四川大学学报（医学版） 2025，56（2）：411－418
J Sichuan Univ（Med Sci）

在线全文

·论 著·

不同分化阶段人气管类器官呼吸道合胞病毒感染后差异性特征*

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【摘要】 **目的** 探讨不同分化阶段人气管类器官呼吸道合胞病毒（respiratory syncytial virus, RSV）感染后的病变程度和免疫应答差异性特征。**方法** 通过构建人胚胎肺类器官和气管类器官模型模拟未成熟和成熟的气道上皮，用免疫荧光染色、电镜和实时荧光定量聚合酶链式反应（real-time fluorescence quantitative PCR, Q-PCR）技术验证上述类器官模型构建成功；用RSV感染不同成熟度的人肺类器官，分别在感染6 h和48 h收集肺类器官样本，通过免疫荧光染色、微滴式数字化聚合酶链式反应（droplet digital PCR, DDPCR）和Q-PCR技术探究RSV感染不同成熟度肺类器官的免疫特点。**结果** 成功构建了性别决定相关基因簇2（sex determining region Y-box transcription factor 2, *SOX2*）和性别决定相关基因簇9（sex determining region Y-box transcription factor 9, *SOX9*）双阳性的胚胎肺类器官以及包含基底细胞、纤毛细胞、club细胞和杯状细胞的成熟气管类器官模型。同时，利用RSV建立了类器官RSV感染模型。DDPCR结果显示，在RSV感染初期气管类器官中的病毒载量高于胚胎肺类器官（$P<0.001$），但RSV感染48 h气管类器官中RSV病毒载量低于胚胎肺类器官（$P<0.05$）。Q-PCR结果显示，气管类器官中RSV感染受体基因表达量高于胚胎肺类器官，如表皮生长因子受体（epidermal growth factor receptor, *EGFR*）、胰岛素样生长因子1受体（insulin-like growth factor 1 receptor, *IGF1R*）、核仁素（nucleolin, *NCL*），差异均有统计学意义（$P<0.001$）。同时在RSV感染48 h后气管类器官中免疫因子表达量较胚胎肺类器官升高，如白细胞介素6（interleukin 6, *IL-6*）、白细胞介素8（interleukin 8, *CXCL8*）、干扰素α（interferon α, *IFN-α*）、粒细胞集落刺激因子（granulocyte colony-stimulating factor, *G-CSF*）、粒细胞-巨噬细胞集落刺激因子（granulocyte macrophage colony-stimulating factor, *GM-CSF*）、肿瘤坏死因子α（tumor necrosis factor α, *TNF-α*），差异均有统计学意义（$P<0.05$）。**结论** 气道发育成熟会增加病毒感染受体蛋白表达，成熟气道上皮细胞比未成熟状态具有更强的免疫应答，抑制RSV病毒复制。

【关键词】 呼吸道合胞病毒 类器官 抗病毒效应

Differential Characteristics of Human Airway Organoids at Different Stages of Differentiation After Respiratory Syncytial Virus Infection

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[**Abstract**] **Objective** To investigate the differences in pathological changes and immune responses of human airway organoids at different stages of differentiation following respiratory syncytial virus (RSV) infection. **Methods** Models of human fetal lung organoids (FLO) and induced airway organoids (iAO) were established to simulate immature and mature airway epithelium. Immunofluorescence staining, electron microscopy, and quantitative polymerase chain reaction (Q-PCR) were used to confirm the successful construction of the lung organoid models. Human lung organoids were infected with RSV, and samples were collected at 6 and 48 hours post-infection. The immune characteristics of immature and mature RSV-infected organoids were assessed using immunofluorescence staining, droplet digital PCR (DDPCR), and Q-PCR. **Results** We successfully generated FLO expressing both the progenitor markers sex determining region Y-box transcription factor 2 (*SOX2*) and sex determining region Y-box transcription factor 9 (*SOX9*), as well as iAO containing basal cells, ciliated cells, club cells, and goblet cells. In addition, organoid models of RSV infection were established. DDPCR results showed that, at the initial stage of RSV infection, the viral load in iAO was significantly higher than that in FLO ($P < 0.001$). However, at 48 hours post-infection, the viral load in iAO was lower than that in FLO ($P < 0.05$). Q-PCR results indicated that the expression of RSV infection receptor genes, including epidermal growth factor receptor (*EGFR*), insulin-like growth factor 1 receptor (*IGF1R*), and nucleolin (*NCL*),

* 国家自然科学基金（No. 82370001）、中央高校基本科研业务费专项（No. SCU2022D022）、四川省自然科学基金（No. 24NSFSC3457）、四川省科技厅重点研究项目（No. 2024YFFK0071）和四川省重大科技专项（No. 2022ZDZX0021）资助

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出版日期：2025-03-20

was significantly higher in iAO compared to that in FLO ($P < 0.001$). RSV infection led to an increase in the expression levels of immune factors, including interleukin 6 (*IL-6*), interleukin 8 (*CXCL8*), interferon α (*IFN-α*), granulocyte colony-stimulating factor (*G-CSF*), granulocyte-macrophage colony-stimulating factor (*GM-CSF*), and tumor necrosis factor α (*TNF-α*), in iAO compared to those in FLO, and the differences were statistically significant ($P < 0.05$). **Conclusion** The expression of RSV infection receptor proteins increases with airway maturation, and mature airway epithelial cells exhibit a stronger immune response than immature ones do, effectively inhibiting RSV replication.

