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论著

CD40L融合改造后的人乳头瘤病毒16型E7基因DNA疫苗的构建及其免疫原性测定

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摘要 摘要：目的 研究针对人乳头瘤病毒（HPV）16型的 DNA疫苗, 测定其免疫原性, 用于治疗HPV16感染及感染相关恶性肿瘤。方法 联合采用基因切割重排、定点突变及密码子优化等策略改造HPV16的转化基因E7, 基因合成法获得改造后的E7基因（mE7）; PCR法将mE7基因与CD40L胞外区编码序列融合, 然后以pVR1012为载体构建pVR1012-mE7 (mE7)及pVR1012-mE7/CD40L (mE7/CD40L)表达质粒; 经肌肉免疫C57BL/6小鼠; ELISA法检测E7特异性血清抗体水平, ELISPOT法分析E7 49-57（H-2b）特异性分泌IFN-γ的CD8+ T细胞活化水平, 胞内染色一流式细胞检测分析E7特异性CD4+ Th细胞活化水平; 并在C57BL/6小鼠体内进行疫苗抗瘤活性检测。结果 与野生型E7基因（wE7）相比, mE7基因诱发产生的E7特异抗体水平（P<0.01）、分泌IFN-γ的CD8+ T细胞数目（P<0.01）及CD4+ Th细胞活化水平(P<0.05)均显著提高; 与mE7基因相比, mE7/CD40L融合基因可进一步显著提高E7特异性分泌IFN-γ的CD8+ T细胞数目(P<0.01), 但对E7特异性抗体产生及CD4+ Th细胞活化水平没有明显影响。疫苗小鼠体内预防性免疫实验中, 经wE7免疫的小鼠接种瘤细胞后2周内全部形成移植瘤, 而所有经mE7及mE7/CD40L免疫的小鼠在瘤细胞攻击后第7周仍未见移植瘤形成; 疫苗体内治疗性免疫实验中, 小鼠在接种wE7后第8天左右全部形成移植瘤, 并呈渐进性生长, 而接种mE7的小鼠移植瘤清除率为30%, 接种mE7/CD40L的小鼠移植瘤清除率增高至45%。对移植瘤的组织学检查结果显示, mE7/CD40L及mE7免疫组小鼠瘤细胞间及瘤组织周围可见大量淋巴细胞浸润, 而wE7组小鼠的瘤细胞呈编织状紧密排列, 未见有淋巴细胞浸润。结论 HPV16型mE7/CD40L融合基因疫苗免疫小鼠后可诱发较强的E7特异性细胞免疫及体内抗瘤活性, 具有较强的免疫原性。

关键词 人乳头瘤病毒 子宫颈癌 DNA疫苗 CD40配体

分类号

Linkage of Modified Human Papillomavirus Type 16 E7 to CD40 Ligand Enhances Specific CD8+ T-lymphocyte Induction and Anti-tumour Activity of DNA Vaccine

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Abstract ABSTRACT:Objective To develop human papillomavirus (HPV)16 DNA vaccine for the treatment of HPV16 infection and its related tumors. Methods HPV16 oncogene E7 was modified by combined approaches including insertion and replication of specific region of E7 gene, murine codon optimization, and point-mutation at transforming regions of the E7 protein. The resulting artificial gene, named as mE7, was obtained by gene synthesis. The mE7 gene was then genetically fused to murine CD40 ligand (CD40L) by overlapping PCR to form the mE7/CD40L fusion gene. The mE7/CD40L gene was inserted into pVR1012 plasmid and then immunized C57/BL6 mice intramuscularly. The E7-specific IFN-γ-secreting CD8+ T cells were analyzed with ElISPOT, and E7-specific antibody was measured by indirect ELISA. FACS assays were performed to analyze the activation of E7-specific Th cells. Mice were vaccinated, followed by tumor challenged or challenged before immunization. Tumor growth was observed. Results The mE7 DNA vaccine elicited an increased E7-specific antibody level (P<0.01), E7-specific IFN-γ-secreting CD8+ T(P<0.01), and CD4+ T cells number (P<0.05), compared with those of mice immunized with wE7 gene. Furthermore, the mE7/CD40L DNA vaccine elicited an increased number of E7-specific IFN-γ secreting CD8+ T cell compared with that of mice immunized with mE7 gene (P<0.01); however, no significant differences were found between mice immunized with the mE7 gene and mE7/CD40L fusion gene in the E7-specific antibody production and Th cell activation. In the preventive experiment, all mice received the mE7 or mE7/CD40L remained tumor-free 7 weeks after challenges with TC-1 tumor cells, while the wE7 group exhibited tumor growth within 2 weeks. In the therapeutic experiment, all the mice in the wE7 group exhibited tumor growth within 8 days, while among mice receiving the mE7 and mE7/CD40L, 30% and 45% of mice remained tumor-free after TC-1 challenge, respectively. HE staining of tumor tissues showed copious lymphocytes infiltration around tumor cells in mE7 and

mE7/CD40L mice with regression of tumor growth. Conclusions The mE7 DNA vaccine increases the E7-specific humoral and cellular immune responses, and the fusion of CD40L to mE7 gene enhances the specific immune responses and anti-tumor effects against HPV16 E7-expressing murine tumors. mE7/CD40L may therefore be a suitable and promising target for HPV16 therapeutic vaccine.

Key words human papillomavirus cervical cancer DNA vaccine CD40 ligand

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