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胆管癌 *P* 53 抑癌基因突变及表达的研究

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提 要 应用免疫组化法及多聚酶链反应和限制性片段长度多态性法(PCR-RFLP),检测32例新鲜组织和石蜡包埋组织胆管癌的p53蛋白过表达,及*P* 53抑癌基因248、249位点的突变。结果表明:胆管癌*P* 53蛋白过表达率(40.6%)显著高于慢性炎症胆管壁粘膜($P<0.05$),*P* 53抑癌基因248、249位点的突变率分别为25%(8/32)和3.1%(1/32),突变和过表达与肿瘤的生长部位、组织学类型和临床分期无关。提示:*P* 53抑癌基因的突变可能是胆管癌发生的后期事件,对维持胆管癌的恶性表型有关。*P* 53 248位点可能是胆管癌*P* 53抑癌基因的突变热点。p53蛋白过表达可作为临床上诊断胆管癌的辅助方法之一。

关键词 胆管癌;免疫组化;*P* 53抑癌基因;突变热点;PCR-RFLP;生物学行为

中图法分类号 R735.8

肿瘤是一种基因病,探索肿瘤细胞的基因损害,在当前肿瘤研究中占主导地位。有关胆管癌的癌基因和抑癌基因的突变及表达的研究,国外文献报道较少。对于胆管癌*P* 53抑癌基因的研究,主要限于肝内胆管癌[1],在肝外胆管癌尚未见*P* 53基因点突变的报道。我们采用聚合酶链反应和限制性片段长度多态性分析(PCR-RFLP)法,探索32例肝内、外胆管癌*P* 53抑癌基因第248、249位点的突变情况,并用免疫组化法研究*P* 53抑癌基因产物的表达情况,以期从分子水平探讨胆管癌发生、发展机理,为临床早期诊断及防治提供依据。

1 材料与方法

1.1 标本及DNA提取

32例我院和解放军第一医院1986~1994年手术切除及活检的标本,25例经常规10%福尔马林固定、石蜡包埋,7例为新鲜组织。10例我院1991~1994年手术切除的胆总管囊肿或慢性胆管炎的胆管壁组织,经10%福尔马林固定、石蜡包埋。胆管癌石蜡包埋组织连续切片,一张行HE染色,以证实为肿瘤组织及组织学分类,同时切4 μm厚免疫组化染色片。对照HE切片,充分剔除周围无关组织,连续切5~8 μm厚蜡片3~5张。新鲜胆管癌组织行冰冻切片HE染色,对照HE片取材。DNA提取用酚、氯仿抽提、蛋白酶K消化法。石蜡组织用大剂量蛋白酶K(800 μg/ml)消化5~6 d,酚、氯仿提取2~3次,加RNA酶,37℃消化3 h,乙醇沉淀、TE溶解。新鲜组织加蛋白酶K消化18~24 h,酚、氯仿提取,乙醇沉淀。