[Key words] Respiratory syncytial virus Organoids Antiviral effect

呼吸道合胞病毒(respiratory syncytial virus, RSV)是全世界范围内引起婴幼儿下呼吸道感染的最常见病原体,往往引起喘息、呼吸困难等严重呼吸道症状[1-3]。目前针对RSV感染仍然缺乏特异性的治疗方案,极大限制婴幼儿患者的临床诊治[4-5]。由于婴幼儿免疫系统和气道上皮发育不成熟,早产、支气管肺发育不良等危险因素可导致RSV感染加重[6-7]。此外,婴幼儿时期严重的RSV感染与后期哮喘和其他形式的支气管疾病的发生息息相关[4,8]。因此探究RSV的致病特点以及人肺上皮损伤修复机制具有极大的临床意义。

细胞系和动物模型是研究RSV感染机制的常用模型[9-10]。然而细胞系并不能有效模拟人体复杂的器官结构,而常见的小鼠、斑马鱼等动物模型与人仍然存在极大的物种差异,部分研究结果仍然无法应用于人体[11]。源于人类肺上皮干细胞的类器官模型有效地解决了上述研究模型的缺陷,并且大量运用于新型冠状病毒的致病特征以及药物筛选研究,已成为呼吸道病毒感染的常用模型[12-14]。

本研究拟构建的以性别决定相关基因簇2(sex determining region Y-box transcription factor 2, SOX2)和性别决定相关基因簇9(sex determining region Y-box transcription factor 9, SOX9)双阳性的胚胎肺上皮祖细胞为主的胚胎肺类器官,经过关键信号通路的调控,诱导分化为成熟气管类器官。同时通过RSV感染不同成熟度肺类器官,评估病毒感染特点以及上皮损伤应答特征。

1 材料与方法

1.1 实验样本

本课题中所有涉及胚胎肺类器官构建的样本使用均已获得四川大学华西第二医院伦理委员会批准,项目批准号为U21A20333,所有操作均严格遵循赫尔辛基宣言相关要求,实验开展前均在充分告知后以书面形式取得患者本人的知情同意。

1.2 方法

1.2.1 人胚胎肺类器官的体外培养

取孕9周、12周和16周的流产胚胎肺(均来源于合并严重妊娠期并发症的胎儿,但不合并肺部畸形及其他肺部疾病相关原因),剥离胎肺尖端后,取其最外侧透光度较高区域,放入原代消化液中:400 U/mL Collagense Ⅰ(Sigma, C0130)、10 μmol/L Y27632(Cell Signaling Technology, 13624)、10 U/mL DNase(Sigma, D5025),在37 ℃摇床以100 r/min的转速消化30 min。消化成单个细胞后,种入基质胶中,在24孔板中加入自我更新培养基进行培养:Advanced DMEM/F12(Gibco, 12634010),1×GlutaMax(Gibco, 35050061),1 mmol/L HEPES(Gibco, 15630080),1×Penicillin/Streptomycin(Gibco, 15140122),1×B27 supplement(Gibco, 17504044),1.25 mmol/L n-Acetylcysteine, 5 mmol/L Nicotinamide, 50 ng/mL recombinant human EGF(Sino Biological, 10605-HNAE),100 ng/mL recombinant human Noggin(R&D, 6057-NG),100 ng/mL recombinant human FGF10(Sino Biologica, 10573-HNAE),100 ng/mL recombinant human FGF7(Sino Biological, 10210-H07E),500 ng/mL recombinent human R-spondin1(R&D, 4645-RS),3 μmol/L CHIR99021(Selleck, S1263)和500 nmol/L A8301(Cell Signaling Technology, 75073)。每4 d更换一次培养基,当类器官生长到直径200 μm时进行传代。

1.2.2 气管类器官的诱导分化

本研究通过调节转化生长因子β(transforming growth factor, TGFβ)、成纤维细胞生长因子(fibroblast growth factor, FGF)、骨形成蛋白(bone morphogenetic protein, BMP)、Wnt等肺近端分化的关键信号通路诱导胚胎肺类器官,能够使其分化为成熟气管类器官[15]。将胚胎肺类器官种在基质胶中用自我更新培养基增殖培养7 d,7 d后加入近端诱导培养基进行3 d的近端诱导:Advanced DMEM/F12, 1×GlutaMax, 1 mmol/L HEPES, 1×Penicillin/Streptomycin, 1×B27 supplement, 1.25 mmol/L n-Acetylcysteine, 5 mmol/L Nicotinamide, 100 ng/mL BMP4(R&D systerms, 314-BPE),100 ng/mL TGFβ(Peprotech, AF-100-21C-UG)。3 d后再加入气道分化培养基诱导14 d:Advanced DMEM/F12, 1×GlutaMax, 1 mmol/L HEPES, 1×Penicillin/Streptomycin, 1×B27 supplement, 1.25 mmol/L

n-Acetylcysteine, 5 mmol/L Nicotinamide, 100 ng/mL recombinant human Noggin, 500 ng/mL recombinant human FGF10, 500 nmol/L A8301, 10 μmol/L Y27632(Cell Signaling Technology, 13624)。期间每4 d更换一次培养基。

