·综 述·

肿瘤类器官的研究进展

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摘要：类器官是通过三维结构培养技术，将组织来源的成体干细胞培养成可自行组织的结构，来源于多能干细胞或离体器官的祖细胞。至今从小鼠和人类干细胞中已成功培养出多种类器官。在三维结构培养体系下，肿瘤类器官可长期扩增、冷冻保存和基因改造，并在扩增和冻存的过程中保持遗传和表型稳定，其优势被广泛地用于各种肿瘤的研究中。该文针对肿瘤类器官的发展及研究进展作综述。

关键词：类器官；肿瘤；三维结构培养

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Research progress of tumor organoids

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Abstract: Organoid is to cultivate tissue-derived adult stem cells into a self-organizing structures through three-dimensional（3D）culture technology, which are derived from pluripotent stem cells or progenitor cells of isolated organs. So far, many organoids have been successfully cultured from mouse and human stem cells. Under the 3D culture system, tumor organoids can be expanded, cryopreserved and genetically modified for a long time, and maintain genetic and phenotypic stability can be maintained in the process of expansion and cryopreservation, which has been widely used in the research of various tumors. This article reviews the development and research progress of tumor organoids.

Key words: organoid; tumor; three-dimensional culture

肿瘤类器官是肿瘤细胞的三维结构培养物，可从诱导的多能干细胞中获得[1]，亦可直接从临床患者的肿瘤组织中提取。直接从肿瘤组织中获得培养的肿瘤类器官更实用，且更贴近临床肿瘤的真实情况。相比其他的肿瘤模型，如建株细胞系、细胞系来源异种移植瘤（cell-line derived xenograft，CDX）和患者来源的异种移植瘤（patients-derived tumor xenograft，PDX）模型等，肿瘤类器官用作疾病模型具有不可替代的优势。本文针对肿瘤类器官的研究进展进行综述。

1 肿瘤类器官的发展

随着体外三维结构培养技术的发展，类器官开辟了肿瘤领域的新模型。该模型可有效地将基础研究和临床治疗相结合，并为肿瘤患者治疗方案的选择提供参考。

1.1 结直肠癌（colorectal cancer）类器官

肠的发育过程中，成纤维细胞生长因子（fibroblast growth factor，FGF）/Wnt 至关重要[2-3]。2011 年，Spence 等[4]通过组合调整 FGF/Wnt 等

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生长因子，成功建立了成熟的促进肠道生长、形态形成和细胞分化的肠类器官培养系统。2015 年，van de Wetering 等[5]成功培养了肠癌类器官，并发现结直肠癌中常见腺瘤样结肠息肉病易感基因（adenomatous polyposis of colon，*APC*）、*TP53* 及鼠类肉瘤病毒癌基因（kirsten rat sarcoma viral ancogene homolog，*KRAS*）等基因突变。2019 年，Yao 等[6]在局部晚期直肠癌（advanced rectal cancer）患者中建立类器官生物库，发现类器官与临床治疗反应的匹配度高达 84.43%，证实类器官在局部晚期直肠癌治疗中具有重要指导价值。

1.2 胰腺癌（pancreatic cancer）类器官

胰腺癌发病隐匿、恶性程度高，且治疗效果不佳，是致死率较高的恶性肿瘤之一[7]。因体外培养的胰腺癌模型较少，故其通路研究一直缺乏可操作性。Boj 等[8]从小鼠和人类的胰腺组织中成功建立胰腺癌类器官模型，研究了胰腺导管癌（ductal carcinoma of cancer）的发病机制。Huang 等[9]在胰腺导管癌患者的肿瘤类器官中发现，其既维持了原发肿瘤的分化状态、组织结构和表型异质性，亦保留了患者特有的生理变化，包括缺氧、耗氧量、表观遗传标记和抑制组蛋白甲基转移酶 zeste 基因增强子同源物 2（enhancer of zeste homolog 2，*EZH2*）敏感性的差异。