1.2 免疫组化染色

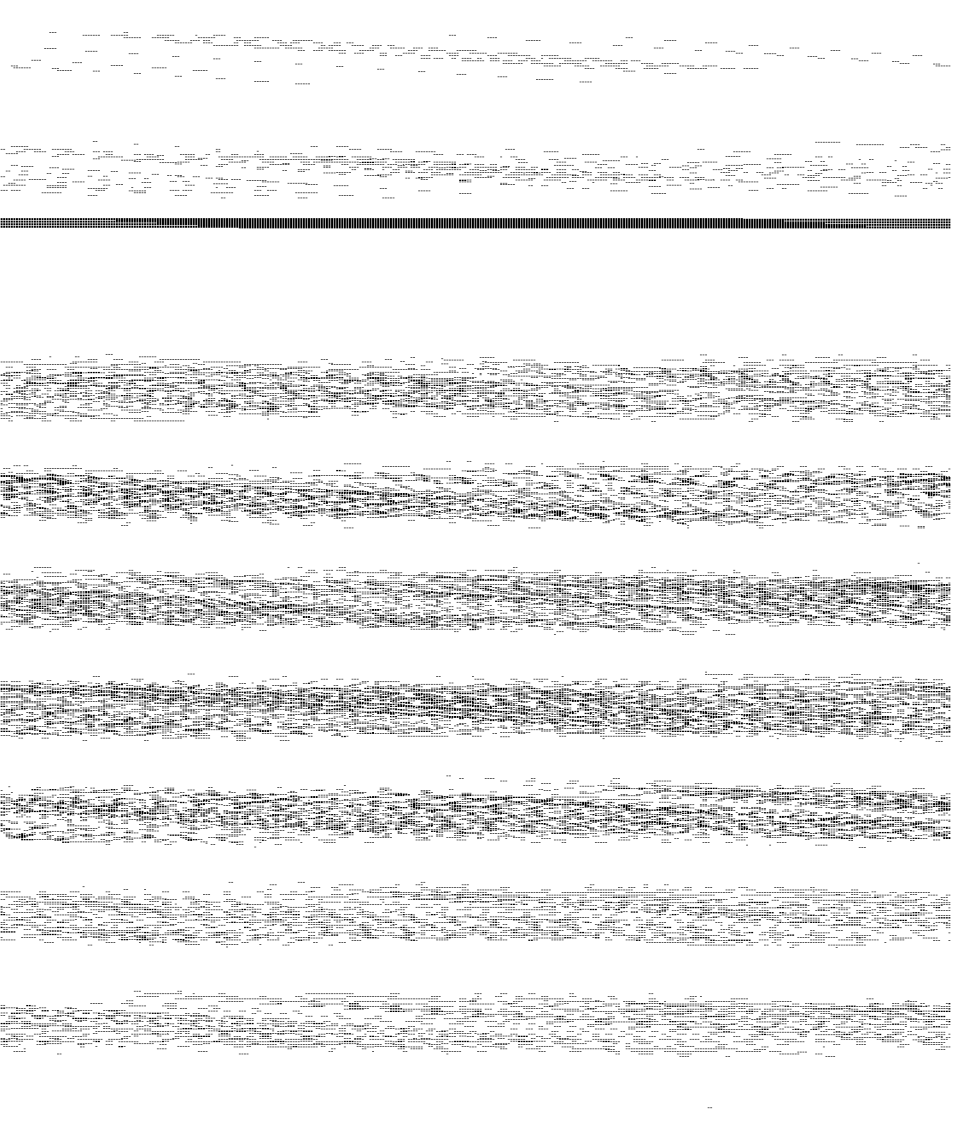
用链菌素亲生物蛋白-过氧化酶免疫组化染色法(SP法)。第一抗体为p53鼠抗人单克隆抗体DO-7(1∶50)(福州迈新生物公司),SP试剂盒及DAB酶、底物试剂盒(福州迈新生物公司)。操作步骤按说明书及有关文献稍作改良。以磷酸生理缓冲液(PBS,0.01 mol/L,pH7.4)代替第一或第二抗体做为阴性对照,用已知p53蛋白阳性胃癌及大肠癌做为阳性对照(亦购自迈新生物公司)。结果判断:p53阳性表达按Teh[2]标准判断染色结果,染色范围分为:<10%、10%~50%、>50%三级,染色强度分:轻度和中度染色及重度染色二组,其中重度染色范围>10%或轻、中度染色范围>50%为强阳性,结果分为三组:阴性、阳性、强阳性。染色范围主要为肉眼光镜下计数,染色强度以肉眼观察与图像分析系统检测的灰度相结合。首先肉眼确定较典型的轻、中、重度染色,然后测其灰度作为标准,在标准附近的染色再划为不同的染色强度组。