1.2.3　RSV感染模型的构建

病毒采用RSV A2株(ATCC, VR-1540)。类器官培养至直径200 μm时, 收集类器官, 200×*g*离心3 min, 去除上清液, 加入1 mL 1×TryLE(Gibco, A1217701)吹打混匀, 放入37 ℃培养箱中消化30 s, 30 s后使用适量类器官洗液: Advanced DMEM/F12, 1×GlutaMax, 1 mmol/L HEPES, 1×Penicillin/Streptomycin终止消化后再次离心去除上清液, 后再清洗两次; 提前配置病毒孵育液: 使用RSV病毒悬液(1×10^9 PFU/mL), 以1∶100稀释配置病毒孵育液(1×10^7 PFU/mL), 加入1 mL病毒孵育液重悬类器官沉淀后, 在37 ℃培养箱中孵育6 h, 去除多余的病毒孵育液, 清洗两次后种入基质胶中继续培养。分别于感染6 h和48 h收集样本, 进行后续验证实验。

1.2.4　实时荧光定量聚合酶链式反应(real-time fluorescence quantitative PCR, Q-PCR)分析

收集类器官, 200×*g*离心3 min, 用类器官洗液洗两次去除基质胶, 按照RNA提取试剂盒说明书(Qiagen, 4004)提取总RNA。再按照反转录试剂盒(Roche, 4897030001)说明书对提取的RNA进行反转录成cDNA, 然后将cDNA按照1∶10稀释进行Q-PCR, 详细引物序列如表1所示, 以甘油醛-3-磷酸脱氢酶(*GAPDH*)为内参基因, 采用$2^{-\Delta\Delta Ct}$法进行定量分析。引物合成公司为生工生物工程有限公司。

表 1　引物序列

Table 1　Primer sequences

Gene	Forward primer sequence (5'-3')	Reverse primer sequence (5'-3')
SOX2	ACATGAACGGCTGGAGCAA	GTAGGACATGCTGTAGGTGGG
SOX9	GGACCACCCGGATTACAAGT	AAGATGGCGTTGGGGGAGAT
KRT5	CTCAGTGGAGAAGGAGTTGGAC	ACTGCTACCTCCGGCAAG
FOXJ1	GGCATAAGCGCAAACAGCCG	TCGAAGATGGCCTCCCAGTCAAA
CC10	CCCCTCCTCCACCATGAAAC	AGGAGGGTTTCGATGACACG
MUC5AC	GCACCAACGACAGGAAGGATGAG	CACGTTCCAGAGCCGGACAT
IL-6	TTCGGTCCAGTTGCCTTCTC	CTGAGATGCCGTCGAGGATG
CXCL8	ACTCCAAACCTTTCCACCCC	TTCTCAGCCCTCTTCAAAAACT
EGFR	GCCTTGACTGAGGACAGCAT	AATCTGCCACTGTTTCCCCC
IGF1R	GGGGCTCTTGTTTACCAGCAT	TCTCCCGCCTCTCTCGAGTT
NCL	ACTCTGGTTCAGTTGGGCTG	TAGTTACAACCCTGGCTGGC
TNF-α	CACAGTGAAGTGCTGGCAAC	AGGAAGGCCTAAGGTCCACT
IFN-α	ACTCATACACCAGGTCACGC	CAGTGTAAAGGTGCACATGACG
G-CSF	TGGTGAGTGAGTGTGCCA	GGTAGAGGAAAAGGCCGCTA
GM-CSF	ACTTCCTGTGCAACCCAGATT	CTCATCTGGCCGGTCTCAC
GAPDH	GACTCATGACCACAGTCCATGC	AGAGGCAGGGATGATGTTCTG
RSVA-N-P	CACCATCCAACGGAGCACAGGAGAT	

SOX2: sex-determining region Y-box 2; *SOX9*: sex-determining region Y-box 9; *KRT5*: keratin 5; *FOXJ1*: forkhead box J1; *CC10*: Clara cell 10-kDa protein; *MUC5AC*: mucin 5AC; *IL-6*: interleukin 6; *CXCL8*: interleukin 8; *EGFR*: epidermal growth factor receptor; *IGF1R*: insulin-like growth factor 1 receptor; *NCL*: nucleolin; TNF-α: tumor necrosis factor α; *IFN-α*: interferon α; *G-CSF*: granulocyte colony-stimulating factor; GM-CSF: granulocyte macrophage colony-stimulating factor; *GAPDH*: glyceraldehyde-3-phosphate dehydrogenase; *RSV*: respiratory syncytial virus.