1.3 前列腺癌（prostatic cancer）类器官

体外培养前列腺癌模型较为困难，在超过 1 000 个经典的肿瘤细胞系中，前列腺癌细胞只有 7 个[10-14]。2014 年，Gao 等[15]成功建立可长期培养的前列腺癌类器官，其具有 *TMPRSS2-ERG* 融合、*SPOP* 突变、丝氨酸蛋白酶抑制剂 Kazal 1 型（serine protease inhibitor Kazal type 1，*SPINK1*）过表达和染色质解旋酶 DNA 结合蛋白 1（chromodomain helicase DNA binding protein 1，*CHD1*）丢失等特性。在此基础上，Wadosky 等[16]将缺乏抑癌基因的前列腺癌基因工程小鼠和三维结构培养技术相结合，为前列腺癌的发展机制研究提供更好的方法。

1.4 肝癌（liver cancer）类器官

经典的肝类器官培养方式由多能干细胞[17-18]或成体干细胞分化形成[19]。2017 年，Broutier 等[20]在肝类器官的培养体系中去除 *Respondin-1*、*Noggin* 和 *Wnt3a*，添加地塞米松（dexamethasone）和 Rho 激酶抑制剂，同时增加肝肿瘤组织的消化时间，成功培养出肝细胞癌（hepatic cellular cancer）、肝内胆管癌（intrahepatic cholangiocarcinoma）和混合型肝癌 3 种常见肝癌类器官。其维持了与体内肿瘤相似的组织结构和甲胎蛋白、上皮细胞黏膜附分子、角蛋白 19、白蛋白及 Ki-67 等相关蛋白的表达。Fong 等[21]使用三维大孔水凝胶代替传统基质凝胶培养 PDX 肝癌类器官，从中培养出肝癌类器官，维持原发肿瘤的遗传和表观信息，并保留肝癌组织的异质性。

1.5 胃癌（gastric cancer）类器官

2009 年，Sato 等[22]用富含亮氨酸重复序列的 G 蛋白偶联受体 5 成功培养出上皮类器官，这为胃类器官的成功培养奠定了基础。2015 年，Bartfeld 等[23]对人类胃黏膜上皮干细胞进行体外扩增和长期培养。2019 年，Seidlitz 等[24]完善胃癌类器官的培养方案，并成功建立可长期培养人类和小鼠的胃癌类器官模型，其具有胃癌常见的 *KRAS*、*TP53*、*APC* 和 *CHD1* 等基因突变。

2 肿瘤类器官模型与常用肿瘤研究模型

通过肿瘤类器官水平评价，可对临床真实病例进行研究，获得更贴近临床的真实结果。以下是目前常用的肿瘤研究模型和肿瘤类器官模型的比较。

2.1 肿瘤细胞系

肿瘤细胞系是经典的肿瘤研究模型，包括已建株细胞的二维结构、三维结构培养和原代培养的肿瘤细胞。其因易获取、易保存、易传代以及操作简便等优势被长期用作抗肿瘤药物筛选的模型，但二维结构细胞模型无法准确模拟疾病组织的生理特征和发育过程[25-26]，且无法展现个体化的生理病理特征[27]。建株细胞的三维结构培养不可恢复其细胞在长期传代和冻存中已丢失的肿瘤原始特性[28]。原代

肿瘤细胞的培养可在一定程度上保留遗传信息的异质性，且可评价药物对多细胞的作用，但长期培养难度大，且缺乏肿瘤相应的微环境[29]。

2.2 动物模型

常见的肿瘤动物模型有 CDX 和 PDX。其包含成纤维细胞、内皮细胞、血细胞和免疫细胞的基质及宿主细胞的相关生理环境中来评价药物的有效性，可通过观察荷瘤小鼠的体质量、有无便血及呕吐等不良反应来评价药物对整个机体的作用。但 CDX 经长期传代，其生物学特性已与原代细胞相差甚远，且增殖较快的肿瘤克隆早已占据优势，故已失去肿瘤的异质性。研究显示，PDX 中的肿瘤细胞，更大程度上保留了原始肿瘤特征[30-33]，即使在连续传代后，其依然与原始肿瘤高度相似[34-37]。

2.3 肿瘤类器官

与上述两类模型相比，肿瘤类器官不仅具有二维结构细胞易保存、易传代等优势，且大多取自于患者肿瘤组织，最大程度上保留了原始肿瘤的遗传和表型特征[9]。此外，三维结构培养体系亦提供了丰富的生长因子和细胞外基质，让 PDX 的生长更接近于体内的微环境[38]。研究显示，胃癌类器官与各种亚型胃癌的分子结构和组织形态学均高度相似，且还可模拟正常胃细胞转化为侵袭性肿瘤细胞的过程[39]。

