

肿瘤基础专题

肺癌恶性胸腔积液来源肿瘤细胞的小鼠 PDX 模型构建及实验验证

王梦婷, 陈怡楠, 轩辕欣阳, 袁海花

上海交通大学医学院附属第九人民医院肿瘤科, 上海 201999

[摘要] **目的**·构建肺癌患者恶性胸腔积液 (malignant pleural effusion, MPE) 肿瘤细胞来源的肿瘤异种移植 (patient-derived tumor xenograft, PDX) 模型, 并进行实验验证。**方法**·从基因表达综合数据集 (Gene Expression Omnibus, GEO) 下载人肺癌伴 MPE 单细胞转录组测序公共数据 GSE131907 和人肺癌实体瘤单细胞转录组测序公共数据 GSE203360, 对数据进行聚类、差异基因本体功能富集分析, 明确应用 MPE 建模的可行性。同时收集肺癌患者的 MPE 样本, 经离心、裂解红细胞等富集细胞操作后, 将其植入非肥胖型糖尿病重症联合免疫缺陷 (non-obese diabetic/severe combined immunodeficient, NOD/SCID) 小鼠皮下, 待移植瘤生长至 1 000 mm³ 时进行瘤体传代及保存。对稳定传代移植瘤进行组织病理学检测, 通过苏木精-伊红染色 (hematoxylin-eosin staining, H-E 染色) 观察细胞组织形态, 免疫组织化学法 (immunohistochemistry, IHC) 检测肺癌标志物表达情况。**结果**·经单细胞数据分析发现 MPE 中肿瘤细胞的增殖功能更强, 提示 MPE 中肿瘤细胞 PDX 建模或具备更佳成瘤效果; 共收集 35 例肺癌 MPE 样本, 成功构建 13 例 PDX 模型, 成功率达 37.14%; 在组织病理学检测中, H-E 染色可见移植瘤组织细胞异型性明显, IHC 检测显示细胞角蛋白 7 (cytokeratin 7, CK7)、甲状腺转录因子 1 (thyroid transcription factor-1, TTF1) 和天冬氨酸蛋白酶 A (Napsin A) 等肺癌标志物均呈阳性表达。**结论**·通过富集肺癌患者 MPE 中的肿瘤细胞, 成功构建了更为简便高效、可实时动态建模的 PDX 模型。该模型保留了肺癌患者肿瘤细胞的恶性特征及蛋白表达特性, 为肺癌伴 MPE 患者的基础研究和临床用药指导提供了重要的实验模型工具。

[关键词] 肺癌; 恶性胸腔积液; 原代细胞培养; 患者来源的肿瘤异种移植模型

[DOI] 10.3969/j.issn.1674-8115.2024.04.003 **[中图分类号]** R734.2 **[文献标志码]** A

Construction and experimental validation of mouse PDX model by malignant pleural effusion-derived tumor cells from lung cancer

WANG Mengting, CHEN Yinan, XUANYUAN Xinyang, YUAN Haihua

Department of Oncology, Shanghai Ninth People's Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai 201999, China

[Abstract] **Objective**·To establish a patient-derived tumor xenograft (PDX) model using tumor cells sourced from malignant pleural effusion (MPE) in patients with lung cancer, and to conduct experimental validation. **Methods**·Gene expression data were downloaded from the Gene Expression Omnibus (GEO), including single-cell RNA sequencing data for lung cancer with MPE (GSE131907) and for solid lung cancer (GSE203360). Data were clustered, and differential gene ontology functional enrichment analysis was performed to ascertain the feasibility of modeling by using MPE. MPE samples from patients with lung cancer were collected and processed through centrifugation and red blood cell lysis to enrich cells. The enriched cells were then implanted subcutaneously into non-obese diabetic/severe combined immunodeficient (NOD/SCID) mice. Tumor growth was monitored, and when tumors reached 1 000 mm³, they were passaged and preserved. Histopathological examination was conducted on stable passaged tumors, the cell morphology was observed via hematoxylin-eosin (H-E) staining and the expression of lung cancer biomarkers was detected by using immunohistochemistry (IHC). **Results**·Single-cell data analysis revealed stronger proliferative functions of tumor cells in MPE, suggesting that PDX modeling using MPE tumor cells may yield better tumor formation. A total of 35 samples of MPE from lung cancer patients were collected, and 13 PDX models were successfully constructed, with a success rate of 37.14%. Histopathological examination showed significant cellular atypia by H-E staining, and IHC result showed positive expression of lung cancer biomarkers such as cytokeratin 7 (CK7), thyroid transcription factor-1 (TTF1), and Napsin A. **Conclusion**·By enriching tumor cells from MPE of lung cancer patients, a more convenient, efficient, and dynamically modelable PDX model is successfully constructed. This model retains the malignant characteristics and protein expression features of tumor cells from lung cancer patients, providing an important experimental model tool for basic research and clinical drug guidance for

[作者简介] 王梦婷 (1997—), 女, 硕士生; 电子信箱: 0_0ncwcnbc@sju.edu.cn。

[通信作者] 袁海花, 电子信箱: 72300611229@shsmu.edu.cn。

[Corresponding Author] YUAN Haihua, E-mail: 72300611229@shsmu.edu.cn。

lung cancer patients with MPE.

[Key words] lung cancer; malignant pleural effusion (MPE); primary cell culture; patient-derived tumor xenograft (PDX)

肺癌是我国乃至世界范围内死亡率最高的恶性肿瘤^[1-2]。肺癌患者中,伴恶性胸腔积液(malignant pleural effusion, MPE)的发生率约为40%;MPE的产生是由于恶性肿瘤在发生胸膜转移后刺激胸腔壁产生炎症反应,进而诱发含恶性肿瘤细胞的胸腔积液^[3-4]。此外,伴MPE的肺癌患者相较于无MPE的肺癌患者,中位总生存期缩短(7.9个月 vs 15.8个月)^[5];并且在接受酪氨酸激酶抑制剂(tyrosine kinase inhibitor, TKI)靶向治疗的表皮生长因子受体(epidermal growth factor receptor, EGFR)突变的晚期肺腺癌患者中,与无MPE的患者相比,伴MPE的患者应答率更低(85.3% vs 62.1%)、预后更差,且缺乏有效的治疗策略^[6-7]。因此,为探究伴MPE的肺癌患者的治疗和预后,建立基于肺癌MPE相关的动物模型具有重大意义。