1.2.5　电镜

收集类器官, 加入2.5%戊二醇固定液(JSK, JS13111)固定类器官, 然后送里来公司进行电镜实验, 采用日本电子生产的JEM-1400FLASH透射电镜对铜网进行图像采集, 每张铜网先于6 000倍下观察, 选择要观察的区域采集图片, 观察具体细胞器结构。

1.2.6　免疫荧光染色

收集类器官, 加入1 mL 4%PFA(Beyotime, P0099)固定类器官, 放置于4 ℃冰箱过夜, 第二天用DPBS(Gibco, c14190500bt)洗两遍类器官, 然后使用3%琼脂糖包裹类器官, 4 ℃孵育30 min, 使用70%乙醇浸泡10 min, 后将其逐渐浸入不同浓度的乙醇溶液中脱水, 脱水后使用二甲苯将乙醇从组织中逐渐置换掉, 使组织逐步浸透至二甲苯中, 完成后利用石蜡包埋类器官, 放于4 ℃冰箱冷藏。第二天使用切片机切片后进行免疫荧光染色, 将切片依次进行脱蜡、抗原修复、5%胎牛血清室温封闭1 h后, 与一抗〔抗角蛋白5(keratin 5, KRT5)抗体(Abcam, ab52635)、抗Clara细胞10-kDa蛋白(Clara cell 10-kDa protein, CC10)

抗体(Santa Cruz, sc-365992)、抗ACE-TUBULIN抗体(Santa Cruz, sc-23950)、抗黏蛋白5AC(mucin 5AC, MUC5AC)抗体(Abcam, ab3649)、抗SOX2抗体(Santa Cruz, sc-365823)、抗SOX9抗体(Abcam, ab5535)、抗肺表面活性蛋白B(surfactant protein B, SFTPB)抗体(Santa Cruz, sc-133143)、抗肺表面活性蛋白C(surfactant protein C, SFTPC)抗体(Abcam, ab3786)]孵育4 ℃过夜,洗涤后与荧光二抗(Alexa Fluor 488, Alexa Fluor 594, Alexa Fluor 647;均1∶400)及三盐酸三苯基荧光素(Hoechst33342, 1∶800)孵育1 h,最后使用抗荧光淬灭剂进行封片,避光静置2 h后,使用奥林巴斯FV3000共聚焦显微镜采集图片。

1.2.7 微滴式数字化聚合酶链式反应(droplet digital PCR, DDPCR)

以逆转录的cDNA为模板,将DDPCR专用混合液(Bio-Rad, 1863023)、RSV探针、RSV引物、cDNA及无酶水配置成20 μL反应体系,加入DG8加样盒对应的孔中,加入70 μL微滴生成油在DG8加样盒对应的孔中,放入微滴生成仪生成微滴。然后将微滴转移至96孔板中进行聚合酶链式反应,完成将96孔板放入微滴读取仪中进行读取。最后将读取的结果按照公式$X\times10\times1\,000\times20/0.5=Y$(copies/ug RNA)计算RSV载量。

1.2.8 统计学方法

采用Graph Pad Prism 8软件进行数据分析和制图,符合正态分布的计量资料采用$\bar{x}\pm s$表示,组间比较采用独立样本t检验,多组间比较采用单因素方差分析,多组间两两比较采用LSD法,双侧检验,检验水准$\alpha=0.05$。

2 结果

2.1 假腺管期胚胎肺类器官构建

利用孕9周、12周和16周的胚胎肺尖端上皮细胞培养形成的胚胎肺类器官,主要表现为空心球状结构(图1A)。在体外可稳定传代至第11代,并保持一致的类器官形态以及干细胞基因表达(图1B)。免疫荧光染色中胎肺类器官细胞中可见假腺管期胎肺祖细胞标志物SOX2和SOX9表达,而气道上皮细胞标志物如KRT5、叉头框蛋白J1(forkhead box J1, FOXJ1)、CC10、MUC5AC以及肺泡上皮细胞标志物如 SFTPC、FTPB几乎不表达(图1C)。

2.2 成熟气管类器官分化诱导

Q-PCR结果显示,相比于胚胎肺类器官,诱导形成的成熟气管类器官中气道上皮细胞标志基因表达升高,差异均有统计学意义($P<0.05$)(图2A);免疫荧光染色结果

