直接注射法制作 ICR 小鼠肝癌原位移植瘤模型 *

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摘要 目的:培养鼠肝癌 H22 细胞,直接注射法制作 ICR 小鼠肝癌原位移植瘤,为后续实验奠定基础。方法:鼠肝癌 H22 细胞体外培养,将调整好的对数生长期的肝癌细胞直接注射小鼠肝脏,2 周后解剖观察,并进行组织 HE 染色。结果:所有实验小鼠均可见肿瘤生长,HE 染色示肝细胞肝癌。结论:直接注射法制作 ICR 小鼠肝癌原位移植瘤模型简便易行,值得推广应用。

关键词:ICR 小鼠;癌,肝细胞;模型,动物

中图分类号:Q95-3,R735.7 **文献标识码**:A **文章编号**:1673-6273(2011)09-1680-03

Making an Orthotopic Tumor Model of Hepatocellular Carcinoma for ICR Mice by Direct Injection*

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ABSTRACT Objective: To cultivate H22 cell line and making an orthotopic tumor model of hepatocellular carcinoma on ICR mouse using direct injection method to lay the foundation for subsequent experiment. Methods: Cultivate H22 cell line in vitro and an orthotopic xenograft tumor model of hepatocellular carcinoma was created by injection of H22 cells in logarithmic growth phase directly into the liver parenchyma of ICR mice. Two weeks later, hepatocellular carcinoma specimens of animals were observed and stained with hematoxylin/eosin. Results: Tumor growth happened on all the experimental mice and was showed as hepatocellular carcinoma by hematoxylin-eosin staining. Conclusion: It is convenient of direct injection method on making an orthotopic tumor model of hepatocellular carcinoma and should be reported and used widely.

Key words: ICR Mouse; Carcinoma, Hepatocellular; Models, Animal

Chinese Library Classification(CLC): Q95-3, R735.7 Document code: A

Article ID:1673-6273(2011)09-1680-03

前言

肝癌发病率高,预后差,严重影响着人类的健康。因此,建立一个理想的类似人肝癌的动物模型,对于研究肝癌的药物实验治疗有着极其重要的意义。将肝癌细胞株直接注射在小鼠皮下建立肝癌皮下移植瘤是常见的制作肝癌模型的方法[1-2],但是这种模型不能代表肝癌的自然发生,同时药物治疗对于皮下和肝内肿瘤组织的药效学和药物代谢动力学也会有很大的不同[3]。ICR 小鼠经济,易饲养,易获得,为此,我们探索了采用直接注射法[4]建立 ICR 小鼠肝癌原位移植瘤的方法。

1 材料和方法

1.1 材料实验动物和试剂

实验用 ICR 小鼠 20 只,雌性,体质量 28 g± 2g,购于南京医科大学实验动物中心,饲养于南京鼓楼医院动物中心无特殊病原菌(SPF)环境中。鼠肝癌细胞株 H22 购于中科院上海生命科学研究院细胞资源中心。RPMI 1640 培养液、胎牛血清、胰蛋白酶(HyClone 美国)。

1.2 实验方法和步骤

1.2.1 细胞培养 鼠肝癌 H22 细胞培养于含 100 mL/L 胎牛血清、青霉素 100 kU/L、链霉素 100 mg/L 的 RPMI 1640 培养基中,37℃,50 mL/L CO_2 条件下常规培养,0.25%胰蛋白酶传代培养。

1.2.2 小鼠肝癌原位移植瘤模型的建立 鼠肝癌原位移植瘤模型,采用直接种植法。收集对数生长期的鼠肝癌 H22 细胞,PBS 调整细胞悬液至 5× 10^7/mL。小鼠腹腔麻醉(3.5 %水合氯醛 300 mg/kg)后,取仰卧位,固定于实验板上,取肋缘下横行切口,逐层剪开皮肤和腹膜,充分暴露肝左叶。用 28 号针抽取 50 μL H22 细胞悬液(含细胞数 2.5× 10^6),30° 角刺入肝脏缓慢注入细胞悬液,注射完毕拔出针头,立即用无菌纱布轻压针孔(1 min)至肝脏表面不再渗血,逐层关腹。

1.2.3 肿瘤组织病理形态学观察 原位移植瘤模型在手术 2 周后剖腹探查观察肿瘤生长情况,然后瘤组织经 40 g/L 甲醛液固定,脱水,石蜡包埋,制片,H&E 染色,光学显微镜下观察其组织病理形态的改变。

* 基金项目:南京市卫生局医学科技发展项目(ZKX09027)

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(收稿日期:2011-02-22 接受日期:2011-03-18)

2 结果

2.1 肝脏肿瘤组织大体观察

模型制作 2 周后处死小鼠，解剖观察肝脏原位移植瘤形态相对不规则，肿瘤呈弥漫性生长或分叶结节状，大部分肿瘤包膜较完整，肿瘤以浸润性生长为主，与周围组织多有粘连，晚期出现肝内局部扩散转移及腹腔淋巴结转移（图 1）。

