合併腫瘤內進行李斯特菌注射與抑制轉型生長因子β1能有效提供皮下肝細胞癌治療效果

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摘要

目的:肝細胞癌(肝癌)是當今非常惡性的癌症，外科手術是目前最有效的治療方法，然而外科手術只限於針對原位小腫瘤才有明顯的治療效果，一旦腫瘤轉移後或是演變成多發性腫瘤，外科手術則不易治療，因此積極尋找治療肝癌是刻不容緩的一項工作。免疫療法具有記憶性與專一性的優勢，因此被視為治療肝癌的明日之星。細胞激素在調節免疫反應上扮演著重要的角色，轉型生長因子(TGF)是一種會抑制免疫反應的細胞激素，大多數的腫瘤細胞都會分泌此蛋白，隨著腫瘤越惡性，腫瘤細胞分泌的轉型生長因子就越多。

近年來許多研究發現，如果將細菌(特別是厭氧菌或是兼性厭氧菌)注射到腫瘤細胞內，誘發發炎反應藉此達成腫瘤抗原被免疫細胞所辨識，產生一個有效的抗腫瘤免疫反應來達到治療腫瘤的效果。李斯特細菌為兼性厭氧菌又具有感染肝細胞癌的能力，本研究是利用核醣核酸干擾技術，來抑制肝癌細胞分泌轉型生長因子，同時合併在腫瘤內注射李斯特菌，藉此產生抗腫瘤免疫反應，來達到清除腫瘤的目的。

方法與結果:我們將設計好的轉型生長因子β1 寡核甘酸構築到一個反轉錄病毒載體 pSM2，並穩定地轉殖到小白鼠肝癌細胞(BNL 1MEA.7R.1)細胞株，轉殖後的細胞命名為 BNL/TGF-β1-RNAi 細胞，同時也將反轉錄病毒載體 pSM2 轉殖到肝癌細胞並命名為 BNL/vector 細胞，作為實驗中的載體控制組細胞，將 BNL/vector 或 BNL/TGF-β1-RNAi 合併李斯特菌同時注射到小鼠(BALB/c)皮下，來觀察腫瘤的生長情形，結果發現到實驗的老鼠中都沒有腫瘤的發生，直到第 70 天，在老鼠另一側皮下再次注射肝癌細胞株，仍然不會有腫瘤產生，相反的，只注射肝癌細胞株的老鼠，在注射完後的 14 天內皮下全部產生腫瘤，表示透過此方法能誘發出一個有效的全身性抗腫瘤免疫反應。進一步我們利用 BNL/TGF-β1-RNAi 合併李斯特菌進行腫瘤治療實驗，結果發現合併治療可以有效的降低腫瘤的生長，利用免疫組織化學染色來分析治療後的腫瘤細胞內免疫細胞的浸潤情形，結果顯示合併治療的腫瘤內顆粒球細胞、$CD4^+$和$CD8^+$T 細胞數目明顯增加。

結論:我們發現對肝癌進行單一治療，不論是抑制腫瘤內轉型生長因子的分泌，或是單獨對腫瘤內注射李斯特菌，都能夠降低腫瘤在老鼠體內的生長，有趣的是，如過合併兩種治療方式，也就是同時抑制腫瘤內轉型生長因子的分泌，再加上對腫瘤細胞注射李斯特菌，腫瘤細胞的治療效果更明顯，引起的免疫反應效果更好。

關鍵字：李斯特菌、轉型生長因子

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Combining Listeria monocytogenes injection with TGF-β1 knockdown provides a synergistic therapeutic effect on the treatment of subcutaneous liver tumors

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Abstract

Objectives: Clinically, the immunosuppression induced by large tumors may represent a major reason for the failure of cancer immunotherapy. Consequently, antitumor activity could be increased by reducing immunosuppressive factors combine with immunotherapy. Transforming growth factor-beta (TGF-*b*) is one of the immunosuppressive factors in human cancers including hepatocellular carcinoma. Blocking TGF-*b* provides a potentially important therapeutic strategy for the treatment of cancer. On the other hand, some anaerobic and facultative anaerobic bacteria represent novel therapeutic agents that have been recently applied in improving cancer immunotherapy. In this study, we investigated the antitumor effects of combining TGF-*b*1 protein knockdown by RNA interference and intratumoral injection of *Listeria monocytegenes* (LM) on subcutaneous hepatocellular carcinoma (HCC) in animals.

Materials and Methods: We evaluate the anti-tumor immunity of animals by co-injection of the mouse hepatoma cell line, BNL 1MEA.7R.1, and LM into syngeneic BALB/c mice. The therapeutic effect of intratumoral injection with LM was evaluated in mice bearing subcutaneous tumors. Using RNAi technology, we successfully cloned the engineered BNL/TGF-*b*1-RNAi cells (TGF-*b*1 protein knockdown). We injected intratumorally LM into animals bearing subcutaneous wild type BNL 1MEA.7R.1 tumors or engineered BNL/TGF-*b*1-RNAi tumors. The combinational therapeutic effect of LM treatment and TGF-*b*1 protein knockdown on a mouse liver tumor model was examined.

Results: Co-injection of BNL 1MEA.7R.1 cells and LM into syngeneic BALB/c mice elicited protective immunity in the animals, which could inhibit the growth of parental tumor cells reinjection. The antitumor immunity induced by intratumoral treatment by LM significantly reduced the growth of preestablished subcutaneous tumors. Results from animal experiments demonstrated

a synergistic antitumor effect induced by the combination of TGF-*b*1 protein knockdown and LM injection. Effector cells analyses, revealed by immunohistochemical staining of tumor infiltrates, indicated that granulocytes, $CD4^+$T and $CD8^+$T cells were more important in the antitumor effects of combined therapy.

Conclusion: We found that TGF-*b*1 protein expression was significantly reduced from BNL 1MEA.7R.1 cells after transferring our designed TGF-*b*1 hairpin oligonucleotide. Silencing of TGF-*b*1 expression in BNL 1MEA.7R.1 cells by RNA interference technology can inhibit the growth of this tumor after being transplanted to BALB/c mice. Compared with the control treatment, intratumoral injection of LM significantly reduced the tumor size. Combined therapy with TGF-*b*1 protein knockdown and intratumoral injection of LM represents a promising immunotherapy strategy for treating hepatocellular carcinoma.

Key words: *L. monocytogenes*, TGF-*b*1 RNA interference, Hepatocellular Carcinoma