1.3 聚合酶链反应限制性片段长度多态性分析

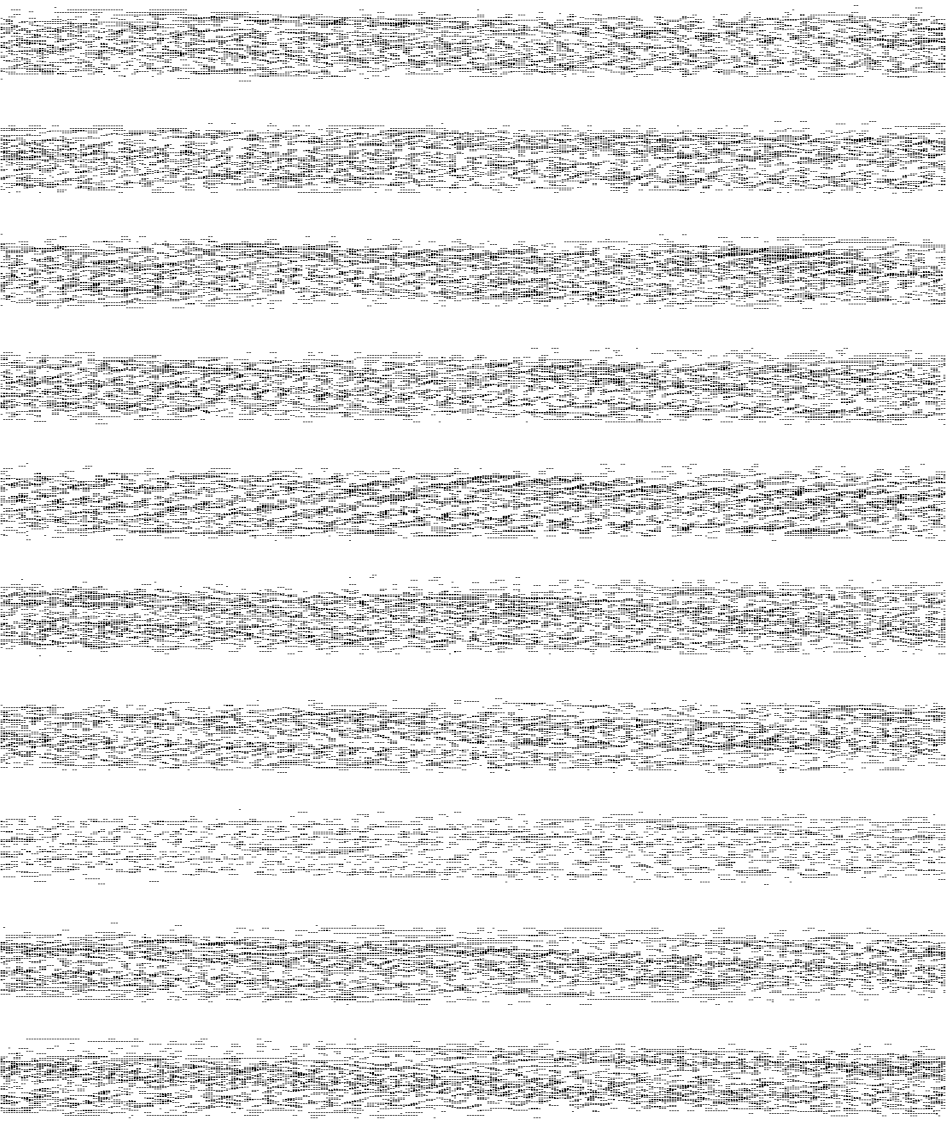
按有关文献稍作改良:取石蜡组织DNA 3~5 μl,新鲜组织DNA 1~2 μl,加入40 μl反应体系中,其中含10 mmol/L Tris-HCl(pH8.3),50 mmol/L KCl,1.5 mmol/L $MgCl_2$,0.2 mmol/L 4×dNTP,1 μmol/L引物,2~5 u Taq DNA聚合酶(华美公司),表面覆盖等体积石蜡后,置PCR循环仪上扩增。首次变性:石蜡组织:93℃ 8 min+100℃ 4 min,新鲜组织:93℃ 8 min+100℃ 8 min。循环参数:95℃ 35 s→55℃ 40 s→72℃ 1 min,循环35次,72℃保温10 min。取5~10 μl扩增产物,用1.5%~2%琼脂糖凝胶电泳检查,EB染色,紫外灯下观察扩增结果。将PCR扩增后的产物用等体积酚、氯仿(1∶1)抽提,4℃ 14,000 r/min离心20 min,加入2.5倍体积无水乙醇,1/10体积3 mol/L醋酸钠(pH4.8),置-20℃冰箱过夜,然后4℃ 14,000 r/min离心20 min。加75%乙醇反应30 min,再4℃ 14,000 r/min离心20 min,倒上清,真空抽干,加消毒去离子水12 μl溶解。用相应的限制性内切酶Hpa Ⅰ/Msp Ⅰ、Hae Ⅲ(华美公司)消化16~36 h(置37℃水浴),8~10%聚丙烯酰胺垂直电泳,EB染色,紫外灯下观察、照像。

