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## Relationship between overexpression of p53 protein and intratumoral microvessel density in osteosarcoma

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**Abstract** **Objective**: To study the relationship between overexpression of p53 protein and intratumoral microvessel density (MVD) and their prognostic significance in osteosarcoma. **Methods**: The expression of p53 protein and MVD count in 80 cases of osteosarcoma were determined with immunohistochemistry and morphometry. **Results**: The overexpression of p53 protein was significantly associated with MVD count and the two parameters were important in evaluation of the prognosis of osteosarcoma. p53 positivity was the most important index to predict the overall survival rate in patients with osteosarcoma. **Conclusion**: It is indicated that p53 protein involves in angiogenesis and its overexpression is an important angiogenic phenotype in neoplasm. Meanwhile, p53 protein and MVD may be useful prognostic indicators for patients with osteosarcoma.

**Key words** bone tumor; p53; angiogenesis

# 反义核酸抑制 TGFβ1表达及对肝纤维化调节的研究* 博士生论文摘要

## Study of inhibition of TGF-β1 expression and regulation of hepatic fibrogenesis by antisense RNA

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肝纤维化是由肝细胞的损伤以及相继持续发生的炎症反应所启动,结果不仅导致细胞外基质(Extracellular matrix, ECM)的堆积,还使 ECM 成分发生永久的分子和组织结构的重新分布,结果出现严重临床并发症。因此,肝纤维化的治疗一直是困绕人们的难题,到目前为止,仍无一种有效的治疗方法。

已经证明,纤维化肝脏的 ECM 主要由 Ito细胞合成。人和动物的肝纤维化肝脏产生的大量转化生长因子-β1(Transforming growth factor, TGFβ1)是刺激 Ito细胞激活、产生过量 ECM 的主要因子。

为了探讨能否通过抑制 Ito细胞和实验性肝纤维化 TGF-β1的产生来抑制 ECM 堆积,我们采用脂质体和逆转录病毒相结合的方法将反义 TGFβ1基因导入 Ito细胞株 LI90及 $CCl_4$诱导的大鼠纤维化肝脏内,以研究反义 TGFβ1在抑制 ECM 产生及抗肝纤维化中的作用。

(1)反义 TGFβ1逆转录病毒载体的构建及其包装和滴度测定:将人的转化生长因子 β1 cDNA(1467bp)反向插入逆转录病毒载体 pLXSN,筛选转化菌,挑取抗性克隆,用限制性内切酶酶切鉴定重组子。用脂质体介导反义 TGFβ1重组逆转录病毒载体和空载体转染包装细胞 PA317,筛选 G418抗性细胞克隆,并用 NIH3T3细胞测定病毒滴度,空载体感染细胞和重组病毒感染细胞分泌的病毒颗粒效价分别为 $4.23\times10^5$ CFU/ml 和 $3.75\times10^6$ CFU/ml

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# Changes of endothelin in plasma and pancreas in rats with acute hemorrhagic necrotizing pancreatitis and their significance

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**Abstract** **Objective**: To measure the content of endothelin (ET) in the plasma and pancreas in rats with acute hemorrhagic necrotizing pancreatitis (AHNP) and determine the role of ET in the pathogenesis of AHNP. **Methods**: After the model of AHNP was established with the injection of 5% sodium taurocholate into the bile-pancreatic duct in rats, the changes of pancreatic blood flow (PBF), levels of ET and 1, 4, 5-trisphosphate (IP3) in pancreatic tissue and the content of $[Ca^{2+}]i$ in pancreatic cells were dynamically observed. Meanwhile, the pathological changes of the pancreatic tissue (PCPT) were also quantitatively analyzed. **Results**: The level of ET in the plasma and pancreatic tissue was significantly increased and its increase in the plasma was slower than that in the pancreatic tissue in the early stage. Significant correlation was found among ET level in the plasma, ET level in the pancreatic tissue, PBF, IP3, $[Ca^{2+}]i$ and PCPT and the first was more closely correlated with the last four than the second. **Conclusion**: ET in the pancreas might mediate pancreatic ischemia in the early stage and that in the plasma aggravate pancreatic injury after AHNP in rats.

**Key words** acute pancreatitis; endothelin; blood circulation; $Ca^{2+}$; rat

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(2)反义 TGFβ1调节贮脂细胞 TGFβ1 mRNA的表达及抑制细胞外基质的合成:将高滴度的重组反义 TGFβ1逆转录病毒载体感染 Ito细胞株 LI90,经 G418筛选,获得抗性克隆并命名为 LIAT;用 Southern Blot杂交证实重组病毒已在转染细胞基因组 DNA内稳定整合,通过 RT-PCR和 Northern Blot杂交检测转染细胞内有反义 TGFβ1RNA存在。进一步用原位杂交及 RT-PCR分析发现转染反义 TGFβ1的培养细胞 LIAT表达的 TGFβ、TβRⅠ TIMP2 CoL1A1和 CoL4A1 mRNA明显低于未转染细胞,进一步用 Western Blot、免疫组化、ELISA和 RIA检测培养 LIAT细胞及培养上清内合成分泌的 TGFβ1及Ⅰ Ⅲ Ⅳ前胶原和 FN明显降低。另外,用免疫组化和原位杂交分析发现 LIAT细胞表达的 MT-MMP明显高于 LI90细胞,提示反义 TGFβ1RNA能有效抑制 Ito细胞内源性 TGFβ1的表达、合成分泌以及胶原等 ECM的合成;并能下调金属蛋白酶抑制剂 TIMP的表达和上调 TβRI及 MT-MMP的表达,加速 ECM的降解。

(3)脂质体和逆转录病毒复合物介导反义 TGFβ1RNA肝脏转移及其对肝纤维化的调节:我们通过门静脉将反义 TGFβ1基因与阳离子脂质体混合后再与重组逆转录病毒一起注入 $CCl_4$诱导的大鼠肝纤维化模型肝脏内,结果用 RT-PCR证实转基因大鼠肝脏有反义 TGFβ1 RNA的转录和表达。同时用原位杂交、Western印迹、免疫组化和 ELISA等方法检测发现,转染反义 TGFβ1的纤维化肝脏内源性 TGFβ1的表达、合成分泌明显低于未转染组。此外,通过免疫组化和原位杂交分析证实转基因组大鼠纤维化肝脏门静脉周围、肝小叶和纤维间隔内的 TβRI TIMP和 FN阳性细胞明显低于未转基因组,但是 MT-MMP MMP2 MMP9 MMP3和 TβR II阳性细胞却高于未转基因组。常规 HE染色和 V-G染色提示转基因组大鼠纤维化肝脏内的胶原纤维间隔较未转基因组减少,进一步通过扫描电镜观察发现转基因大鼠纤维化肝脏贮脂细胞内的微管及致密颗粒明显较未转基因组少。结果显示反义 TGFβ1RNA能通过抑制内源性的 TGFβ、TIMP1和 FN的表达以及刺激金属蛋白酶的产生而降低 ECM在肝脏的堆积。

总之,我们的结果提示反义 TGFβ1RNA能减缓或改善肝脏纤维化进程,对于慢性肝脏纤维化的治疗具有潜在的应用价值。

关键词 肝硬变;转化生长因子β1;反义核酸
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