构建肿瘤动物模型有2种传统的方法。一种是采用肿瘤细胞系进行皮下接种。但由于肿瘤细胞系是在体外建立且脱离了体内微环境,其遗传不稳定且不能概括患者肿瘤的异质性。另一种是采用患者体内获取的新鲜肿瘤组织,经处理后移植到非肥胖型糖尿病重症联合免疫缺陷(non-obese diabetic/severe combined immunodeficient, NOD/SCID)小鼠上,依靠小鼠提供生长环境,高度模拟人体内肿瘤的发生发展,建立患者来源的肿瘤异种移植(patient-derived tumor xenograft, PDX)模型。该模型极大保留了原代肿瘤的基因谱表达水平、肿瘤异质性和肿瘤微环境等特征,为肿瘤的机制研究、治疗和药物筛选提供了理想的体内模型。但由于该模型需行有创手术取得患者肿瘤组织,随后在极短时间内完成移植,患者接受度有限;同时还需选取活性高的部位才能获得较高的移植成功率,使其应用受到限制^[8]。

应用MPE构建动物模型则能够更好地弥补以上缺点。临床上,肺癌伴MPE发生率高^[3-4,9],样本易取得;同时MPE需经胸腔引流治疗,方式简易且对患者损伤较小,可实时动态获得组织样本,患者接受度高;MPE中含患者新鲜活性肿瘤细胞,故可考虑将其中肿瘤细胞富集作为患者源性肿瘤进行PDX模型建立。因此,本研究拟通过富集MPE中的肿瘤细胞,将其植入NOD/SCID小鼠皮下进行PDX模型的

构建,同时检验PDX是否保留原肿瘤特异性,为进一步研究伴MPE肺癌患者胸腔积液易复发、靶向药物应答率低等的机制及治疗药物筛选奠定基础。

1 材料与方法

1.1 人体组织标本

新鲜MPE取自2019—2021年上海交通大学医学院附属第九人民医院肿瘤内科就诊的35例伴MPE的肺癌患者。患者均经病理科确诊为肺癌。将患者胸腔积液引流至引流袋,将其置于装有冰袋预冷的保温箱内,送至实验室,在超净台无菌环境中进一步处理。

1.2 实验动物

6~8周龄雄性NOD/SCID小鼠(浙江维通利华实验动物技术有限公司),体质量19~23 g,饲养于上海市浦东新区爱迪生路332号SPF(specific pathogen free)区。饲养条件:温度20~27℃,湿度40%~70%,换气次数10~15次/h,空气清洁度7级,昼夜明暗交替12 h:12 h。实验动物生产许可证号:SCXK(浙)2019-0001。实验动物使用许可证号:SYXK(沪)2020-0029。

1.3 主要试剂与仪器

RPMI-1640(Invitrogen,美国),5%胎牛血清(Gibco,美国),红细胞裂解液(Solarbio,北京),基质胶(Corning,美国),抗细胞角蛋白7(cytokeratin 7, CK7)抗体、抗甲状腺转录因子1(thyroid transcription factor-1, TTF1)抗体、抗天冬氨酸蛋白酶A(Napsin A)抗体(Cell Signaling Technology,美国)。

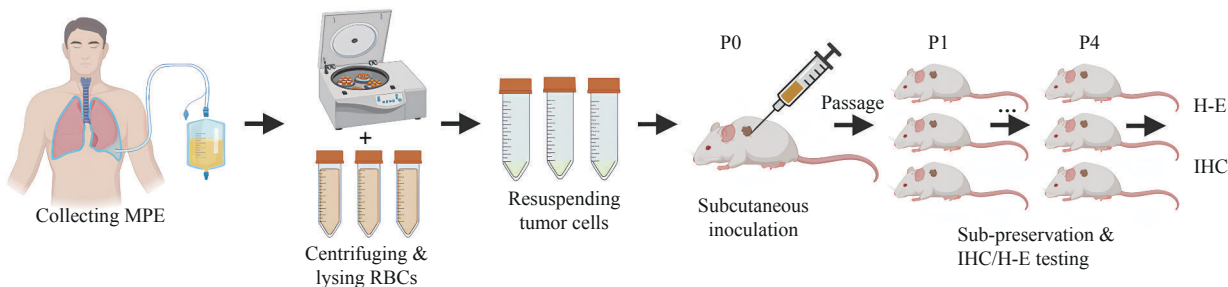
全景组织切片扫描分析系统(Olympus,日本),倒置荧光显微镜(Olympus,日本),高速离心机(Eppendorf,德国)。

1.4 方法

1.4.1 单细胞公共数据挖掘 5例人肺癌伴MPE以及6例人肺癌原位实体瘤单细胞转录组测序数据,来自基因表达综合数据集(Gene Expression Omnibus, GEO; <https://www.ncbi.nlm.nih.gov/geo/>),检索号分别为GSE131907、GSE203360,测序平台均为10×

Genomics。使用Seurat软件包(版本4.3.0)进行分析。数据经质量控制、整合及降维聚类,严格遵循Seurat的标准流程,计算单个细胞中检测到的基因数量(nFeature)和分子总数(nCount)。通过LogNormalize函数对质量控制后的数据进行标准化后,再归一化表达进行缩放;通过FindVariableFeatures函数筛选出前2 000个高变异基因,随后进行主成分分析。分别选取排名前20和前15的主成分作为输入,使用 t 分布随机近邻嵌入(t -distributed stochastic neighbor embedding, t -SNE)进行降维^[10]。使用FindNeighbors函数和FindClusters函数执行聚类分析;随后,利用FindAllMarkers函数识别各聚类中的标志基因,并注释细胞类型;同时使用FindMarkers函数识别MPE和实体瘤样本中肿瘤细胞群体的差异基因,并对基因进行基因本体(Gene Ontology, GO)功能富集分析^[11]。富集通路分为细胞组分(cell component, CC)、生物学过程(biological process, BP)及分子功能(molecular function, MF)3个部分展示。选取显著性阈值均 $P<0.05$ 且排名前10的代表性功能展示。Counts代表该词条下包含的基因数,值越大表示该词条下基因数越高;颜色从蓝到红代表富集的显著性,红色表示富集程度更高。

1.4.2 肺癌PDX建模



Note: RBC—red blood cell; H-E—hematoxylin-eosin; IHC—immunohistochemistry.