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基因的突变及 p53 蛋白过表达有较密切的联系。*P* 53 基因的突变可能在胆管癌的发生和演进中占据重要地位，并且可能是胆管癌发生的后期事件，对维持肿瘤的恶性表型有关。*P* 53 基因 248 位点可能是胆管癌 p53 抑癌基因的突变热点之一。p53 蛋白的过表达，可作为临床上诊断胆管癌的依据之一。*P* 53 基因点突变及 p53 蛋白过表达与胆管癌生物学行为关系不明显，尽管 *P* 53 248 位点是胆管癌较重要的基因损害，但它们并不是唯一影响胆管癌临床病理特征的因素。

参考文献

1 Kiba T, Tsuda H, Pairojkuk C *et al*. Mutation of the p53 tumor suppressor gene and the ras gene family in intrahepatic cholangiocellular carcinomas in Japan and Thailand. Mol Carcinog, 1993; 8(4): 312

2 Teh M, Wee A, Raju GC *et al*. An immunohistochemical study of *P*53 protein in gallbladder and extrahepatic bile duct/ampullary carcinomas. Cancer, 1994; 74(5): 1542

3 Henson DE, Albores-Savedra J, Corle D *et al*. Carcinoma of the extrahepatic bile ducts: histologic types, stage of disease, grade, and survival rates. Cancer, 1992; 70(6): 1498

4 Hollstein M, Sidransky D, Vogelstein B *et al*. p53 mutations in human cancers. Science, 1991; 2353: 49

5 Finlay CA, Hinds PW, Levine AJ *et al*. The *P*53 Proto-oncogene can act as a suppressor of transformotion. Cell, 1989; 57: 1083

6 Chang F, Syrjanen S, Tervahauta A *et al*. Tumorigenesis associated with the p53 tumor suppressor gene. Br J Cancer, 1993; 68: 653

7 Choi SW, Hytiroglou P, Geller SA *et al*. The expression of p53 antigen in primary malignant epithelial tumors of the liver: an immunohistochemical study. Liver, 1993; 13: 172

8 Bodner SM, Minna JD *et al*. Expression of mutant *P*53 proteins in lung cancer correlates with the class of p53 gene mutation. Oncogene, 1992; 7: 743

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Study of mutation and expression of antioncogene *P53* in cholangiocarcinoma

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Abstract The expression of p53 protein and its mutation at codons 248 and 249 were observed in 32 specimens of fresh and formalin-fixed tissue of cholangiocarcinoma with immunohistochemistry and PCR-RFLP. It was found that p53 was positive in 40.6% cases of cholangiocarcinom, which was significantly higher than that in chronic inflammatory cholangiomucosa ($P < 0.05$). Mutation of *P*53 at codons 248 and 249 was found in 25% (8/32) and 3.1% (1/32) of cases respectively. There was no relationship of the mutation of *P*53 with the location of the cancer, its histological types or its clinical staging. These findings indicate that mutation of *P*53 might occur in the late stage of cholangiocarcinoma and is related to maintain the phenotype of the carcinoma, codon 248 of *P*53 may be the hot spot to have mutation and over-expression of p53 may be used as a supplementary procedure to diagnose cholangiocarcinoma.

Key words cholangiocarcinoma; immunohistochemistry; PCR-RFLP; *P*53; mutation; expression; behavior/biological

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