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Studies of the effects of recombinant human tumor necrosis factor on human colon carcinoma cell line SW-480 in vitro

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Abstract The cytotoxic and colony inhibiting effects of recombinant human tumor necrosis factor (rhTNF) on human colon carcinoma cell line SW-480 were observed in vitro with modified MTT and ^{3}H-TdR incorporation. It was found that rhTNF 60 000 u/ml exerted obvious cytotoxic effect on SW-480 cells and the effect was not dosage-dependent. The SW-480 cells seemed to be low sensitive to rhTNF. However, the colony inhibiting test revealed that rhTNF 10 u/ml was capable of inhibiting the growth of SW-480 cell colonies in a dosage=dependent manner. When the dosage of rhTNF was larger than 150 u/ml, the inhibition was increased with the prolongation of time.

The ultrastructural changes of SW-480 cells after rhTNF treatment were small holes on the cell membrane in the initial stage, then degeneration and lysis of the mitochondria, pyknosis, karyolysis and eventually cytolysis.

On the basis of the fact that the curve of the cytotoxic effect of rhTNF determined with MTT and that of the colony inhibiting effect determined with ^{3}H-TdR incorporation were consistent, it is believed that the lethal effects of rhTNF on the SW-480 cells were mainly on the inhibition of the DNA synthesis of intra- and extranuclear chromosomes of the cells.

Key words tumor necrosis factor; colonic neoplasms; SW-480 cells

原发性肝癌 P^{53} 基因249编码区点突变的研究

Study of the P^{53} gene mutation at codon 249 in primary hepatocellular carcinoma

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本文采用 PCR－RFLP 法对原发性肝细胞癌 P^{53} 基因第249编码区的点突变进行分析，探讨其在肝癌发生中的作用。

1 材料和方法

25例肝癌组织为我院手术切除标本，均经病理证实。手术切下肿瘤后立即收集癌组织，剔除出血坏死部分后置液氮中，－70℃贮存。按文献提取基因组 DNA[1]。

PCR 引物由中国科学院上海细胞生物所合成，上游引物序列：5′－CACAGTCTTTATTCTTCAGCG－3′；下游引物序列：5′－CGAAGAGTGAAGTGCACAGG－3′。取约1 μg 组织 DNA 溶于40 μl PCR 缓冲液中(10 mmol/L Tris－HCl，50 mmol/L KCl，1.5 mmol/L $MgCl_2$，200 μmol/L dNTP，1 μmol/L 引物，2～3u Taq DNA 多聚酶)，PCR 扩增仪(Perkin Elmer Cetus)上操作：93℃变性35 s，55℃退火40 s，72℃延伸1 min。循环35次后，72℃延伸10 min。取7 μl 扩增产物在2%琼脂糖凝胶电泳上确定扩增效果。

PCR 扩增产物用酚/氯仿抽提，乙醇沉淀，真空抽干，去离子水溶解，加 Hae Ⅲ 消化，37℃过夜。酶解产物8%聚丙烯酰胺电泳分离，EB 染色，紫外光下观察并照像。

结果判定：DNA 经 PCR 扩增后，扩增片段长164 bp，用 Hae Ⅲ 酶切后野生型被切为90，46和27 bp 三个片段，突变型则为136，27 bp 两个片段[1]。

2 结果

在25例肝癌中有5例出现136 bp带，即249位密码子发生突变。

3 讨论

抗癌基因 P^{53} 与细胞增殖分化的调节有关，因其蛋白产物对几种细胞生长负调控基因有调节作用，它的失活将导致细胞的异常增殖。而 P^{53} 基因突变是其失活的主要途径之一，也是许多肿瘤发生的一个共同现象[2]。尤其是位于高度保守区的249位密码子突变是当今肝癌 P^{53} 基因突变研究的热点所在。在黄曲霉毒素 B_1(AFB_1)污染严重的地区，如我国的启东以及南非地区，该位点在肝细胞癌的突变率可高达50%，并认为 P^{53} 基因第249位密码子为 AFB_1 作用的靶位点[3,4]。最近 Aguilar[5] 在体外实验也证实 AFB_1 作用的特异位点为 P^{53} 基因第249位密码子。然而，肝癌的发生是受多因素影响的，不同地区的肝癌因病

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Canges of myocardial calcium, oxygen free radicales and neutrophil infliration of ischemic and reperfused heart in rabbits

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Abstract The content of myocardial calcium (Ca^{++}) and malodialdehyde (MDA) and the density of neutrophil infiltration were increased in the ischemic period of the heart and became more severe in the reperfusion period. The content of Ca^{++} and MDA reached the peak in the first half hour to 1.5 hours after reperfusion but neutrophil infiltration was increased gradually and no decrease was observed even in the 6th hour after reperfusion. These findings suggest that the damages of the myocardium resulting from the 3 harmful factors occur in different time intervals after reperfusion and they can injure the myocardium not only in the ischemic period but also in the reperfusion period.

Key words reperfusion injury; calcium overloading ; free radicals; superoxide dismutase; neutrophils;

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因不一样，其 P^{53} 基因249位密码子突变率也不一样。主要病因为HBV的我国西北地区肝癌，该位点的突变率极低(2.2%)[16]，受同样病因作用的日本肝癌 P^{53} 基因的突变分散于基因序列高度保守区，而无突变热点所在[7]。本实验病例均来自西南地区，以往的实验表明该地区肝癌的发生多与 HBV 和 HCV 感染有关，尚无 AFB_1 的资料。本实验发现20%的原发性肝细胞癌 P^{53} 基因249位密码子突变，较 AFB_1 高污染区的西北地区明显增高。形成该特点的原因是否与西南地区肝癌受 HBV，HCV 和 AFB_1 双重因素的作用有关，尚需进一步扩大研究的样本量和对 AFB_1 在西南地区污染的流行病学调查。

关键词 肝肿瘤；肿瘤基因；突变

中图法分类号 R394

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