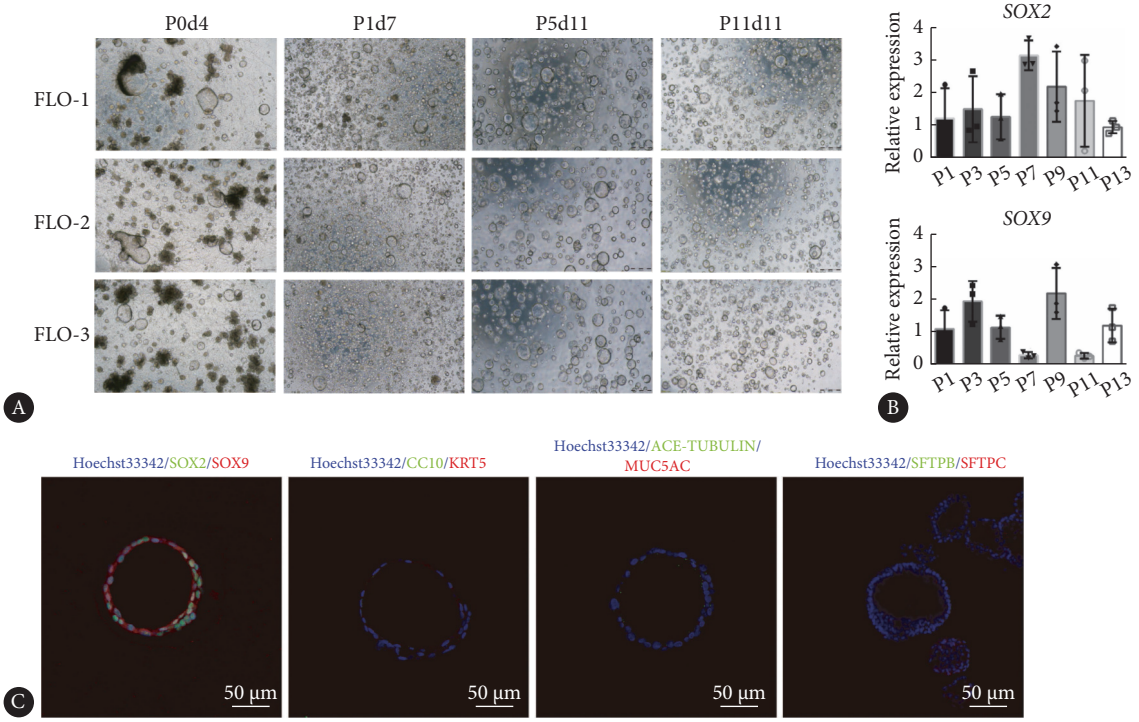

图 1 胚胎肺类器官自我更新维持培养

Fig 1 Self-renewal and maintenance culture of fetal lung organoids

FLO: fetal lung organoids; P: passage number of the organoids; d: days of culture for the current generation of organoids; FLO1: 9 pregnancy chronological week; FLO2: 12 pregnancy chronological week; FLO3: 16 pregnancy chronological week. A, Bright-field image of cultured FLO; B, Q-PCR results of FLO for fetal lung progenitor cell-specific gene expression, $n = 3$; C, immunofluorescence staining results of lung epithelial cell marker protein expression in FLO.

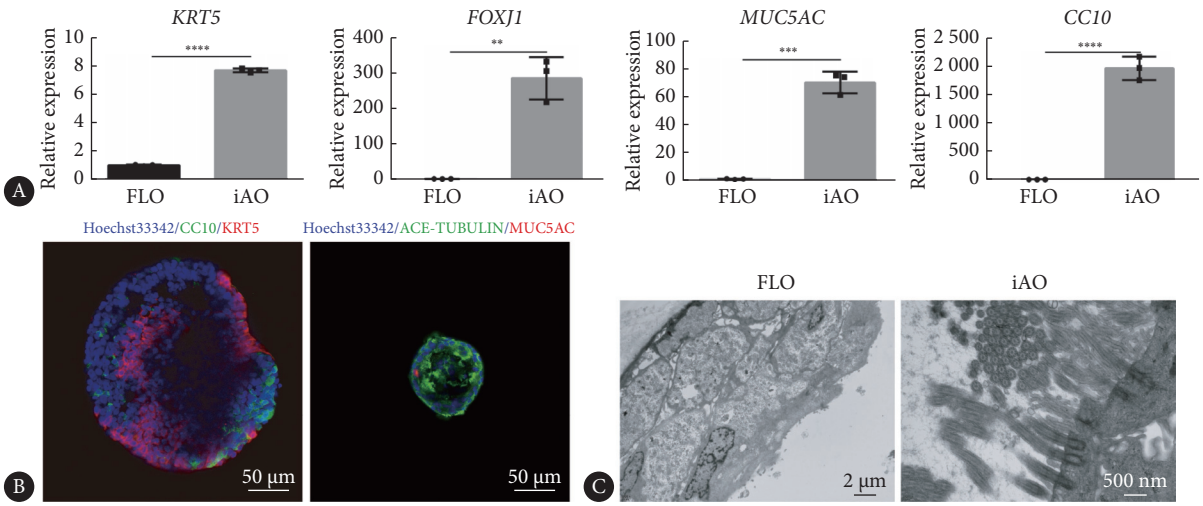

图 2 胚胎肺类器官分化为成熟气管类器官

Fig 2 Fetal lung organoids differentiate into mature airway organoids

FLO: fetal lung organoids; iAO: induced airway organoids. A, Q-PCR results of airway epithelial cell marker gene expression in FLO and airway organoids; $n = 3$, $^{**}P < 0.01$, $^{***}P < 0.001$, $^{****}P < 0.000\,1$; B, immunofluorescence staining results of airway epithelial cell marker protein expression in airway organoids; C, ultrastructural characteristics of FLO and iAO as observed by electron microscopy.

显示成熟气管类器官包含纤毛细胞、Club细胞、杯状细胞和基底细胞(图2B)；电镜下可见大量具有完整的“9+2”结构的纤毛分布在类器官内侧(图2C)。

2.3 不同分化阶段人气管类器官RSV感染特征

RSV感染后的类器官病毒载量结果显示，与感染6 h相比，感染48 h胚胎肺类器官和成熟气管类器官病毒基因组复制量均增加，差异均有统计学意义($P< 0.05$)(图3)。通过免疫荧光染色共定位，本研究发现纤毛细胞、Club细胞、杯状细胞、假腺管期胚胎肺祖细胞和基底细胞均能被RSV感染，且分析被感染细胞与未被感染细胞比例可见，相较于类器官中其他细胞，胚胎肺祖细胞被RSV感染的比例最高，其次是纤毛细胞，而基底细胞被感染的比例最低(图4)。

