26575.
- [92] Mcqualter JL, Yuen K, Williams B, et al. Evidence of an epithelial stem/progenitor cell hierarchy in the adult mouse lung [J]. *Proc Natl Acad Sci U S A*, 2010, 107: 1414-1419.
- [93] Tanaka Y, Yamaguchi M, Hirai S, et al. Characterization of distal airway stem-like cells expressing N-terminally truncated p63 and thyroid transcription factor-1 in the human lung [J]. *Exp Cell Res*, 2018, 372: 141-149.
- [94] Chen H, Matsumoto K, Brockway BL, et al. Airway epithelial progenitors are region specific and show differential responses to bleomycin-induced lung injury [J]. *Stem Cells*, 2012, 30: 1948-1960.
- [95] Lee JH, Bhang DH, Beede A, et al. Lung stem cell differentiation in mice directed by endothelial cells *via* a BMP4-NEFATc1-thrombospondin-1 axis [J]. *Cell*, 2014, 156: 440-455.
- [96] Shiraishi K, Shichino S, Ueha S, et al. Mesenchymal-epithelial interactome analysis reveals essential factors required for fibroblast-free alveolosphere formation [J]. *iScience*, 2019, 11: 318-333.
- [97] Yamamoto Y, Gotoh S, Korogi Y, et al. Long-term expansion of alveolar stem cells derived from human IPS cells in organoids [J]. *Nat Methods*, 2017, 14: 1097-1106.
- [98] Shiraishi K, Nakajima T, Shichino S, et al. *In vitro* expansion of endogenous human alveolar epithelial type II cells in fibroblast-free spheroid culture [J]. *Biochem Biophys Res Commun*, 2019, 515: 579-585.
- [99] Albert RK, Schwartz DA. Revealing the secrets of idiopathic pulmonary fibrosis [J]. *N Engl J Med*, 2019, 380: 94-96.
- [100] Nureki SI, Tomer Y, Venosa A, et al. Expression of mutant sftpc in murine alveolar epithelia drives spontaneous lung fibrosis [J]. *J Clin Invest*, 2018, 128: 4008-4024.
- [101] Rock JR, Onaitis MW, Rawlins EL, et al. Basal cells as stem cells of the mouse trachea and human airway epithelium [J]. *Proc Natl Acad Sci U S A*, 2009, 106: 12771-12775.
- [102] Carrington R, Jordan S, Pitchford SC, et al. Use of animal models in IPF research [J]. *Pulm Pharmacol Ther*, 2018, 51: 73-78.
- [103] Fatehullah A, Tan SH, Barker N. Organoids as an *in vitro* model of human development and disease [J]. *Nat Cell Biol*, 2016, 18: 246-254.