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MUC1基因免疫抑制H22肝癌生长的实验研究

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Inhibitory effect of MUC1 gene immunization on H22 hepatocellular carcinoma growth

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Abstract

AIM: To investigate the special anti-H22 hepatocellular carcinoma growth effect of the MUC1 gene immunization.

METHODS: Balb/c mice were immunized intramuscularly with 100 μg MUC1 cDNA 3 times at 3-weekly intervals. Three weeks after the last immunization, tumor challenge experiments were performed by using MUC1 expressing tumor cell line H22. Tumor growth inhibition and body protection were observed two weeks later. After 43d of challenge experiments, all mice were killed and tumors were weighed. Histological analysis of tumor tissue was carried out with HE staining.

RESULTS: After 43 d of challenge experiments, the volumes of H22 hepatocellular carcinoma in MUC1cDNA, pcDNA3.1 (+) and NS groups were 547±59 mm^3, 1 185±84 mm^3 and 1 220±95 mm^3 (P <0.01), respectively. The average mass of H22 hepatocellular carcinoma in the three groups was 1.87±0.96 g, 4.19±1.34 g and 4.23±1.32 g (P <0.01), respectively . Tumorigenic rate was only 50 % in MUC1cDNA group, and was 100 % in pcDNA3.1(+) and NS group. H22 hepatocellular carcinoma growth in mice of MUC1cDNA group was significantly suppressed (P <0.01), and a significant body protective effect was found in mice of MUC1cDNA group (P <0.05), compared with control group. Histological analysis showed that the H22 hepatocellular carcinoma tissues were markedly necrosed in mice of MUC1cDNA group compared with that in control group.

CONCLUSION: MUC1 gene immunization can significantly suppress H22 hepatocellular carcinoma growth.

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摘要

目的: 观察MUC1基因免疫对H22肝癌生长的特异性抑制作用.

方法: 采用股四头肌肌肉注射法将构建的MUC1基因疫苗pcDNA3.1-MUC1免疫Balb/c小鼠, 每次100 μg, 3 wk/次, 共3次. 最后一次基因免疫后3 wk, 接种表达MUC1的H22肝癌细胞. 2 wk后观察、记录肿瘤的生长情况. 于肿瘤细胞接种后43 d, 处死全部动物, 称肿瘤的质量. 荷瘤小鼠的瘤组织常规HE染色.

结果: 肿瘤细胞接种后43 d, MUC1预防组, 质粒pcDNA3.1对照组及生理盐水阴性对照组H22肝癌大小分别为547 ± 59 mm^3, 1185 ± 84 mm^3和1220 ± 95 mm^3 (P <0.01);平均瘤质量分别为1.87 ± 0.96 g, 4.19 ± 1.34 g和4.23 ± 1.32 g (P <0.01); pcDNA3.1对照组和生理盐水阴性对照组100 %可见瘤体形成, 肿瘤生长, 而MUC1基因疫苗预防组仅见50 % (5/10)的小鼠有瘤体形成, 与对照组相比, MUC1预防组H22肝癌生长受到明显抑制(P <0.01); MUC1预防组小鼠免疫保护有显著差异(P <0.05). 病理学检查结果显示, 与pcDNA3.1对照组相比, MUC1 DNA疫苗预防组鼠H22肝癌组织大量坏死.

结论: MUC1基因免疫显著抑制H22肝癌生长.

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0 引言

尽管化放疗等有效的抗肿瘤治疗使癌症患者生存率不断提高[1-4], 然而, 大多数癌症转移患者因不能被治

愈而死亡[5-7]. 因此, 探索有效的治疗肿瘤的新途径十分必要[8-10]. DNA疫苗以其简单而独特的免疫方式和良好的动物免疫效果而倍受关注[11,12]. 人MUC1基因的编码产物Mucin是一种Ⅰ型跨膜蛋白. 研究发现, MUC1在乳腺癌及肝癌等多种肿瘤中异常表达且由于糖基化不全, 导致新的抗原肽表位暴露,是肿瘤主动特异性免疫治疗理想的靶分子[13-17]. 我们探讨MUC1基因疫苗是否可以特异性抑制H22肝癌生长.