图1 肺癌MPE来源肿瘤细胞的小鼠PDX模型构建流程

Fig 1 Workflow of establishing mouse PDX model of tumor cells derived from MPE of lung cancer

1.4.3 移植瘤组织苏木精-伊红染色 将取材所得的移植瘤块经4%多聚甲醛固定,常规脱水、石蜡包埋,制备5 μm 切片。苏木精-伊红染色(hematoxylin-eosin staining, H-E染色)过程:切片常规脱蜡后,苏木精染核3 min,流水冲洗反蓝;盐酸乙醇分化2 s,流水冲洗;伊红染色40 s,流水冲洗;常规脱水及透明;中性树胶封片。扫片机于20倍视野下拍照观察表达。

1.4.4 移植瘤组织免疫组织化学法检测 采用免疫组织化学法(immunohistochemistry, IHC)进行检

(1) 标本处理 将收集到的MPE颠倒混匀,分装至50 mL离心管中;400 \times g离心7 min,收集上清液;将沉淀细胞和管中上清液分别置于2个离心管中,上清液再次400 \times g离心7 min,以充分获取上清液中的细胞;弃上清液,存有沉淀细胞的管中各加红细胞裂解液10~15 mL,常温裂解5~10 min;400 \times g离心7 min,弃上清液;沉淀细胞各加10 mL 1 \times PBS或RPMI 1640重悬清洗;400 \times g离心7 min,弃上清液;用100 μL PBS重悬沉淀细胞500万~1 000万个,分装至1.5 mL EP管中,用冰盒送至动物房。

(2) 皮下接种与传代 将MPE离心裂解后肿瘤细胞悬液与基质胶按1:1混合,将混合物注射到NOD/SCID小鼠左前肢皮下,注射体积为100 μL ,细胞数量 $(0.5\sim1.0)\times10^7$ 个。每2周用游标卡尺测量肿瘤体积。当PDX的肿瘤体积达到1 000 mm^3 时,手术剥离肿瘤组织,并对小鼠实施安乐死。将剥离的肿瘤组织用手术刀片切成2 mm \times 2 mm \times 2 mm的组织块,无菌接种到新一代NOD/SCID小鼠左前肢皮下。采用相同方法将肿瘤细胞传至P4进行后续实验。对表现出皮肤溃疡、驼背姿势、体质量减轻、发声、易怒或缺乏毛发梳理的小鼠进行仔细监测,必要时进行安乐死。MPE来源肿瘤细胞PDX建模流程具体见图1。

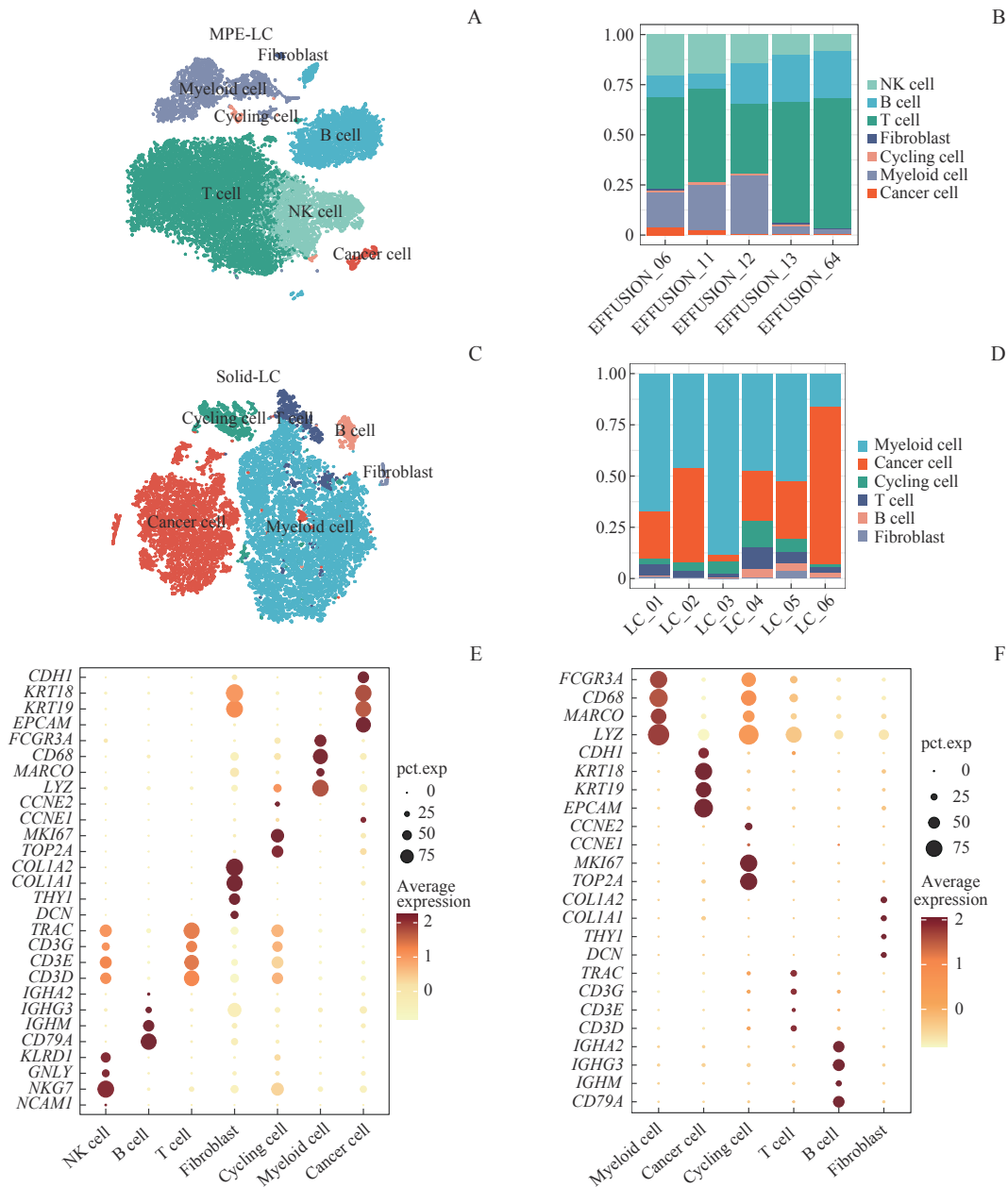
测。将5 μm 切片常规脱蜡后,3% H_2O_2 室温孵育10 min,蒸馏水漂洗;枸橼酸缓冲液微波炉加热10~15 min,室温冷却,PBS漂洗;5%胎牛血清室温孵育10 min;滴加一抗(1:200~1:50)37 $^\circ\text{C}$ 孵育1~2 h,PBS漂洗;滴加辣根过氧化物酶(horseradish peroxidase, HRP)标记的二抗(1:100 000~1:1 000)37 $^\circ\text{C}$ 孵育20 min,PBS漂洗;DAB显色,水洗,苏木精复染3~5 min;常规脱水及透明;中性树胶封片。扫片机于20倍视野下拍照观察表达。

