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表皮生长因子受体通路底物8疫苗对乳腺癌细胞的抑制效应及其可能机制 点此下载全文

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摘要：

目的：探索表皮生长因子受体通路底物8（epidermal growth factor receptor substrate 8，EPS8）疫苗对乳腺癌细胞的抑制效应及其可能的机制。方法:Western blotting检测EPS8蛋白在小鼠乳腺癌4T1细胞株中的表达。通过基因重组、表达和纯化等技术制备特异性的小鼠源性EPS8，以EPS8蛋白为靶点制备抗肿瘤疫苗并免疫BALB/c小鼠，间接ELISA测定免疫前后不同时间小鼠血清内抗EPS8抗体效价；流式细胞术检测免疫前后的脾T淋巴细胞亚群比例。建立乳腺癌4T1细胞荷瘤小鼠模型并接种EPS8疫苗，接种佐剂作为对照，比较2组荷瘤小鼠的生存期、肿瘤体积、肿瘤重量，计算EPS8疫苗抑瘤率；流式细胞术检测荷瘤小鼠脾T淋巴细胞亚群比例，LDH法检测其细胞毒性T细胞（CTL）杀伤率。结果：EPS8蛋白在乳腺癌4T1细胞株中高表达。成功构建的EPS8蛋白疫苗免疫小鼠后产生抗EPS8抗体，且随着免疫次数的增加，小鼠体内抗EPS8抗体滴度呈上升趋势。乳腺癌4T1细胞接种于经EPS8疫苗或佐剂免疫后的小鼠后，与佐剂对照组相比，EPS8疫苗组小鼠生存期显著升高\[中位生存时间：44(38~50) vs 37 (34~40) d; t =2.477, P =0 043\]，肿瘤质量显著降低\[（2.21±0.35） vs （3 31±0.88）g; t =3.574, P =0.009\]，EPS8疫苗的抑瘤率为33.23%。EPS8疫苗组小鼠脾CD4 +T比例和CD4 +/CD8 +比值均显著高于佐剂对照组（ P <0.001），EPS8疫苗组CD4 +CD25 +Treg/CD4 +T细胞的比值显著低于佐剂对照组（ P <0.001）。效靶比为20〖DK〗：1时，EPS8疫苗组CTL对靶细胞的杀伤活性即显著高于佐剂对照组\[(19.05±4.41)% vs (13.36±3.10)%; t =2 263, P =0.040\]。结论：EPS8疫苗不仅具有诱导小鼠产生体液免疫应答的功能，还能够降低荷瘤小鼠体内Treg细胞比例，激活机体内T细胞免疫功能；EPS8疫苗可抑制肿瘤的生长、有效延长荷瘤小鼠的生存期。

关键词：肿瘤疫苗 表皮生长因子受体通路底物8 乳腺癌 4T1细胞

Epidermal growth factor receptor substrate 8 vaccine-mediated breast cancer cell growth inhibition and the underlying mechanisms Download Fulltext

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Abstract:

Objective : To study the inhibiting effect of a vaccine against epidermal growth factor receptor substrate 8 (EPS8) on the growth of breast cancer cells and the possible underlying mechanisms. Methods: Recombinant mouse EPS8 protein was prepared through gene recombination, expression and purification. A vaccine was generated using this recombinant EPS8 protein and BALB/c mice were immunized with this vaccine (n =8) or an adjuvant (n =8). The titer of anti-EPS8 antibody before and at different time points after immunization was assessed by indirect ELISA. Proportion of T lymphocyte subsets in the spleen of immunized mice was determined through flow cytometry before and after immunization. Seven days after the third immunization with ESP8 vaccine or the adjuvant, mice were injected with 4T1 breast cancer cells. In the two groups of animals, survival time, tumor volume, and tumor weight were assessed and the rate of tumor growth inhibition was accordingly calculated. In tumor-bearing animals, T lymphocyte subsets in the spleen were analyzed by flow cytometry, and CTL killing rate was measured by LDH assay. Results: High levels of Eps8 protein were detected in 4T1 cells. Anti-EPS8 antibody was produced inmice immunized with the EPS8 vaccine; its titer was increasing with the frequency of immunization. Survival time was significantly higher mice (P <0.05), tumor weight was significantly lower (\[2.21±0.35\] g vs \[3.31±0.88\] g, P <0.05), the proportion of CD4 +T cells and ratio of CD4 +/CD8 +in the spleen were significantly higher (P <0.001), those control animals and the CD4 +CD25 +Treg/CD4 +T cell ratio in the spleen was significantly lower (P <0.001) in mice immunized against ESP8 than in control animals. There was no difference in the percentage of CD8 + T cells in the spleen between two groups (P >0.05). EPS8 vaccination resulted a significant increase in the killing activity of CTLs as compared with the control (\[19.05±4.41\]% vs \[13.36±3 10\]%, P <0.05). Conclusion: EPS8 vaccination may induce the mice functional humoral immune response, reduce the proportion of Treg cells, and enhance the cytotoxicity of T cells in mice with breast cancer, thereby suppressing tumor growth and prolong survival.

Keywords:tumor vaccine epidermal growth factor receptor substrate 8 (EPS8) breast cancer 4T1 cell

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