DOI:10.16016/j.1000-5404.1999.07.002

Hammerhead ribozyme-mediated cleavage of mdrl mRNA with different length*

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Abstract Objective: To explore the new methods to reverse the multidrug resistance of bladder cancer. Methods: A hammerhead ribozyme was designed to cleave the GUC sequence at codon 1959 of mdrl mRNA after the analysis of the secondary structure of mdrl mRNA by computer. The hammerhead ribozyme 1 gene was synthesized and cloned into the *BamH* I /*EcoR* I sites of pGEMEX-1 called pEX-RZ1. The 864 bp fragment of mdr1 cDNA was subcloned into the *Hind* Ⅲ/*EcoR* I sites of pGEMEX-1 called pMDR-864. The 1383 bp fragment of mdr1 cDNA was subcloned into the *EcoR* I site of pGEMEX-1 called pMDR-1383 (+5). The pEX-RZ1 and pMDR-864 were lineralized with *Hind* Ⅲ while the pMDR-1383(+5) was lineralized with *BamH* I and these lineralized plasmids were transcribed with T3 promotor *in vitro*. Results: The ribozyme 1 showed strong cleavage activity after heing mixed with the transcribed RZ1 and its substrates for 2 hours at 42℃ and 52℃. Conclusion: The designed ribozyme can be used as a tool to reverse the multidrug resistance of bladder cancer. the cleavage activity of the ribozyme is not associated with the length of its substrate but depended on the location of the cutting site.

Key words multidrug-resistance gene; ribozyme; gene transfer; bladder cancer

* This project was supported by the National Natural Science Foundation of China, No. 39670824

个案与短篇

成功救治脑干出血1例

A case report for curing hemorrhage of brain stem successfully

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患者,男,68岁,因烦躁,谵妄1 d于1998年5月18日入院。患者于4 d前早上散步时突发意识障碍倒地,右额部跌伤,当地医院予脱水、止血、补液等对症治疗。1 d前,患者出现烦躁、谵妄,意识呈昏睡状态,为进一步救治,转入我院。既往无高血压史。查体:血压16/9 kPa,昏睡状态,双侧瞳孔不等大,右侧约3 mm,左侧约2 mm,光反射灵敏。心、肺(-)。四肢肌张力正常,肌力Ⅴ级。腱反射(++),巴氏征(-)。头颅CT两个层面示中脑被盖部中线左侧血肿,大小约1.2 cm×0.9 cm,双侧额颞部硬膜下积液。查血常规:白细胞11.1×10^9/L,N 90%,L10%。红细胞4.92×10^{12}/L,血色素149 g/L,血小板191×10^9/L。生化肾功能正常,血糖7.2 mmol/L。患者病情危重,入院后意识障碍逐步加重,入院后第2天呈昏迷状态。患者入院时血压偏低为16/9 kPa,此后血压渐下降,波动于9.33～12/4.67～7.33 kPa,积极予多巴胺,阿拉明升血压并加大补液量,输血浆等治疗。第5天后患者血压平稳,波动于13.3～16/8～9.33 kPa。患者于入院后第3天出现持续高热,体温波于39～40.8℃。血常规:白细胞24.0×10^9/L,N90%,L10%。胸片示右上肺大叶性肺炎,予舒普深抗炎3 d,病情无明显好转,痰检见霉菌,未找到抗酸杆菌,换用泰能抗感染,并应用大蒜素抗霉菌等治疗3 d,病情好转,继续用罗氏芬抗炎等治疗4 d,患者病情明显好转,无明显发热,复查血常规正常,胸片示右上肺浸润性肺结核,血沉97 mm/h,进一步查胸部CT示右上肺浸润性肺结核,部分纤维化。遂加用口服抗痨药治疗。患者入院后多次测血糖均高于正常值,最高达14.64 mmol/L。经予胰岛素治疗后,血糖基本控制在正常范围。经积极脱水、补液、升压、抗感染及加强营养支持等综合治疗下,患者病情逐渐好转,意识障碍减轻,于第7天呈嗜睡状态,12 d以后逐渐恢复清醒。治疗43 d后病情明显好转,能扶床下地行走,遂出院带药治疗。

