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Effects of acute hypoxia on the functions of the diaphragm in dogs

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Abstract In order to observe the functions of the diaphragm during acute hypoxia, 15 dogs were equally and randomly divided into the hypoxic group, salbutamol-treated group and control group. It was found that 15 minutes after hypoxia, the transdiaphragmatic pressure (TDP) was progressively decreased with all kinds of frequency of stimulation, and the central frequency (FC), the medium frequency (FM) and the high/low frequency ratio (H/L) were all significantly lower than the baseline in the hypoxic group. After the treatment with salbutamol, TDP was larger than the baseline value but FC, FM and H/L were not affected by the administration of salbutamol. It is concluded that hypoxia can damage the functions of the diaphragm in dogs and the administration of salbutamol can improve the contractility of the diaphragm.

Key words diaphragm; anoxemia; electromyography*; transdiaphragmatic pressure*; sulbutamol*

人胎肝 cDNA 文库构建及肝细胞生长因子 cDNA 克隆筛选和活性研究*

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本研究从人胎肝细胞提取总 RNA 并纯化出 mRNA,经反转录合成第一链、第二链 cDNA,去除小于200 bp 的 cDNA 片段后,连接 EcoRI 人工接头,与去磷酸化的 λgtll 噬菌体长、短臂连接,体外包装后感染 E. coli Y1090,成功构建含4.2×10⁶重组子的人胎肝 cDNA 表达文库。重组子平均外源插入片段约1.5kb,适合用于筛选低丰度 mRNA 的克隆。

用高纯度 HCF 免疫大白兔,成功地制备出特异性抗 HGF 多克隆抗体。酶联免疫法证明该抗体可检测到的 HGF 最小剂量为100 pg,蛋白免疫印迹法可检测到10 pg HGF;此抗体还能与人胎肝细胞 mRNA 体外翻译产物活性部分(MW 1～3万 Da 组)反应,并可完全阻抑 HGF 对 SMMC－7721肝癌细胞 DNA 合成的促进作用。经大肠杆菌粗提物孵育后,与仅表达载体序列的 E. coli Y1090菌反应,不产生阳性信号。

用制备的特异性抗 HGF 多克隆抗体筛选构建的人胎肝 cDNA 文库,筛选约10⁶重组子后得8株免疫反应阳性克隆,用一株抗-HGF 单克隆抗体进一步筛选出3株反应阳性克隆;插入片段亚克隆于表达质粒 PSVK3中,DEAE-葡聚糖法转染 cos-7细胞,用³H-TdR 掺入法检测其表达产物的活性,发现其中1株克隆表达产物可促进 SMMC－7721肝癌细胞增殖;部分序列测定表明该克隆的序列与已知可促进肝细胞增殖的细胞活性因子不同;提取肝部分切除术后大鼠肝细胞 RNA,与该克隆 cDNA 杂交,结果表明肝部分切除术后24 h 其基因表达明显增加,说明该基因表达产物在肝再生调控中可能起重要作用。

用原代培养肝细胞技术,本文还观察了人胎肝细胞条件培养液对肝细胞增殖的影响,并检测了重型病毒性肝炎患者外周血促肝细胞增殖的活性,结果发现人胎肝细胞条件培养液可促进培养大鼠肝细胞 DNA 的合成,提示人胎肝细胞本身可分泌产生某种(些)促进肝细胞增殖的活性物质;重型病毒性肝炎患者外周血促肝细胞增殖活性较正常人、慢性肝炎及急性肝炎患者明显增高,并与病情严重程度呈正相关,对它的检测可作为判断患者预后的指标。

关键词 cDNA*文库*;肝细胞生长因子*;cDNA 克隆*

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