2.4 成熟气管类器官在RSV感染后存在更强的免疫应答

成熟气管类器官RSV感染48 h后的病毒载量低于胚胎肺类器官($P< 0.05$)，同时RSV感染6 h病毒载量高于胚

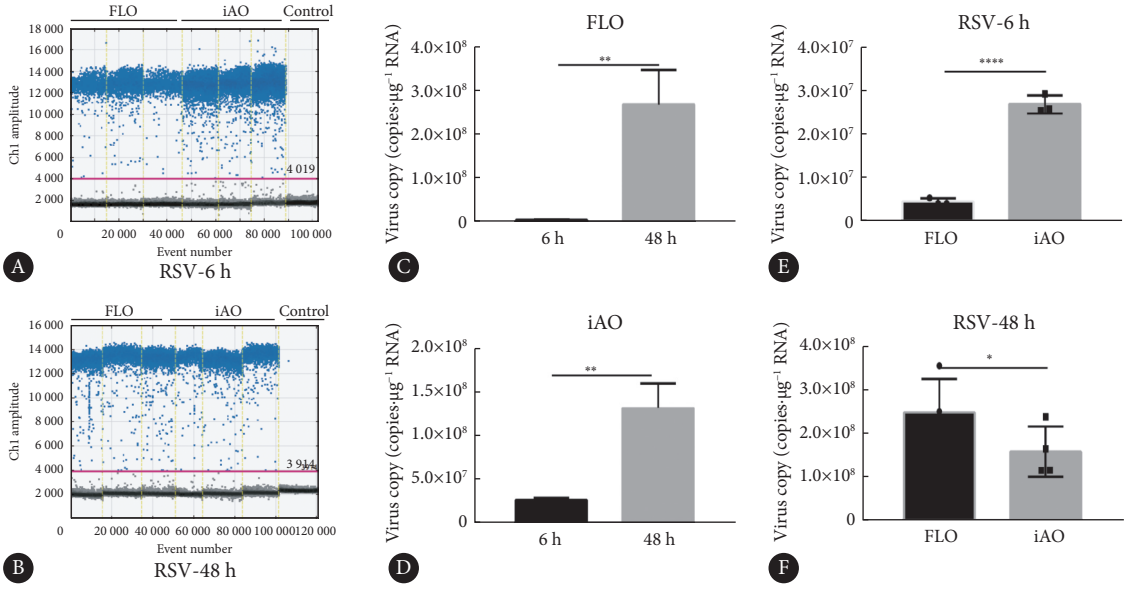

图 3 胚胎肺类器官和气管类器官RSV感染6 h和48 h后病毒载量

Fig 3 The viral load in FLO and airway organoids at 6 h and 48 h post-RSV infection

The abbreviations are explained in the note to Fig 2. A, DDPCR results for viral load in the organoids 6 h after RSV infection; B, DDPCR results for viral load in the organoids 48 h after RSV infection; C, the viral load in FLO; D, the viral load in iAO; E, the viral load at 6 h after RSV infection; F, the viral load at 48 h after RSV infection. $n = 3$, $^{*}P < 0.05$, $^{**}P < 0.01$, $^{****}P < 0.000\,1$.

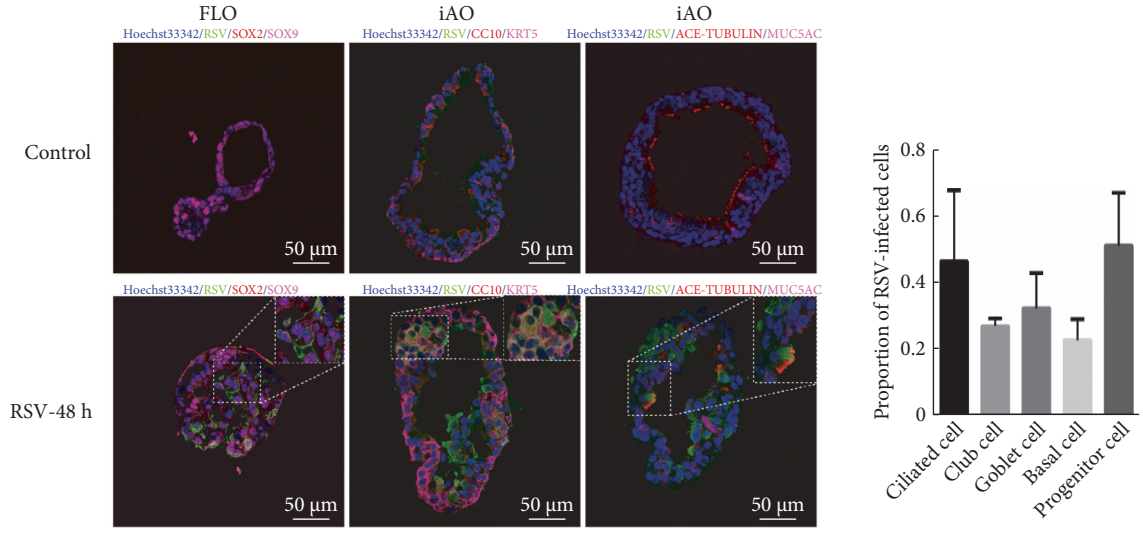

图 4 RSV感染后48 h胚胎肺类器官、气道类器官及未感染类器官中RSV免疫荧光染色共定位

Fig 4 Co-localization of RSV immunofluorescence staining in FLO and iAO at 48 hours post-RSV infection and in uninfected organoids

Control: uninfected organoids; RSV-48 h: organoids at 48 hours post-RSV infection; the other abbreviations are explained in the note to Fig 2.