1 材料和方法

1.1 材料 小鼠肝癌细胞H22为本室保存. Balb/c小鼠20只, 雌雄不限, 4-6 wk, 15-20 g, 第四军医大学实验动物中心提供. 含32 tandem repeats的MUC1质粒pVax-MUC1由英国Imperial Cancer Research Fund的Taylor-Papadimitriou教授惠赠, *E.coli* DH5 α由本室保存. E.Z.N.A^R plasmid Miniprep Kit,购自Omega公司; DMEM购自Gibco公司; RPMI1640培养液(Sigma公司); 小牛血清购自杭州四季清生物工程材料研究所; MUC1单克隆抗体为Antibody Diagnostica公司产品; 链霉素-卵白素-生物素-过氧化物酶复合物(SABC)试剂盒、二氨基联苯胺(DAB)试剂盒(武汉博士德生物工程有限公司).

1.2 方法 MUC1基因疫苗pcDNA3.1(+)-MUC1构建及表达见文献[18]. 碱裂解法大量制备重组质粒pcDNA3.1-MUC1及空载体pcDNA3.1, 采用聚乙二醇沉淀法(PEG法)纯化. 无菌生理盐水溶解质粒, 紫外分光光度计测A_{260}及A_{280}计算浓度, A_{260}/A_{280}值达1.8-2.0, 定量, 用无菌生理盐水稀释成浓度为1 g/L, -20 ℃保存. 免疫组织化学法检测H22肝癌细胞MUC1表达 取少许H22肝癌细胞, 浓度以形成单细胞层为宜, 分别滴于多聚赖氨酸处理过的载玻片上, 经空气干燥后, -20 ℃冷丙酮固定5 min, 接着在蒸馏水中洗一下, 空气中干燥, 4 ℃保存. ABC法免疫染色, 封片待检. Balb/c小鼠20只随机分为3组, pcDNA3.1-MUC1实验组(MUC1组)10只, pcDNA3.1空白对照组(pcD3.1组)5只, 灭菌生理盐水阴性对照组(NS组) 5只. 每只小鼠100 μL (1 g/L)质粒, 小鼠股四头肌肌肉丰厚处肌肉注射. 3 wk/次, 共3次, 最后一次基因免疫后3 wk, 实验组和对照组小鼠于右臀及大腿部皮下接种0.2 mL (10^{10}/L) H22肝癌细胞, 进行保护实验. 每周观察记录1次肿瘤的生长情况, 用游标卡尺测量肿瘤的长、短径. 肿瘤体积按公式$V(mm^3)=0.4ab^2$计算[19], a=长(mm), b=宽(mm), 比较肿瘤大小并绘制生长曲线. 于肿瘤细胞接种后43 d, 处死全部存活动物, 称肿瘤的质量. 解剖并分离荷瘤Balb/c小鼠的瘤组织, 用40 g/L甲醛固定, 常规石蜡包埋, 切片, HE染色镜检, 观察肿瘤的组织细胞结构.

统计学处理 采用SPSS11.0软件进行方差分析和χ^2检验.

2 结果

2.1 肿瘤生长 接种H22肝癌细胞后43 d, MUC1预防组、质粒pcDNA3.1对照组及生理盐水阴性对照组H22肝癌大小分别为547 ± 59 mm^3, 1185 ± 84 mm^{3}和1220 ± 95 mm^3, 3组肿瘤生长速度有非常显著的差异(F=198.29, P=0.0 001, 图1), 差别主要在MUC1预防组与pcDNA3.1对照组和MUC1预防组与NS对照组之间(P<0.01), MUC1预防组H22肝癌生长受到明显抑制. MUC1 DNA免疫对小鼠H22肝癌的瘤体质量为1.87 ± 0.96 g, 比pcD3.1组4.19 ± 1.34 g和NS组4.23 ± 1.32 g轻(P<0.01).

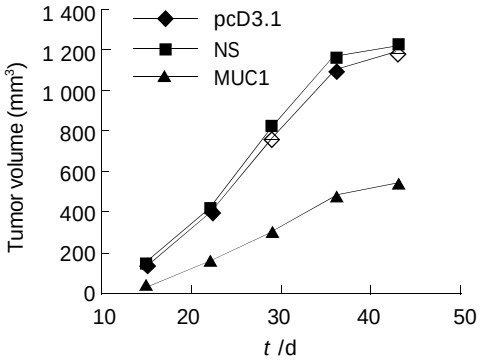

图1 小鼠H22肝癌生长曲线.