讨论

脑干出血在临床上较为常见,是危险性、死亡率高的疾病之一。有报道,若血肿直径大于脑干横径的50%或直径大于2 cm则预后不良。本例患者头颅CT两个层面示中脑被盖部中线左侧血肿,大小约1.2 cm×0.9 cm,双侧额颞部硬膜下积液,诊断明确,且年龄大,合并糖尿病、肺结核及硬膜下积液,使病情变得复杂,增加了治疗上的困难,有一些救治经验值得总结[2]。

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（收稿：1998-09-21；修回：1999-03-14）

（编辑　刘　鹏）

Effects of retrovirus-mediated human interferon-γ gene on cholangiocarcinoma cell line

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Abstract　**Objective**: To investigate the changes of the immunogenicity of the cholangiocarcinoma cell line QBC 939 transduced with human interferon-γ gene. **Methods**: Retroviral vector pZIP-IFN-γ carrying human IFE-γ cDNA was constructed and transduced to QBC 939 cells. The expression of MHC class I antigen and the changes of the immunogenicity of the transduced QBC 939 cells were studied with Western blot, mixed lymphocyte and tumor cell reaction (MLTR) and assay of cytotoxicity on lymphocytes respectively. **Results**: The IFN-γ activity secreted by the G418 positive QBC939-IFN-γ cells was 0～9.51 IU/10^6 cells/48 h. Southern blot and Slot blot confirmed the presence of the incorporation and expression of the target gene. The expression level of MHC class I antigen was 1.78 times higher in the transduced cells than in the untransduced ones. MLTR revealed marked proliferation of lymphocytes in the peripheral blood of patients with cholangiocarcinoma. Enhancement of lymphocytic lysis was found in HLA A2 matched patients. **Conclusion**: The immunogenicity of QBC 939 cells was enhanced after the transduction interferon-γ gene.

Key words　cholangiocarcinoma cell line; gene therapy; interferon-γ; tumor vaccine; immunogenecity

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①积极脱水降颅压，减轻脑水肿，并处理好与低血压、低血容量之间的矛盾。为避免脱水所致血压波动和血容量不足，采用了人体白蛋白与血浆交替脱水的疗法，并加大补液量。临床治疗效果佳。②控制好血压，保证脑灌流。本例患者血压低，血容量不足，为避免低血容量所致脑灌流不足，脑损伤加重，在加大补液量的同时，予多巴胺，阿拉明升血压，保持血压稳定在适当范围内。③积极预防和治疗肺部感染。患者为老年人，先后合并肺部细菌、真菌感染，予舒普深、泰能抗炎、大蒜素抗霉菌等治疗后，复查胸部 CT 提示右上肺浸润性肺结核，并予口服抗痨药物治疗，病情复杂危重，救治及时。从本例患者治疗中体会，我们只有早期联合用药，并注意舒普深、泰能等强抗生素用药 3 d 都会出现霉菌感染，才能有效控制肺部感染。④积极控制血糖并处理好胰岛素用量的问题。患者虽为高血糖状态，在救治中仍要适当补充糖水和能量。高血糖状态易致酸碱平衡失调而加重脑细胞损害，也可发生酮症酸中毒而导致死亡。故控制好血糖具有极为重要的意义。胰岛素用量不足，仍为高血糖状态，胰岛素用量过大，易致低血糖昏迷加重脑损伤，这就要求胰岛素用量个体化，在用药的同时不断监测患者血糖变化，随时调整剂量，以保证血糖控制在适当范围。⑤加强营养支持，维持机体水、电解质和酸碱的平衡，保证脑部内环境的稳定。

关键词　脑干出血；治疗

中图法分类号　R743.34

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（收稿：1999-01-15；修回：1999-05-12）

（编辑　刘　鹏）

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