胎肺类器官($P<0.000\,1$)(图3E、3F)。为了探讨RSV在成熟气管类器官中受到抑制的原因,本研究对RSV感染受体mRNA表达量的进行了检测,结果表明成熟气管类器官RSV感染受体基因如表皮生长因子受体(epidermal growth factor receptor, *EGFR*)、胰岛素样生长因子1受体(insulin-like growth factor 1 receptor, *IGF1R*)和核仁素(nucleolin, *NCL*)表达量高于胚胎肺类器官,差异均有统计学意义($P<0.000\,1$)(图5A)。进一步检测类器官中免疫

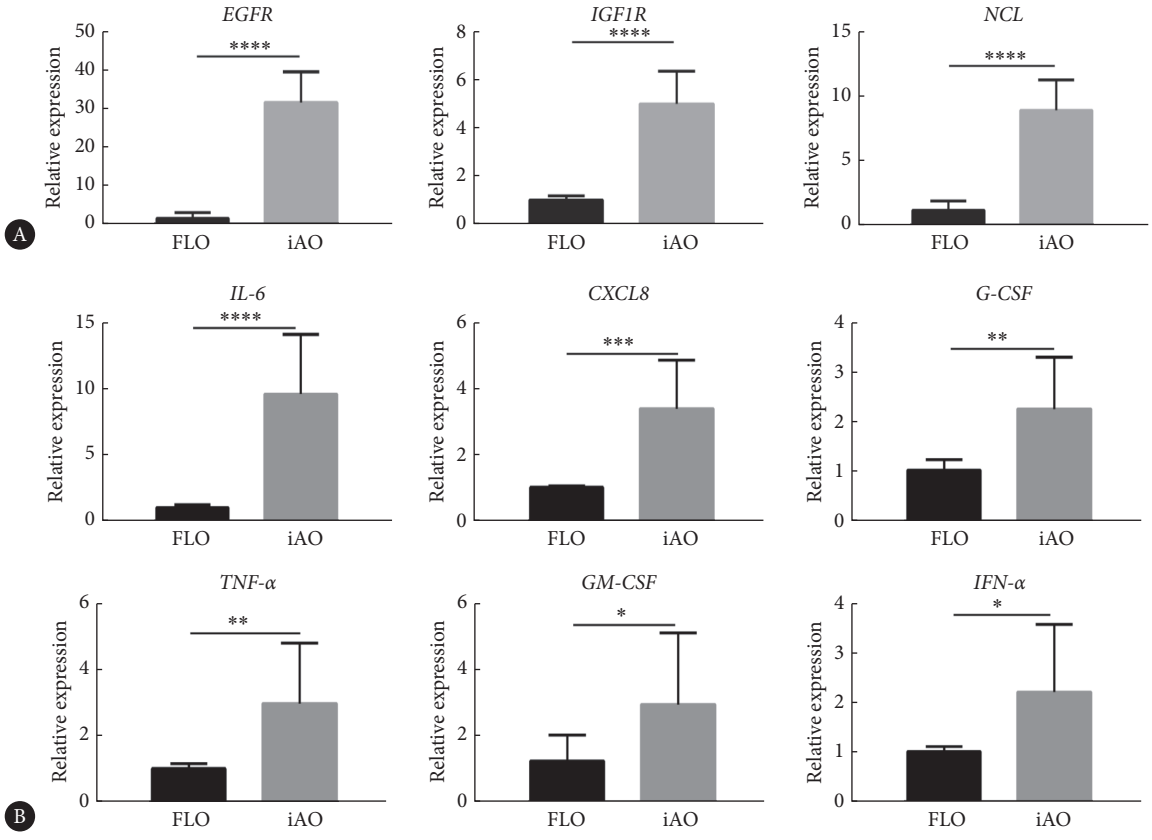

图 5 胚胎肺类器官和气管类器官中RSV感染相关基因表达Q-PCR结果

Fig 5 Q-PCR results of the expression of RSV infection-related genes in FLO and iAO

The abbreviations are explained in the note to Fig 2. A, Q-PCR results of RSV entry receptor gene expression in FLO and iAO; B, Q-PCR results of immune factor-related gene expression in FLO and iAO at 48 hours post-infection. $n=3$, $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$, $^{****}P<0.000\,1$.

因子mRNA表达情况，结果显示成熟气管类器官中免疫因子如白细胞介素6（interleukin 6, *IL-6*）、白细胞介素8（interleukin 8, *CXCL8*）、干扰素α（interferon α, *IFN-α*）、粒细胞集落刺激因子（granulocyte colony-stimulating factor, *G-CSF*）、粒细胞-巨噬细胞集落刺激因子（granulocyte macrophage colony-stimulating factor, *GM-CSF*）和肿瘤坏死因子α（tumor necrosis factor α, *TNF-α*）的mRNA表达量也高于胚胎肺类器官，差异均有统计学意义（$P<0.05$）（图5B）。