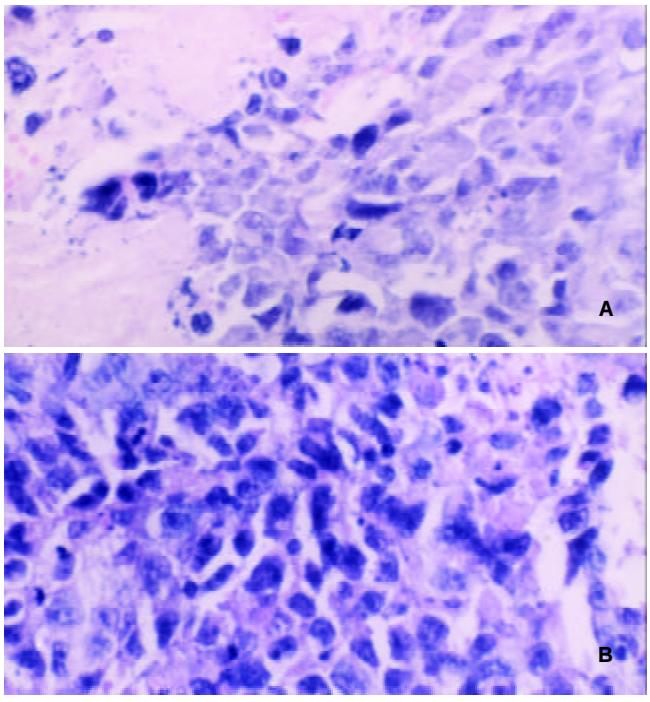

图2 MUC1疫苗组H22肝癌组织细胞坏死.
A: MUC1 cDNA组; B: 对照组.

2.2 体内免疫保护 小鼠接种H22肝癌细胞后观察43 d, MUC1基因疫苗预防组仅见50%(5/10)的小鼠有瘤体形成, 而pcDNA3.1对照组及生理盐水阴性对照组100 %可见瘤体形成, 肿瘤生长. 与对照组相比, MUC1预防组小鼠免疫保护有显著差异(校正χ^2=4.27, P<0.05). 与对照组相比, MUC1 DNA疫苗预防组鼠H22肝癌组织中大量肿瘤细胞变性和坏死(图2). H22肝癌细胞滴片

免疫组化呈阳性(图 3)

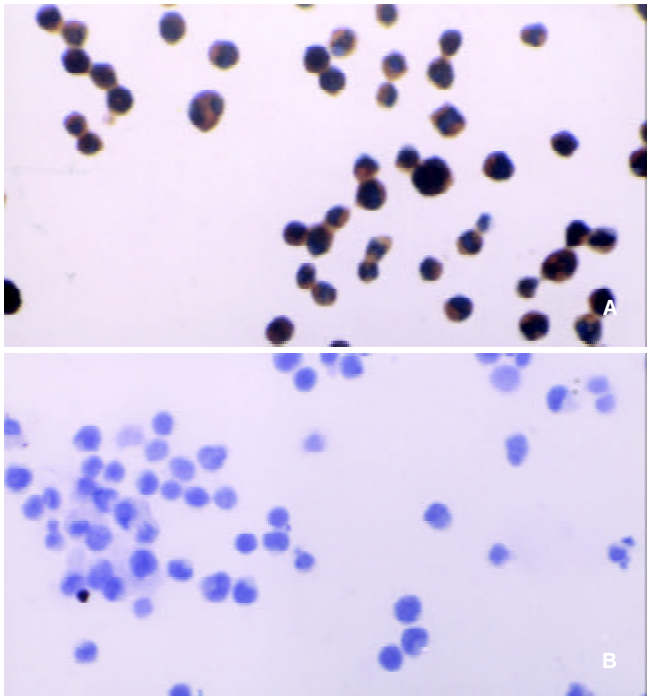

图 3 H22 肝癌细胞 MUC1 免疫组化染色.
A: Positive; B: Negetive.