2 结果

2.1 单细胞数据分析肺癌原位实体瘤与MPE中细胞组成异质性

单细胞转录组测序数据显示, 来自肺癌伴MPE的

20 280个细胞被聚类为7个细胞簇, 各自占比如图2A、B所示; 来自肺癌原位实体瘤的19 391个细胞被聚类为6个细胞簇, 各自占比如图2C、D所示; 可见肺癌MPE中肿瘤细胞占比相较实体瘤少, 而免疫及间充质类细胞比例较高。通过标志基因识别细胞群体(图2E、F)为自然杀



Note: A. Clustering and dimension reduction identified 7 cell subtypes in 5 malignant pleural effusion lung cancer (MPE-LC) samples. B. Stacked percentage bar charts for MPE-LC show the cell proportions in each subtype. C. Clustering and dimension reduction revealed 6 cell subtypes in 6 solid lung cancer (Solid-LC) samples. D. Stacked percentage bar charts for Solid-LC illustrate the cell proportions in each subtype. E. Bubble plots for MPE-LC display the expression of classic marker genes within each cell subtype. F. Bubble plots for Solid-LC depict the expression of classic marker genes within each cell subtype. *KLRD1*—killer cell lectin-like receptor D1; *GNLY*—granulysin; *NKG7*—natural killer cell granule protein 7; *NCAM1*—neural cell adhesion molecule 1; *IGHA2*—immunoglobulin heavy constant $\alpha 2$; *IGHG3*—immunoglobulin heavy constant $\gamma 3$; *IGHM*—immunoglobulin heavy constant μ ; *CD79A*—CD79a molecule; *TRAC*—T cell receptor α constant; *CD3G*—CD3 γ subunit of T-cell receptor complex; *CD3E*—CD3 ϵ subunit of T-cell receptor complex; *CD3D*—CD3 δ subunit of T-cell receptor complex; *COL1A2*—collagen type I $\alpha 2$ chain; *THY1*—thy-1 cell surface antigen; *DCN*—decorin; *CCNE2*—cyclin E2; *MKI67*—marker of proliferation Ki-67; *TOP2A*—DNA topoisomerase II α ; *FCGR3A*—Fc γ receptor IIIa; *MARCO*—macrophage receptor with collagenous structure; *LYZ*—lysozyme; *CDH1*—cadherin 1; *KRT18*—keratin 18; *EPCAM*—epithelial cell adhesion molecule.