3 肿瘤类器官的研究进展

3.1 肿瘤发病机制

与细胞模型相比，肿瘤类器官的基因易修饰、改造。Roper 等[40] 在人的结直肠癌类器官上应用 CRISPR-Cas9 基因编辑系统，编辑 *APC*、*KRAS* 及 *Trp53* 等位点的突变，将 *Trp53* 基因缺失的肿瘤类器官移植到 NSG 小鼠身上研究结直肠癌的发病机制，且证实肿瘤类器官可用来评价体内的基因功能。基因编辑可研究单个基因的致瘤能力，揭示了 *Trp53* 在肿瘤转移侵袭过程中扮演重要角色[41]。Giroux 等[42] 在类器官中选择性清除 *Notch1* 基因，证实 *Notch1* 在早、晚期食管鳞状细胞癌（esophageal squamous cell carcinoma）上皮 - 间质转化过程中发挥重要作用。胃癌类器官可证实钙 / 磷脂结合蛋白 ANX10 与胃癌病情进展及不良预后有关[43]。以上均说明肿瘤类器官有助于深入探究肿瘤发病机制。

3.2 药物筛选

肿瘤类器官可高度保留原代肿瘤组织的遗传和表型特征，且不会影响肿瘤间的异质性，是用作药物筛选的最佳模型。

Sachs 等[44] 建立的乳腺癌（breast cancer）类器官库包含不同类型的乳腺癌，证实类器官与原发肿瘤的组织病理学、激素受体状态、人类表皮生长因子受体 -2 状态及基因突变均吻合，且药物筛选的结果与患者临床反应一致。Gao 等[45] 在前列腺癌类器官上探究去势抵抗性前列腺癌（castration-resistant prostate cancer，CRPC）3 种治疗药物的作用效果，结果发现不同突变基因的 CRPC 类器官对药物的反应亦不同。胃肠道肿瘤类器官模型还被用于药敏试验。研究表明，肿瘤类器官可预测患者用药结果，为个体化治疗提供依据[46]。Yao 等[6] 证实局部晚期直肠癌患者的放疗反应与直肠癌类器官的放疗反应高度一致。

3.3 不良反应研究

与肿瘤细胞系和动物模型相比，肿瘤类器官模型能更好地预测药物对人体的不良反应。有学者应用肾类器官探究顺铂的不良反应，发现近端小管会内吞葡聚糖并开始凋亡[47]。Mun 等[48] 用肝类器官对临床因肝脏不良反应而撤回的药物开展研究，结果在类器官上也获得相同反应。肿瘤类器官在药物筛查、不良反应检测以及减轻暴露风险等方面均有较好的应用价值。

3.4 药物研发

类器官可在健康组织或肿瘤组织中生成，其遗传表型稳定，该优势让肿瘤类器官在药物研发领域也得到广泛运用。van de Wetering 等[5] 研究表明 Wnt 蛋白生成抑制剂 2 可明显抑制突变型结直肠癌肿瘤类器官的生长，这为结直肠癌治疗药物的靶点

研究提供新的方向。Seino 等[49]通过对胰腺导管癌类器官研究发现，*GATA6* 可调节胰腺导管癌对 Wnt 的依赖性，为胰腺癌靶向药的研发提供新思路。在乳腺癌方面，具有黏性的乳腺癌类器官可以帮助研究者更好的地了解乳腺小叶组织学特征，为寻找潜在治疗靶点提供可能。如乳腺癌类器官中，E- 钙黏蛋白会丧失功能[50]。

4 结语

肿瘤类器官的发展扩大了肿瘤基础和临床研究领域，不仅能在临床上作为良好的药物筛选模型，亦能为肿瘤机制研究作出贡献[51]。未来肿瘤类器官培养方法仍会不断优化，让药敏检测更精准。同时，未来的标准化肿瘤类器官生物库以及优化药物筛选平台均是肿瘤类器官应用于临床的关键。

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