3 讨论

基因免疫也称基因疫苗或 DNA 疫苗，是将编码某种抗原的基因片段克隆到真核表达质粒，再用该质粒 DNA 免疫动物，使之在机体细胞内持续表达具有天然构象的抗原蛋白，并刺激机体产生抗原特异的体液免疫和细胞免疫应答. 他的突出优点是能通过不同途径诱导产生细胞毒 T 淋巴细胞(CTL)，因此基因免疫在诱导抗肿瘤免疫方面比常规疫苗更具有优势. 肿瘤基因疫苗是将肿瘤相关抗原(TAA)或肿瘤特异抗原作为抗肿瘤免疫攻击的靶点[20]. 人 MUC1 基因的编码产物 Mucin，他的多肽骨架由胞外段、跨膜段和胞内段 3 部分组成, 含有抗原表位的重复序列位于胞外段，是细胞表面最先与机体免疫系统接触的膜表面分子，可诱发特异性抗乳腺癌的 CTL 免疫应答[21-26]. Takao *et al* [16]报道，MUC1 在肝内胆管癌 65 % 高表达，且与癌灶肝转移及预后不良相关. Cao *et al* [17]研究发现，人肝细胞癌(HCC)MUC1 阳性反应，可作为 HCC 的预后指标. MUC1 基因免疫是否可以抑制肝癌生长尚不清楚. 我们用构建的编码 Mucin 蛋白的基因疫苗 pcDNA3.1-MUC1 质粒免疫 Balb/c 小鼠后，用表达 MUC1 的 H22 肝癌细胞接种，证实 MUC1 基因免疫可以在小鼠体内特异性抑制 H22 肝癌生长.

动物实验前，我们用免疫组织化学法检测 MUC1 在 H22 肝癌细胞表达. 结果表明，H22 肝癌细胞滴片免疫组化均呈阳性染色结果. 在其基础上，我们将 2×10^6 个 H22 肝癌细胞接种在 Balb/c 小鼠右臀及大腿部皮下，接种肿瘤细胞后 10 d 左右即可摸到皮下的瘤结节，肿瘤在不同个体的生长速度均匀，成瘤率 100 %. 稳定的 H22 肝癌荷瘤动物模型的建立，为下一步的免疫预防研究提供了条件.

基因免疫结果显示，接种 H22 肝癌细胞后 43 d，MUC1 预防组、质粒 pcDNA3.1 对照组及生理盐水阴性对照组 H22 肝癌大小分别为 $547 \pm 59\ mm^3$，$1185 \pm 84\ mm^3$，$1220 \pm 95\ mm^3$，与对照组相比，MUC1 预防组肿瘤生长速度显著降低($P<0.01$). pcDNA3.1(pcD3.1)组和生理盐水(NS)对照组的肿瘤生长速度未见显著性差异($P>0.05$). 小鼠接种 H22 肝癌细胞后观察 43 d，MUC1 基因疫苗预防组仅见 50 % (5/10)的小鼠有瘤体形成，而 pcDNA3.1 对照组及生理盐水阴性对照组 100 % 可见瘤体形成，肿瘤生长. 与对照组相比，MUC1 预防组小鼠免疫保护有显著差异($P<0.05$). 生长曲线、平均瘤重和病理学检查结果均表明，与对照组相比，MUC1 预防组 H22 肝癌生长受到明显抑制($P<0.01$).

总之，初步研究表明 MUC1 基因疫苗免疫接种，可显著抑制 H22 肝癌生长，对于 Balb/c 小鼠荷瘤有免疫保护作用. 肝癌在我国及亚洲国家常见[27-35]，因此，本研究具有广阔前景.