3　讨论

本研究利用假腺管期胚胎肺组织构建了胚胎肺类器官，并诱导其分化为成熟的气管类器官。胚胎肺类器官的细胞构成主要为SOX2和SOX9双阳性的假腺管期胚胎肺祖细胞，气管类器官的细胞构成主要为KRT5阳性的基底细胞、ACE-TUBULIN阳性的纤毛细胞、CC10阳性的Club细胞以及MUC5AC阳性的杯状细胞。在人胚胎肺发育的整个过程中，纤毛细胞、杯状细胞和基底细胞的标志性基因在假腺管期近端气道上皮细胞中逐渐开始表达，而后相关功能性细胞逐渐发育成熟[16-17]，同时伴随着病毒感染受体表达量的增加，病毒感染初期RSV病毒载量相应增加，而成熟的气管类器官细胞具有更强的免疫应答，使得在后续感染过程中病毒复制受到了极大的抑制。

目前模型动物、人源细胞系与人类在细胞及器官结构上仍然存在重要差异，极大限制了相关研究进展及成果的临床转化[18]。有研究使用棉鼠模型进行RSV感染，在低年龄组和高年龄组棉鼠中，分别模拟幼年和老年群体，幼年棉鼠存在更高滴度的RSV病毒，同时引起了严重的病理改变和以*TNF-α*和γ干扰素（interferon γ, IFN-γ）为主的炎性因子反应，而成年棉鼠相关反应则较轻[19]，然而小鼠的小气道不含基底细胞和杯状细胞[20]；RAYAVARA等[21]利用人源小气道上皮细胞系进行RSV感染，发现RSV诱导的氧化应激激活气道内的先天免疫受体，如Toll样受体（Toll-like receptors, TLR），可促进细胞外基质蛋白的交联，导致炎症增强，然而人源2D细胞系无法真实地模拟细胞在体内经历的复杂微环境。新冠病毒流行期间，肺类器官模型逐渐成熟，被广泛应用于肺部病原体感染的相关研究。成熟的气道类器官可以作为新冠病毒的感染及抗体干预模型，探讨新冠疫苗对不同突变体新冠病毒的抵抗作用[22]；利用胚胎肺尖组织诱导分化出成熟的肺泡细胞，可用于研究新冠病毒感染肺泡上皮细胞的感染及免疫应答特征[23]。本研究中使用的诱导分化成熟的人源气管类器官模型可以更好模拟气道上皮的多种细胞类型组成以及3D空间结构，以有效地模拟气道在发育不同阶段的生物学特征。

RSV的感染开始于G蛋白与细胞表面受体的结合[24]，随后由F蛋白介导与细胞融合，将遗传物质释放到细胞质中，进行进一步的转录和翻译[25]。RSV和受体的结合与感染的严重程度密切相关。EGFR可与RSV F蛋白相互作用，促进宿主与病毒膜融合[26]。同时，在体外实验中发现RSV通过F蛋白与宿主细胞的NCL相互作用，并特异性结合到顶端细胞表面的NCL上，从而介导病毒进入感染细胞[27]。最新的研究发现细胞表面IGF1R也可以协助RSV进入感染细胞，是一种新的RSV入胞受体[28]。随着上皮细胞的成熟，细胞因子的表达量也随之升高[29]，与本研究结果一致，成熟气管类器官中*EGFR*、*NCL*和*IGF1R*的表达量显著高于未成熟的胚胎肺类器官，可能是RSV感染初期气道类器官的病毒负荷明显高于胚胎肺类器官的重要原因，同时也表明诱导成熟的体外类器官模型在细胞基因表达特征方面可以有效模拟气道上皮发育特征。

RSV感染呼吸道后，会触发Toll样受体途径和RIG-Ⅰ途径，启动信号级联反应，导致IFN-α产生，启动病毒防御机制[30]，进一步激活针对RSV的先天免疫应答，促进IL-6和TNF-α等免疫因子的产生，以促进呼吸道黏液分泌[31]，肺上皮细胞随之分泌嗜酸性粒细胞活化驱化因子，进一步招募更多的粒细胞到感染部位，以及GM-CSF和G-CSF以影响募集的细胞后续存活、分化和发挥功能，从而抵抗RSV的侵袭[31-32]。本研究结果显示，在RSV感染48 h后，气管类器官中免疫应答相关因子如IL-6、CXCL8、TNF-α、IFN-α、GM-CSF、G-CSF都显著高表达于胚胎肺类器官，因此气管类器官具有更强的免疫效应，以抑制RSV病毒的复制。

总的来说，胚胎肺类器官向气道上皮发育成熟的过程中会增加病毒感染受体基因的表达，导致气道上皮更易受到感染，同时成熟气道上皮细胞比胚胎肺上皮细胞具有更强的免疫应答，抑制RSV病毒复制。

*　　*　　*

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Author Contribution　LUO Jiaxin is responsible for conceptualization, data curation, formal analysis, and writing--original draft. YANG Wenhao is responsible for project administration, supervision, and writing--review and editing. HU Yanan is responsible for software and writing--review and

editing. LU Danli is responsible for investigation and methodology. CHEN Lina and LIU Hanmin are responsible for funding acquisition, resources, and supervision. All authors consented to the submission of the article to the Journal. All authors approved the final version to be published and agreed to take responsibility for all aspects of the work.

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Declaration of conflicting interests LIU Hanmin is a Vice Chair of the Editorial Board of the journal. All processes involved in the editing andreviewing of this article were carried out in strict compliance with the journal's policies and there was no inappropriate personal involvement by the author. Other than this, all authors declare no competing interests.

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(2024 – 12 – 05收稿, 2025 – 01 – 25修回)

编辑 汤 洁