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Therapeutic effects of superselective intracranial arterial infusion of VM26 in combination with intravenous dropping of BCNU on brain malignant tumor

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Abstract Objective: To investigate the therapeutic effects of superselective intracranial arterial infusion of VM26 in combination with intravenous dropping of BCNU on brain malignant tumor. Methods: After the excision of brain malignant tumor, 57 patients were treated with superselective intracranial arterial infusion of VM26 in combination with intravenous dropping of BCNU. Then the changes of the size and clinical symptoms of the tumor after the chemotherapy were observed. Results: The average size of the tumor was significantly smaller after the chemotherapy (8.17± 8.80 cm^3) than before it (14.6± 7.63 cm^3, $P < 0.01$). The total effective rate was 66.7% and the complete remissive rate 21.1%. The one-year survival rate was 73.2% and the two-year one 41.1%. None of the patients were found to exhibit vision decrease and serious neural intoxication. Conclusion: The superselective intracranial arterial infusion of VM26 in combination with intravenous dropping of BCNU is better than carotid internal arterial infusion or intravenous chemotherapy alone in treating the brain malignant tumor.

Key words superselective; intracranial artery; chemotherapy; brain tumor

人源性噬菌体抗体库的构建及抗肺癌抗体基因的筛选、表达和核苷酸序列分析

Construction of human phage antibody library and selection, expression, DNA seguencing of antibodies against lung cancer

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为探索出一条制备人源性抗肿瘤抗体的有效途径,本课题研究了以噬菌体抗体库技术制备人源性抗肿瘤抗体 Fab段的可行性。

以硫氰酸胍-酸酚一步法提取肺癌患者淋巴结总 RNA,以此为模板,经逆转录-聚合酶链反应扩增人免疫球蛋白重链 Fd段(VH+ CH1)和 κ 轻链基因,以噬菌粒 pComb3H为载体,构建人源性噬菌体抗体库。用2株肺癌细胞交替作为抗原对噬菌体抗体库进行 6轮亲和筛选,以捕获能与肺癌细胞膜表面抗原结合的噬菌体抗体,再以人成纤维细胞对筛选后的噬菌体抗体库进行 2轮吸附,以尽可能除去既能与肺癌细胞结合又能与成纤维细胞结合的噬菌体抗体。将经过筛选和吸附后的噬菌体抗体库 DNA中的 gIII基因切除,进行可溶性抗肺癌抗体的表达,以蛋白质变性电泳和斑点免疫印迹试验检测可溶性 Fab段的表达情况,以细胞酶联免疫吸附试验对抗肺癌抗体 Fab段的活性作出初步鉴定。对3个克隆的抗肺癌抗体可变区基因进行核苷酸序列分析,并进行基因同源性检索。

提取了3例肺癌患者淋巴结总 RNA,经变性电泳证明 RNA无明显降解。以3例患者淋巴结总 RNA为模板,均成功地扩增出免疫球蛋白轻链基因,有2例扩增出重链 Fd段基因,1例未扩增出 Fd段基因。选择其中 1例 Fd段和轻链基因扩增效果均较好的免疫球蛋白基因,构建成人源性噬菌体抗体库,库容达到 7.5× 10^7个克隆,重链 Fd段和轻链基因与载体 DNA的重组率分别为 90% (9/10)和 100% (10/10)。以 2株肺癌细胞进行 6轮亲和筛选,再以人成纤维细胞进行 2轮吸附后,切除噬菌体抗

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Effects of hypoxia on secretion of vascular activated peptide by cultured endocardial endothelial cells

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Abstract Objective: To explore the effects of hypoxia on secretion of vascular activated peptide by cultured endocardial endothelial cells (EECs). Methods: After EECs were extracted from the right ventricule with scraping and cultured, the content of vascular activated peptides in the culture liquid was determined. Results: At the 24th h after hypoxia, the secretion of endothelin-1 (ET-1) and atrial natriuretic peptide (ANP) by EECs was significantly increased ($P < 0.01$) to levels which were respectively 1.9 and 1.6 fold of those under normoxia. The secretion of endogenous digitalis-like factor (EDLF) by EECs was also markedly increased ($P < 0.01$) to a level which was 1.7 fold of that under normoxia but the secretion of angiotensin II (AT II) was decreased by 12%. Conclusion: Hypoxia results in changes of the secretion of various vascular activated factors by EECs.

Key words hypoxia; endocardial endothelial cell; atrial natriuretic peptide; endothelin-1; angiotensin II; endogenous digitalis-like factor

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体基因中的 gIII基因,随机取 10个克隆进行可溶性抗肺癌抗体 Fab段的表达和鉴定。细胞 ELISA结果显示,10个克隆表达的 Fab段均能与 2株肺癌细胞结合,而不与人淋巴细胞结合,其中仅 2个克隆表达的 Fab段与人成纤维细胞和 1株肝癌细胞也能结合,表明大多数克隆表达的 Fab段有较好的特异性。对此 10个克隆中的 9个克隆表达的 Fab段进行了蛋白质变性电泳和斑点免疫印迹试验,结果显示 8个克隆可见新增的蛋白带,分子量约 50 kDa,与 Fab段分子量相符。同时这 8个克隆斑点免疫印迹试验显示较强的阳性结果。有 1个克隆未见明显的新增蛋白带,可能为 Fab段表达量不足或表达的 Fd段和轻链未在细菌周质腔中组装成 Fab段。对上述 10个克隆中的 Clone 2、Clone 5和 Clone 7等 3个克隆进行抗体可变区基因序列分析,结果显示 3个克隆的重链可变区基因和轻链可变区基因均不相同,以各互补决定区 (CDR区)差异最大,其中以 CDR 3区的核苷酸序列差异最大。基因同源性检索结果显示,3个克隆的抗体可变区基因序列均与人免疫球蛋白可变区基因同源,其中, Clone 2和 Clone 7的重链可变区基因属 VH 1基因家族,轻链可变区基因属 VK 1基因家族。Clone 5的重链可变区基因属 VH 3基因家族,轻链可变区基因属 VK4基因家族。

以噬菌体抗体库技术制备人源性抗肿瘤抗体是可行的。尽管淋巴结中的结缔组织难以被裂解,以硫氰酸胍-酸酚一步法提取淋巴结总 RNA,可以保证 RNA的完整性。且经逆转录-聚合酶链反应从肿瘤患者局部引流淋巴结总 RNA扩增 Fd段和轻链基因是完全可行的。以活肿瘤细胞为抗原对噬菌体抗体库进行亲和筛选,再以人成纤维细胞进行吸附,可筛选出抗肿瘤抗体。在目前尚未发现肿瘤特异性抗原和令人满意的肿瘤相关抗原的情况下,以这种方法筛选抗肿瘤抗体很有价值。不同克隆抗肺癌抗体可变区基因序列的差异主要存在于各 CDR区,以 CDR 3区差异最明显。本研究通过基因序列分析鉴定的 3个克隆抗肺癌抗体中,有 2个克隆的重链可变区基因属 VH 1基因家族,轻链可变区基因属 VK 1基因家族;另 1个克隆的重链可变区基因属 VH3基因家族,轻链可变区基因属 VK 4基因家族。本研究结果再次证明,肿瘤患者局部引流淋巴结内存在着抗肿瘤体液免疫。

关键词　噬菌体抗体;人源性抗体;肺肿瘤;重组,遗传
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