4 参考文献

1 Tang ZY. Hepatocellular carcinoma–cause, treatment and metastasis. *World J Gastroenterol* 2001;7:445-454
2 Shimoda M, Bando T, Nagata T, Shirosaki I, Sakamoto T, Tsukada K. Prophylactic chemolipiodolization for postoperative hepatoma patients. *Hepatogastroenterology* 2001;48:493-497
3 Huang YH, Wu JC, Lui WY, Chau GY, Tsay SH, Chiang JH, King KL, Huo TI, Chang FY, Lee SD. Prospective case-controlled trial of adjuvant chemotherapy after resection of hepatocellular carcinoma. *World J Surg* 2000;24:551-555
4 Pacella CM, Bizzarri G, Cecconi P, Caspani B, Magnolfi F, Bianchini A, Anelli V, Pacella S, Rossi Z. Hepatocellular carcinoma: long-term results of combined treatment with laser thermal ablation and transcatheter arterial chemoembolization. *Radiology* 2001;219:669-678
5 Itamoto T, Katayama K, Fukuda S, Fukuda T, Yano M, Nakahara H, Okamoto Y, Sugino K, Marubayashi S, Asahara T. Percutaneous microwave coagulation therapy for primary or recurrent hepatocellular carcinoma: long-term results. *Hepatogastroenterology* 2001;48:1401-1405
6 Zheng N, Ye SL, Sun RX, Zhao Y, Tang ZY. Effects of cryopreservation and phenylacetate on biological characters of adherent LAK cells from patients with hepatocellular carcinoma. *World J Gastroenterol* 2002;8:233-236
7 Wakabayashi H, Ishimura K, Okano K, Izuishi K, Karasawa Y, Goda F, Maeba T, Maeta H. Is preoperative portal vein embolization effective in improving prognosis after major hepatic resection in patients with advanced-stage hepatocellular carcinoma? *Cancer* 2001;92:2384-2390
8 Huang JZ, Xia SS, Ye QF, Jiang HY, Chen ZH. Effects of p16 gene on biological behavious in hepatocellular carcimoma cells. *World J Gastroenterol* 2003;9:84-88
9 Sun L, Zhou YX, Hao CQ, Feng ZH, Zhao J, Hu PZ, Fu Y, Ma FC, Chang JQ, Wang JP, Nie QH. Effect of DNA vaccine on anti-HCV infection in mice with subcutaneous inoculating tumor. *Shijie Huaren Xiaohua Zazhi* 2003;11:165-168
10 Ikeda K, Arase Y, Saitoh S, Kobayashi M, Suzuki Y, Suzuki F, Tsubota A, Chayama K, Murashima N, Kumada H. Interferon beta prevents recurrence of hepatocellular carcinoma