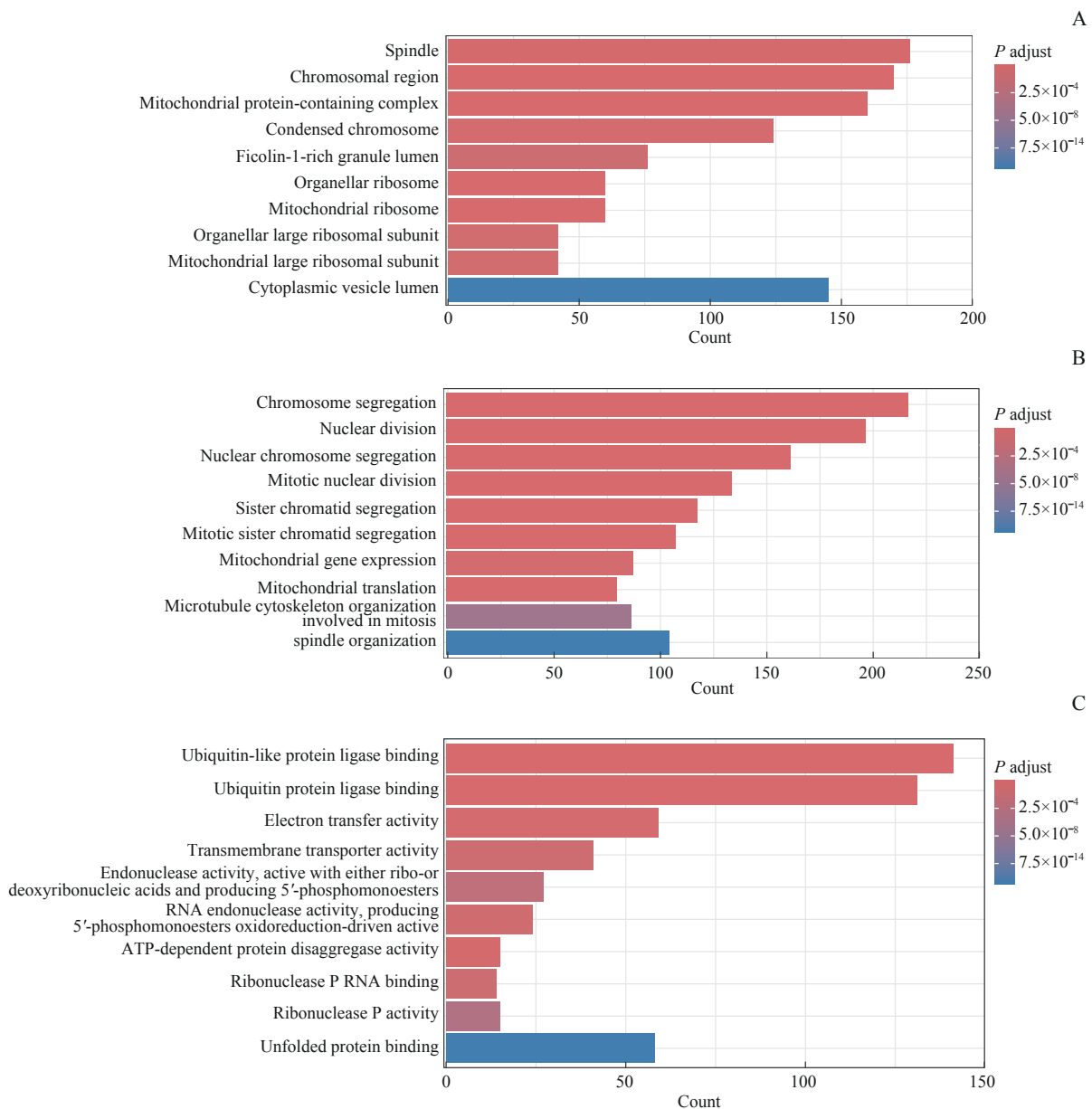
图2 肺癌MPE与原位实体肿瘤细胞的异质性

Fig 2 Heterogeneity of cell subpopulations in MPE and solid tumor of lung cancer

伤 (natural killer, NK) 细胞 (标志基因 *KLRD1*、*GNLY*、*NG7*和*NCAM1*, 实体瘤中不表达上述标志基因)、B 细胞 (标志基因 *IGHA2*、*IGHG3*、*IGHM*、*CD79A*)、T 细胞 (标志基因 *TRAC*、*CD3G*、*CD3E*、*CD3D*)、成纤维细胞 (标志基因 *COL1A2*、*COL1A1*、*THY1*、*DCN*)、周期细胞 (标志基因 *CCNE2*、*CCNE1*、*MKI67*、*TOP2A*)、髓系细胞 (标志基因 *FCGR3A*、*CD68*、*MARCO*、*LYZ*)、肿瘤细胞 (标志基因 *CDH1*、*KRT18*、*KRT19*、*EPCAM*)。

肿瘤组织经聚类分群后, 筛选出 MPE 和实体瘤的肿瘤细胞群体的差异基因, 进行 GO 功能富集分析

结果 (图 3) 显示: CC 主要涉及纺锤体、含线粒体蛋白的复合物、核糖体、囊泡等; BP 主要涉及核染色质分离、姐妹染色单体分离、有丝分裂、线粒体翻译、线粒体基因表达、微管细胞骨架组织等; MF 主要涉及泛素样蛋白连接酶结合、跨膜转运蛋白活性、ATP 依赖性蛋白解聚酶活性等。我们发现 MPE 肿瘤细胞 CC、BP 及 MF 中富集增殖、代谢相关 (染色体分离、有丝分裂、线粒体功能活跃) 等功能更多, 提示其体外成瘤能力并不弱, 这为 MPE 来源的肿瘤细胞 PDX 建模奠定研究基础。



Note: A. Cellular component (CC) terms of GO enrichment analysis. B. Biological process (BP) terms of GO enrichment analysis. C. Molecular function (MF) terms of GO enrichment analysis.

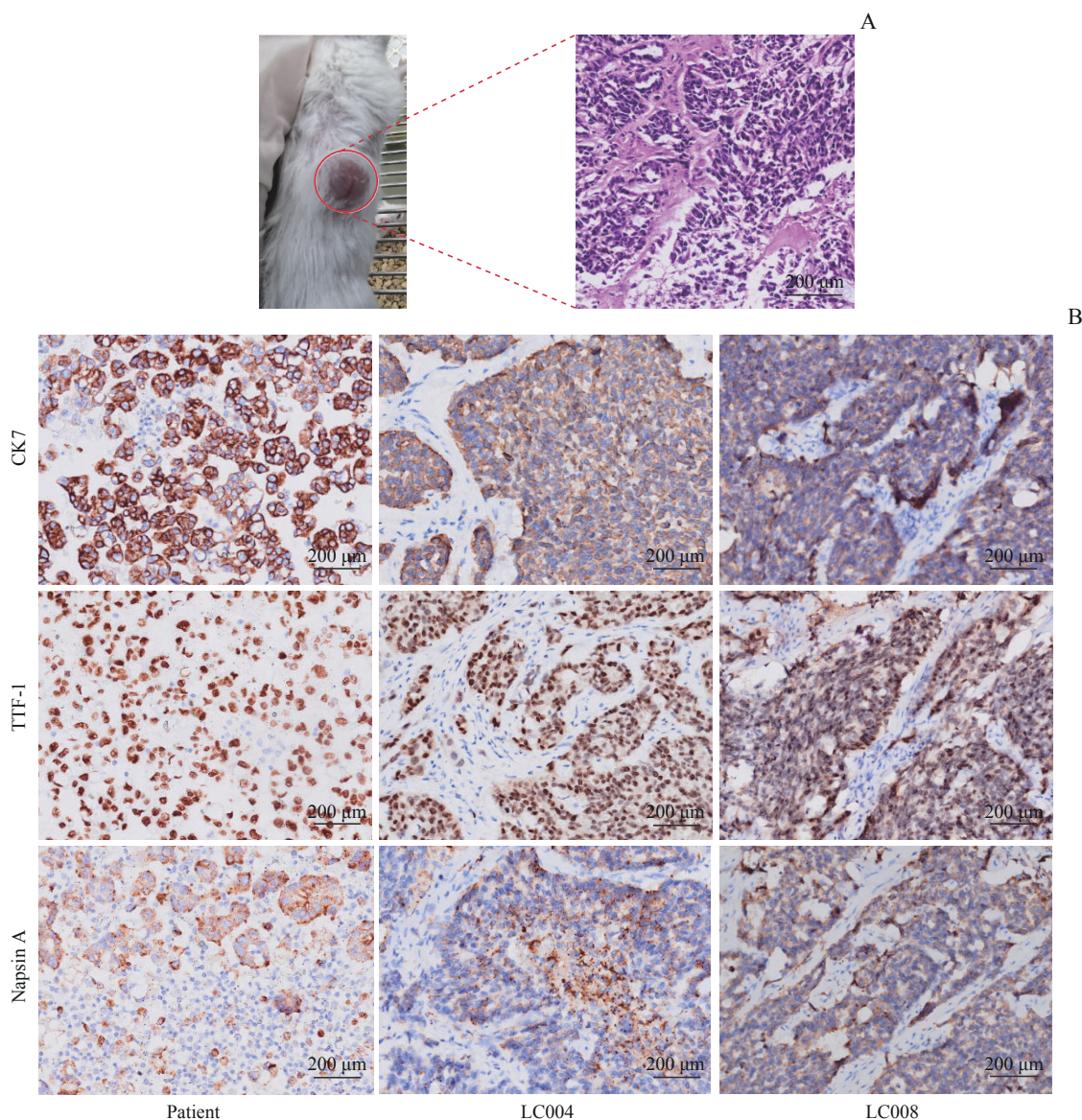
图3 肺癌 MPE 与实体瘤的肿瘤细胞亚群差异基因 GO 富集分析

Fig 3 Differential expression GO enrichment analysis of tumor cell subsets of MPE and solid tumors in lung cancer