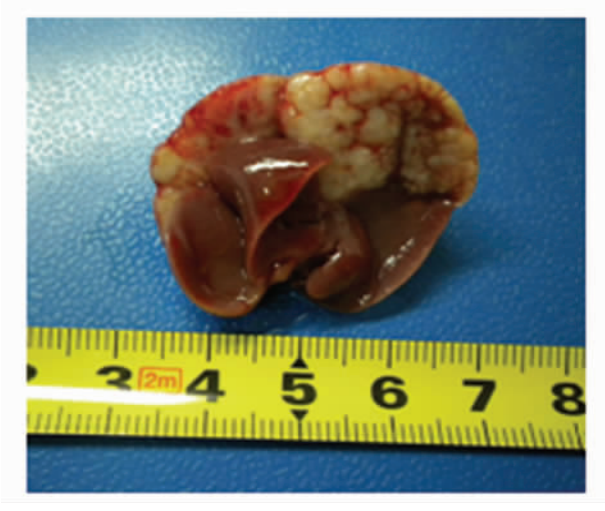

图 1 小鼠肝癌原位移植瘤（2 周）

Fig. 1 An orthotopic xenograft tumor model of hepatocellular carcinoma of mouse (2 weeks)

2.2 肝脏肿瘤组织病理形态学特征

取小鼠肝脏，脱水、石蜡包埋、制片、HE 染色，肿瘤组织 HE 染色镜下：肝小叶结构清晰，部分肝小叶被结节状的肿瘤组织取代（尤其是靠近肝包膜的肝小叶），周边的肝小叶受压质地变实，肝窦扩张充血。瘤区肿瘤细胞呈类圆形，异型性明显，细胞核大，染色深，病理性核分裂像易见，肿瘤细胞呈团块状分布，有的瘤组织内有不同程度的凝固性坏死，呈红染区，有的坏死不彻底区可见明显的核固缩和和核碎裂（图 2）。

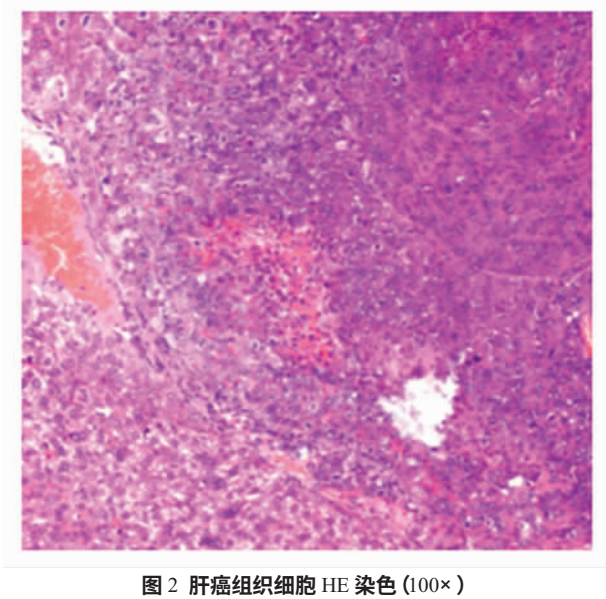

图 2 肝癌组织细胞 HE 染色（100×）

Fig. 2 Hepatocellular tissue cell stained by hematoxylin/eosin (100×)

3 讨论

自 20 世纪初小鼠获得自发性肝癌动物模型以来，人们对肝癌动物模型的研究不断深入，逐渐建立了诱发性肝癌动物模型、移植性肝癌动物模型[5]及转基因动物肝癌模型[6]。诱发性肝癌动物模型起病隐匿、病程较长，肿瘤多为弥漫结节型，诱导周期长，常需 3～5 个月或 1～2 年，在诱癌的过程中死亡率较高，因此多用于肝癌病因学、发生学、发病机理、遗传及生物学方面研究[7-9]。转基因肝癌动物模型[10]是指借助基因工程手段将特定的外源基因导入动物的染色体中，使其发生整合、遗传繁殖，并由此培养出携带外源基因的转基因动物所形成的肝癌模型，转基因肝癌动物模型不仅是研究癌基因活性和肝癌发生的一种极为重要的方法，同时也为肝炎病毒相关性肝癌的研究提供了新的途径[11-12]，但此模型制作技术要求极高，价格昂贵，国内开展尚少。

移植性肝癌动物模型是指用肝癌（源于动物或人）移植到动物体内（肝脏、肝外组织或器官），或非肝脏来源地恶性肿瘤（如乳腺癌[13]、鳞癌[14]或结肠癌[15]）移植到动物的肝脏所形成的荷肝癌动物模型。用移植法制作的肝癌模型周期一般较短，肿瘤的大小和位置比较容易控制，但瘤源多种多样，有自发的、诱发的、切除的肝癌标本或肝癌细胞株，常用的受体动物有小鼠或大鼠，我们采用直接种植法制作 ICR 小鼠肝癌原位移植瘤模型。

该模型制作需要注意以下几点：⑴ 肝癌细胞悬液的浓度调整为 50 μL 细胞悬液含有细胞数 2.5×10^6 细胞一次性注入，肿瘤细胞数量过多容易流进腹腔造成腹腔内肿瘤广泛种植转移，数量过少，成瘤性差；⑵ 穿刺进针时要注意进针的角度和深度，30 度角刺入肝脏包膜 1cm 后，稍稍挑起缓慢注入细胞悬液较好；⑶ 完善的麻醉和熟练地手术技巧是保证模型制作成功的关键，肝癌细胞悬液注射完毕后拔出针头，常规用无菌纱布轻压针孔（1 min）肝脏止血即可。ICR 小鼠对饲养环境要求比裸小鼠要低，且经济易获得，采用肝癌细胞株直接种植法，只要熟练掌握操作方法，造模的成功率还是很高的，值得应用推广。

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