after complete resection or ablation of the primary tumor -A prospective randomized study of hepatitis C virus-related liver cancer. *Hepatology* 2000;32:228-232
11 Bronte V. Genetic vaccination for the active immunotherapy of cancer. *Curr Gene Ther* 2001;1:53-100
12 Bhattachary R, Bukkapatnam R, Prawoko I, Soto J, Morgan M, Salup RR. Efficacy of vaccination with plasmid DNA encoding for HER2/neu or HER2/neu-eGFP fusion protein against prostate cancer in rats. *Int Immunopharmacol* 2002;2: 783-796
13 Mukherjee P, Madsen CS, Ginardi AR, Tinder TL, Jacobs F, Parker J, Agrawal B, Longenecker BM, Gendler SJ. Mucin 1-specific immunotherapy in a mouse model of spontaneous breast cancer. *J Immunother* 2003;26:47-62
14 Rahn JJ, Dabbagh L, Pasdar M , Hugh JC. The importance of MUC1 cellular localization in patients with breast carcinoma: an immunohistologic study of 71 patients and review of the literature. *Cancer* 2001;91:1973-1982
15 von Mensdorff-Pouilly S, Snijdewint FG, Verstraeten AA, Verheijen RH, Kenemans P. Human MUC1 mucin: a multifaceted glycoprotein. *Int J Biol Markers* 2000;15:343-356
16 Takao S, Uchikura K, Yonezawa S, Shinchi H, Aikou T. Mucin core protein expression in extrahepatic bile duct carcinoma is associated with metastases to the liver and poor prognosis. *Cancer* 1999;86:1966-1975
17 Cao Y, Karsten U, Otto G, Bannasch P. Expression of MUC1, Thomsen-Friedenreich antigen, Tn, sialosyl-Tn, and alpha2, 6-linked sialic acid in hepatocellular carcinomas and preneoplastic hepatocellular lesions. *Virchows Arch* 1999;434: 503-509
18 Yuan SF, Li KZ, Han W, Yan ZH, Zhang YQ. Construction of eukaryoticexpression vectors containing coding of human full length MUC-1 gene and its expression in COS-7. *Di Si Junyi Daxue Xuebao* 2002;23:1231-1234
19 Carr-Brendel V, Markovic D, Ferrer K, Smith M, Taylor-Papadimitriou J, Cohen EP. Immunity to murine breast cancer cells modified to express MUC-1, a human breast cancer antigen, in transgenic mice tolerant to human MUC-1. *Cancer Res* 2000;60:2435-2443
20 Mahvi DM, Shi FS, Yang NS , Weber S, Hank J, Albertini M, Schiller J, Schalch H, Larson M, Pharo L, Gan J, Heisey D, Warner T, Sondel PM. Immunization by particle-mediated transfer of the granulocyte-macrophage colony-stimulating factor gene into autologous tumor cells in melanoma or sarcoma patients: report of a phase I/IB study. *Hum Gene Ther* 2002;13:1711-1721
21 Yuan SF, Li KZ, Yan ZH, Han W, Zhang YQ. Suppressing effect of MUC1 gene vaccine on EMT6 breast cancer growth. *Di Si Junyi Daxue Xuebao* 2002;24:327-330
22 Snijdewint FG, von Mensdorff-Pouilly S, Karuntu-Wanamarta AH, Verstraeten AA, Livingston PO, Hilgers J, Kenemans P. Antibody-dependent cell-mediated cytotoxicity can be induced by MUC1 peptide vaccination of breast cancer patients. *Int J Cancer* 2001;93:97-106
23 Johnen H, Kulbe H, Pecher G. Long-term tumor growth suppression in mice immunized with naked DNA of the human tumor antigen mucin (MUC1). *Cancer Immunol Immunother* 2001;50:356-360
24 Musselli C, Ragupathi G, Gilewski T, Panageas KS, Spinat Y, Livingston PO. Reevaluation of the cellular immune response in breast cancer patients vaccinated with MUC1. *Int J Cancer* 2002;97:660-667
25 Miles DW, Taylor-Papadimitriou J. Therapeutic aspects of polymorphic epithelial mucin in adenocarcinoma. *Pharmacol Ther* 1999;82:97-106
26 Soares MM, Mehta V, Finn OJ. Three different vaccines based on the 140-amino acid MUC1 peptide with seven tandemly repeated tumor-specific epitopes elicit distinct immune effector mechanisms in wild-type versus MUC1-transgenic mice with different potential for tumor rejection. *J Immunol* 2001; 166:6555-6563
27 Yuen MF, Cheng CC, Lauder IJ, Lam SK, Ooi CG, Lai CL. Early detection of hepatocellular carcinoma increases the chance of treatment: Hong Kong experience. *Hepatology* 2000; 31:330-335
28 Chen TH, Chen CJ, Yen MF, Lu SN, Sun CA, Huang GT, Yang PM, Lee HS, Duffy SW. Ultrasound screening and risk factors for death from hepatocellular carcinoma in a high risk group in Taiwan. *Int J Cancer* 2002;98:257-261
29 Fan J, Wu ZQ, Tang ZY, Zhou J, Qiu SJ, Ma ZC, Zhou XD, Ye SL. Multimodality treatment in hepatocellular carcinoma patients with tumor thrombi in portal vein. *World J Gastroenterol* 2001;7:28-32
30 Yang YL, Dou KF, Li KZ. Correlation of UPAR and VEGF expression with invasion and metastasis in human hepatocellular carcinoma. *Shijie Huaren Xiaohua Zazhi* 2002;10:381-383
31 Li XM, Tang ZY, Qin LX, Zhou J, Sun HC. Serum vascular endothelial growth factor is a predictor of invasion and metastasis in hepatocellular carcinoma. *J Exp Clin Cancer Res* 1999;18:511-517
32 Niu Q, Tang ZY, Ma ZC, Qin LX, Zhang LH. Serum vascular endothelial growth factor is a potential biomarker of metastatic recurrence after curative resection of hepatocellular carcinoma. *World J Gastroenterol* 2000;6:565-568
33 Zhou J, Tang ZY, Fan J, Wu ZQ, Li XM, Liu YK, Liu F, Sun HC, Ye SL. Expression of platelet-derived endothelial cell growth factor and vascular endothelial growth factor in hepatocellular carcinoma and portal vein tumor thrombus. *J Cancer Res Clin Oncol* 2000;126:57-61
34 Sun HC, Tang ZY, Li XM, Zhou YN, Sun BR, Ma ZC. Microvessel density of hepatocellular carcinoma: its relationship with prognosis. *J Cancer Res Clin Oncol* 1999;125:419-426
35 Shuto T, Hirohashi K, Kubo S, Tanaka H, Yamamoto T, Higaki I, Takemura S, Kinoshita H. Treatment of adrenal metastases after hepatic resection of a hepatocellular carcinoma. *Dig Surg* 2001;18:294-297