2.2 MPE 来源肿瘤细胞PDX病理组织学验证

小鼠经接种后正常饮食饮水, 活动、毛发均正常。图4A左侧可见NOD/SCID小鼠皮下移植瘤生长情况; 图4A右侧为移植瘤稳定生长情况, P4代移植瘤H-E染色可见细胞核异质型明显, 形态不一、大小不等, 排列成索状或巢状, 失去正常结构; 核分裂增多, 可见多核细胞。表明稳定生长及传代的移植瘤, 其主要构成成分为肿瘤细胞, 而非其他免疫细胞或基

质细胞。同时对随机选取的肺癌MPE来源移植瘤(编码为LC004、LC008)进行IHC检测, 并与临床患者肺癌MPE的肿瘤细胞IHC结果对比。移植瘤LC004、LC008的肿瘤标志物CK7、TTF1和Napsin A与临床患者结果一致, 均呈阳性表达(图4B); 表明移植瘤组织相对成功地保留了肺癌MPE中肿瘤细胞的病理组织学特性。



Note: A. The growth of subcutaneous MPE-PDX in NOD/SCID mice (left); P4 generation PDX with H-E staining ($\times 200$) (right). B. IHC testing ($\times 200$) of transplanted tumors compared with clinical samples of MPE tumor cells from patients.

图4 肺癌MPE来源肿瘤细胞小鼠PDX模型的组织病理学验证

Fig 4 Histopathological validation on mouse PDX of tumor cells derived from MPE of lung cancer

2.3 MPE 来源肿瘤细胞PDX构建成瘤率

本研究2019—2021年共收集肺癌伴MPE 35例, 成功构建PDX 13例。具体信息, 如组织学分型、

IHC标志物、TNM分期以及突变情况见表1。PDX构建成率达37.14%。

表1 肺癌MPE来源已建成PDX信息

Tab 1 Information of established MPE-PDX

No.	Histologic subtype	Tumor marker				TNM stage	Mutation
		CK-7	TTF1	Napsin A	p40		
LC001	AD	/	/	/	/	TxN3M0	/
LC002	AD	+	+	+	/	TxN3M1	EGFR L858R
LC003	NSCLC	/	/	/	/	T4N1M1	/
LC004	AD	+	-	+	/	TxN3M1	EGFR Exon20ins
LC005	AD	/	/	/	/	rTNM	EGFR L858R
LC006	AD	/	/	/	/	T4N3M1c	ALK, ROS1
LC007	AD	+	+	+	/	T1N0M0	PD-L1, TP53
LC008	AD	/	/	/	/	TxN1M2	EGFR Exon19del
LC009	AD	+	+	+	/	TxN1M1c	EGFR Exon18
LC010	NSCLC	/	+	/	+	TxN3M1	No mutation
LC011	NSCLC	/	/	/	/	TxN3M1	/
LC012	SCC	/	/	/	/	T3N0M1c	/
LC013	NSCLC	+	-	-	-	TxN3M1c	/

Note: TNM stage—tumor, node, metastasis; AD—adenocarcinoma; NSCLC—non-small cell lung cancer; SCC—squamous cell carcinoma; ALK—Anaplastic lymphoma kinase; ROS1—ROS1 proto-oncogene receptor tyrosine kinase; EGFR L858R—epidermal growth factor receptor exon 21 no. 858 leucine to arginine; EGFR Exon20ins—epidermal growth factor receptor exon 20 insertion mutations; PD-L1—programmed cell death-ligand 1; TP53—tumor protein p53; EGFR Exon19del—epidermal growth factor receptor exon 19 deletion; EGFR Exon18—epidermal growth factor receptor exon 18.

3 讨论

建立合适的临床前模型对于癌症转化研究至关重要。而PDX作为一种临床前模型，能够更真实地模拟患者的癌症生物学特征，为每位患者量身定制个性化治疗方案提供了可能性；同时，PDX模型在测试药物有效性和安全性等方面具有多重优势^[12-15]。然而，对于肺癌PDX模型的建立，以往的研究主要采用切除或活检的肿瘤组织构建，较少使用肺癌患者的MPE。或许由于MPE中的肿瘤细胞相对较少，一些研究者主观上认为体外建模难度大。但是，由于肺癌手术肿瘤实体原料有限，切除的肿瘤组织需要迅速进行移植处理，因此相对应可构建移植肿瘤数量受到限制。

值得注意的是，一些研究^[16-17]指出，肺癌患者MPE中的肿瘤细胞可以轻松有效地分离、扩增和培养；尽管在肺癌伴MPE的PDX模型构建过程中可能存在一些挑战，例如MPE中非肿瘤细胞的比例相对较高以及存在红细胞浸润，但同时考虑到肺癌伴MPE的高发率（约40%）^[3-4]、易复发及缺乏有效治疗方式的临床现况，我们仍认为应用MPE构建PDX模型具备可行性，且在实时动态建模方面具有优势，可为临床肺癌伴MPE患者用药指导提供参考。

通过对比MPE及实体瘤细胞的异质性，以及查阅其他文献报道^[18-19]，我们发现MPE的成分包括肿瘤细胞、免疫细胞（T细胞、B细胞、NK细胞、髓系细胞）以及基质细胞（成纤维细胞）等，其中非肿瘤细胞占比远大于肿瘤细胞本身。通过对MPE和实体瘤肿瘤细胞进行GO功能分析，我们观察到MPE中的肿瘤细胞具有更强的增殖功能，这提示MPE富集的肿瘤细胞具备更强的成瘤能力。另外，由于收集的MPE中存在不同程度的红细胞浸润，颜色呈现出洗肉水样到血性胸腔积液的血红色变化；同时此前的文献^[20]报道称，红细胞通过积累缺氧诱导因子1-α（hypoxia-inducible factor 1-α, HIF-1α）可抑制肿瘤生长。因此在本实验中，对MPE进行离心并裂解红细胞的方式获得MPE中的细胞悬液，所得建模成功率高达37.14%，与历年文献报道中应用原位实体瘤建模的成功率17%~38.5%^[21-28]相差不大。这说明在MPE和实体瘤模型构建中，肿瘤细胞的绝对数量或非肿瘤细胞成分高低并非成瘤的必要条件，恶性程度才是关键。虽然在本文中未探索MPE去除非肿瘤细胞成分如免疫细胞，是否能提升PDX的成瘤率，但对比文献中应用外周血单个核细胞（peripheral blood mononuclear cell, PBMC）制备方法去除MPE中免疫细胞的成瘤率（35.48%）^[29]，我们推测免疫细胞对成

