樹突細胞對自體肝癌細胞作用之前臨床試驗

Preclinical study on the effect of dendritic cells against autologous hepatocellular carcinoma

中文摘要

關鍵詞： 肝癌 Hepatocellular carcinoma (HCC)
樹突細胞 Dendritic cell
腫瘤裂解物 Tumor lysate
自體 Autologous

肝癌是臺灣地區癌症死亡原因中的前二名，而目前對於末期的患者仍然沒有經臨床試驗證實有效的治療方法。所以發展新穎的治療來改善生活品質及延長壽命有其必要性。而樹突細胞是目前已知功能最強的抗原呈現細胞，也扮演引發先天性及後天性免疫反應之重要角色。假若能於體外讓樹突細胞在最有利之條件下攝取並處理腫瘤抗原，應該能夠刺激產生或活化對抗腫瘤之殺手 T 細胞反應，進而抑制腫瘤之生長。

雖然腫瘤疫苗在動物腫瘤模型已經取得了令人興奮的結果，但是成功的臨床試驗尚不多見，其中因素有腫瘤病人本身的問題，例如免疫缺陷或病人 T 細胞信號傳導缺陷或腫瘤逃避生物體的免疫監視。另外腫瘤疫苗的效價也會受到腫瘤抗原成分的異質性及多樣性所影響 。腫瘤裂解物含有很多的腫瘤抗原，被認爲是一個很好的腫瘤抗原來源。本實驗的目地是研究肝癌患者自體周邊血液單核球培養來的樹突細胞，與自體的肝癌細胞裂解物刺激之後，於體外觀察啓動毒殺性 T 淋巴細胞對抗自體肝癌細胞的能力。腫瘤細胞得自肝癌患者手術後的肝癌組織細胞，另外用自體周邊血液單核球經 rhGM-CSF 及 rhIL-4 來培養成未成熟的樹突細胞。之後再將這些細胞與腫瘤裂解物共培養，並用細胞激素來熟成。我們用流式細胞儀來分析腫瘤裂解物刺激後之樹突細胞的成熟度。然後用 carboxyfluorescein diacetate succinimidyl ester (CFSE) stain 來檢測樹突細胞對混合淋巴細胞的活化與增生。另外用 Trypan blue 來評估經過樹突細胞活化後的混合淋巴細胞其對肝癌細胞的抑制能力。結果顯示細胞激素可以很明顯地提高 CD83 的表現量，從 15 % 上升到 64 % ($p < 0.05$)，顯示肝癌病人單核球可經適當培養爲成熟的樹突細胞。經腫瘤裂解物刺激後之樹突細胞，可以看到刺激自體淋巴細胞增生的能力，但若使用正常淋巴球裂解物來刺激樹突細胞就沒有淋巴細胞增生的效果，顯示此樹突細胞可引發肝癌專一性淋巴球反應。在腫瘤抑制率方面，使用腫瘤裂解物刺激之後的樹突細胞，可以使腫瘤抑制率從 2.4 % 上升到 73.6 %。若與未經腫瘤裂解物刺激的樹突細胞相比，樹突細胞經過腫瘤裂解物刺激之後會下調 DC-SIGN 的表現量，但不會影響 CD83 的表現。我們的結論是自體樹突細胞經自體腫瘤裂

解物刺激之後，可以很顯著地活化肝癌專一性淋巴細胞來對抗肝癌細胞。

英文摘要

Key Words: Hepatocellular carcinoma (HCC)
Dendritic cell
Tumor lysate
Autologous

Hepatoma ranks the first two of the cancer mortality in Taiwan, and it remains that there are no effective treatment for advanced HCC. Therefore, novel medical intervention is needed to improve the survival and quality of life for those patients. Dendritic cells are the most potent antigen presenting cells in the human body which involved in the regulation of both innate and adaptive immune responses. It is assumed that matured antigen presenting cells pulsed in vitro with appropriate tumor associated antigens under optimal activation conditions might generate or activate a cytotoxic T lymphocyte response against tumor cells and thereby inhibit tumor growth.

Although there are exciting results for tumor vaccine in animal models, but successful clinical results are lacking. There are some problems needed to be resolved such as immune deficiency of the cancer patients, and defect of T cell receptors or the immune evasion of tumor. The efficacy of tumor vaccine is mainly affected by both heterogenicity of tumor cells and complexity of tumor antigens. Tumor lysates includes multiple antigens, which are supposed to be a good source of tumor antigens. The purpose of this study is to investigate the ability of autologous peripheral blood monocyte-derived dendritic cells (DCs) from the hepatoma patients pulsed with autologous tumor lysate to elicit T cells cytotoxicity against hepatoma cells ex vivo. Tumor cells were cultured from the excised hepatoma tissues from 8 patients. DCs were derived from peripheral blood monocytes by triggering differentiation with recombinant human granulocyte macrophage colony stimulating factor (rhGM-CSF) and interleukin-4 (rhIL-4) to immature DCs. Immature DCs were pulsed with autologous hepatoma cell lysates and matured by using a cytokine cocktail containing TNF- . Surface molecule expression on DCs was analysed by flow cytometry. The ability of the pulsed DCs to stimulate autologous T cell proliferation was assessed by using carboxyfluorescein diacetate succinimidyl ester (CFSE) staining. The cytotoxicity of DC-stimulated T cells against primarily cultured hepatoma cells was estimated by using trypan blue exclusion test. The results showed that the cytokine cocktail greatly increased the expression of CD83 from 15 % to 64 % ($p < 0.05$), indicating a significant maturation of DC from hepatoma patients. DCs pulsed with

tumor cell lysates, but not normal lymphocyte lysates, which stimulate the autologous T lymphocyte proliferation. Viability of primarily cultured hepatoma cells was marked and inhibited by co-culture with T cells stimulated with tumor lysates pulsed DC (73.6 % inhibition) but not un-pulsed DC (2.4 % inhibition). Compared with un-pulsed DCs, tumor lysates down regulated the DC-SIGN expression and did not interefere the CD83 expression on pulsed DCs. Our conclusion is that DCs pulsed hepatoma lysates preferentially and efficiently stimulate the lymphocyte cytotoxicity against hepatoma cells.