瘤的影响可能不大。以上均可说明本研究使用的仅离心与裂解红细胞的MPE建模方法简单易操作,成瘤率相对较高,为应用于非小细胞肺癌基础研究与机制探索,以及晚期肺癌患者个性化指导用药提供了相对较好的模型基础。

许多研究^[15,22,30]证实PDX能够很好地保存患者原有肿瘤特征和蛋白表达模式,与我们的实验结果一致。在本研究中,我们在已建成13例PDX中随机选取了2例患者的P4移植瘤进行H-E染色,同时应用IHC检测相应肿瘤标志物,与临床患者MPE肿瘤细胞的IHC对比分析。肺癌标志物包括CK7、TTF-1和Napsin A。CK7作为肺腺癌最敏感的免疫学标志物,阳性率接近100%,是鉴别原发性肺腺癌的首选;肺腺癌也通常表现为TTF-1与Napsin A阳性。本研究结果发现,MPE中富集的肿瘤细胞所形成的实体瘤,出现典型的癌巢,符合肿瘤细胞病理学特征;同时,结合移植瘤IHC结果,CK7、TTF-1和Napsin A均为阳性,我们确定PDX组织为典型的肺腺癌,且与临床患者MPE中肿瘤细胞IHC特征一致,表明应用MPE构建的PDX可保留患者肿瘤异质性。此外,我们团队已在早前研究^[31]中应用MPE构建PDX对Janus激酶1(Janus kinase 1, JAK1)抑制剂Itacitinib的反应性进行实验验证,进一步证明了应用MPE构建PDX模型的临床转化实用性。

然而,本研究也存在一定的局限性。首先,我们并未使用短串联重复序列(short tandem repeat, STR)技术对患者肿瘤组织与MPE建模移植瘤进行比对,以加强验证PDX保留患者肿瘤异质性的可信性。在后续实验中,我们将同时应用H-E染色、IHC以及STR技术作为与患者比对的硬性指标。其次,本研究仅收集了35例MPE样本,样本量相对较少。为获得更为可信的成瘤率数据,我们将在后续的研究中持续进行样本收集,扩大样本规模,提高研究的统计效能,以助于更全面地评估MPE来源PDX模型的建模效果,并为未来的研究提供更可靠的数据支持。

综上所述,PDX模型样本的H-E染色、IHC与患者样本对比均显示出相似的组织病理学特征,表明采用MPE构建的PDX保留了患者肿瘤异质性。利用MPE中的肿瘤细胞作为建模材料来源,不仅取材和建模过程简单,而且患者对取材的过程接受度高。这种建模方式可实时动态构建不同进展期肿瘤组织,成本低且成功率也有所保障。相较于实体瘤PDX建模而言,该方法更为简易快捷。本研究结果证实MPE中的肿瘤细胞是构建PDX的良好来源,为肺癌伴MPE患者的治疗用药和机制研究提供了优良的模型参考。

利益冲突声明/Conflict of Interests

所有作者声明不存在利益冲突。

All authors disclose no relevant conflict of interests.

伦理批准和知情同意/Ethics Approval and Patient Consent

本研究涉及的所有实验均已通过上海交通大学医学院附属第九人民医院人类伦理委员会的审核批准(文件号:SH9J-2019-T279-3)。受试对象均已经签署知情同意书。

All experimental protocols in this study were reviewed and approved by the Ethical Committee of Shanghai Ninth People's Hospital, Shanghai Jiao Tong University School of Medicine (Approval Letter No. SH9J-2019-T279-3). Consent letters have been signed by the research participants.

作者贡献/Authors' Contributions

王梦婷、陈怡楠、轩源欣阳、袁海花参与设计整个课题;王梦婷参与生物信息学分析、建模组织实验验证及文章撰写;陈怡楠、轩源欣阳参与生物信息分析和文章撰写。所有作者均阅读并同意了最终稿件的提交。

WANG Mengting, CHEN Yinan, XUANYUAN Xinyang and YUAN Haihua participated in the design of the entire research project. WANG Mengting contributed to the completion of bioinformatics analysis, modeling, experimental validation of the model, and writing of the manuscript. CHEN Yinan and XUANYUAN Xinyang were involved in bioinformatics analysis and manuscript writing. All authors have read the final manuscript and approved the submission.

• Received: 2023-10-30

• Accepted: 2024-03-25

• Published online: 2024-04-28

参 · 考 · 文 · 献

- [1] SIEGEL R L, MILLER K D, FUCHS H E, et al. Cancer statistics, 2022[J]. CA Cancer J Clin, 2022, 72(1): 7-33.
- [2] ZHENG R S, ZHANG S W, ZENG H M, et al. Cancer incidence and

mortality in China, 2016[J]. J Natl Cancer Cent, 2022, 2(1): 1-9.

- [3] XU P W, TANG K, MA J W, et al. Chemotherapeutic tumor microparticles elicit a neutrophil response targeting malignant pleural



- effusions[J]. *Cancer Immunol Res*, 2020, 8(9): 1193-1205.
- [4] PORCEL J M, GASOL A, BIELSA S, et al. Clinical features and survival of lung cancer patients with pleural effusions[J]. *Respirology*, 2015, 20(4): 654-659.
- [5] SHIBAKI R, MURAKAMI S, SHINNO Y, et al. Malignant pleural effusion as a predictor of the efficacy of anti-PD-1 antibody in patients with non-small cell lung cancer[J]. *Thorac Cancer*, 2019, 10(4): 815-822.
- [6] SATOH K, MORISAWA S, OKUYAMA M, et al. Severe pleural effusion associated with nilotinib for chronic myeloid leukaemia: cross-intolerance with tyrosine kinase inhibitors[J]. *BMJ Case Rep*, 2021, 14(9): e243671.
- [7] WANG T F, CHU S C, LEE J J, et al. Presence of pleural effusion is associated with a poor prognosis in patients with epidermal growth factor receptor-mutated lung cancer receiving tyrosine kinase inhibitors as first-line treatment[J]. *Asia Pac J Clin Oncol*, 2017, 13(4): 304-313.
- [8] JIN K T, TENG L S, SHEN Y P, et al. Patient-derived human tumour tissue xenografts in immunodeficient mice: a systematic review[J]. *Clin Transl Oncol*, 2010, 12(7): 473-480.
- [9] ROSCILLI G, de VITIS C, FERRARA F F, et al. Human lung adenocarcinoma cell cultures derived from malignant pleural effusions as model system to predict patients chemosensitivity[J]. *J Transl Med*, 2016, 14: 61.
- [10] SUN S Q, ZHU J Q, MA Y, et al. Accuracy, robustness and scalability of dimensionality reduction methods for single-cell RNA-seq analysis[J]. *Genome Biol*, 2019, 20(1): 269.
- [11] ASHBURNER M, BALL C A, BLAKE J A, et al. Gene ontology: tool for the unification of biology. The Gene Ontology Consortium[J]. *Nat Genet*, 2000, 25(1): 25-29.
- [12] WAKEFIELD C E, DOOLAN E L, FARDELL J E, et al. The avatar acceptability study: survivor, parent and community willingness to use patient-derived xenografts to personalize cancer care[J]. *EBioMedicine*, 2018, 37: 205-213.
- [13] HEINRICH M A, MOSTAFA A M R H, MORTON J P, et al. Translating complexity and heterogeneity of pancreatic tumor: 3D *in vitro* to *in vivo* models[J]. *Adv Drug Deliv Rev*, 2021, 174: 265-293.
- [14] MAKIMOTO G, OHASHI K, TOMIDA S, et al. Rapid acquisition of alectinib resistance in ALK-positive lung cancer with high tumor mutation burden[J]. *J Thorac Oncol*, 2019, 14(11): 2009-2018.
- [15] PARK B, JEONG B C, CHOI Y L, et al. Development and characterization of a bladder cancer xenograft model using patient-derived tumor tissue[J]. *Cancer Sci*, 2013, 104(5): 631-638.
- [16] STOCKHAMMER P, HO C S L, HEGEDUS L, et al. HDAC inhibition synergizes with ALK inhibitors to overcome resistance in a novel *ALK* mutated lung adenocarcinoma model[J]. *Lung Cancer*, 2020, 144: 20-29.
- [17] 杨晓乐. 肺癌胸水肿瘤细胞的体外培养、鉴定及其对常规化疗药物敏感性研究[D]. 郑州: 郑州大学, 2017.
- YANG X L. Establishment and identification of malignant pleural effusion-derived lung cancer primary cell culture and its chemosensitivity[D]. Zhengzhou: Zhengzhou University, 2017.
- [18] RUAN H Y, WANG Z, SUN Z W, et al. Single-cell RNA sequencing reveals the characteristics of cerebrospinal fluid tumour environment in breast cancer and lung cancer leptomeningeal metastases[J]. *Clin Transl Med*, 2022, 12(6): e885.
- [19] KIM N, KIM H K, LEE K, et al. Single-cell RNA sequencing demonstrates the molecular and cellular reprogramming of metastatic lung adenocarcinoma[J]. *Nat Commun*, 2020, 11(1): 2285.
- [20] KAO T W, BAI G H, WANG T L, et al. Novel cancer treatment paradigm targeting hypoxia-induced factor in conjunction with current therapies to overcome resistance[J]. *J Exp Clin Cancer Res*, 2023, 42(1): 171.
- [21] KANG H N, CHOI J W, SHIM H S, et al. Establishment of a platform of non-small-cell lung cancer patient-derived xenografts with clinical and genomic annotation[J]. *Lung Cancer*, 2018, 124: 168-178.
- [22] CHEN X M, SHEN C, WEI Z, et al. Patient-derived non-small cell lung cancer xenograft mirrors complex tumor heterogeneity[J]. *Cancer Biol Med*, 2021, 18(1): 184-198.
- [23] CHEN Y, ZHANG R, WANG L, et al. Tumor characteristics associated with engraftment of patient-derived non-small cell lung cancer xenografts in immunocompromised mice[J]. *Cancer*, 2019, 125(21): 3738-3748.
- [24] WANG D, PHAM N A, TONG J F, et al. Molecular heterogeneity of non-small cell lung carcinoma patient-derived xenografts closely reflect their primary tumors[J]. *Int J Cancer*, 2017, 140(3): 662-673.
- [25] KITA K, FUKUDA K, TAKAHASHI H, et al. Patient-derived xenograft models of non-small cell lung cancer for evaluating targeted drug sensitivity and resistance[J]. *Cancer Sci*, 2019, 110(10): 3215-3224.
- [26] STEWART E L, MASCAUX C, PHAM N A, et al. Clinical utility of patient-derived xenografts to determine biomarkers of prognosis and map resistance pathways in *EGFR*-mutant lung adenocarcinoma[J]. *J Clin Oncol*, 2015, 33(22): 2472-2480.
- [27] FICHTNER I, ROLFF J, SOONG R, et al. Establishment of patient-derived non-small cell lung cancer xenografts as models for the identification of predictive biomarkers[J]. *Clin Cancer Res*, 2008, 14(20): 6456-6468.
- [28] ZHANG X C, ZHANG J C, LI M, et al. Establishment of patient-derived non-small cell lung cancer xenograft models with genetic aberrations within *EGFR*, *KRAS* and *FGFR1*: useful tools for preclinical studies of targeted therapies[J]. *J Transl Med*, 2013, 11: 168.
- [29] LIAO H, ZHOU S X, CHEN S, et al. Establishment and characterization of patient-derived xenograft model of non-small-cell lung cancer derived from malignant pleural effusions[J]. *Cancer Manag Res*, 2023, 15: 165-174.
- [30] XIN Y Q, LI S Y, JIANG Q K, et al. Establishment of a jaw fibrosarcoma patient-derived xenograft and evaluation of the tumor suppression efficacy of plumbagin against jaw fibrosarcoma[J]. *Front Oncol*, 2020, 10: 1479.
- [31] HUANG T L, CHEN B Y, WANG F, et al. Rab1A promotes IL-4R/JAK1/STAT6-dependent metastasis and determines JAK1 inhibitor sensitivity in non-small cell lung cancer[J]. *Cancer Lett*, 2021, 523: 182-194.

[本文编辑] 包 玲

