行政院國家科學委員會專題研究計畫　成果報告

趨化激素受體 CXCR4 於基底細胞癌過度表現之研究

計畫類別：個別型計畫
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一. 中英文摘要

Chemokines are a superfamily of small, cytokine-like secreted proteins. Although most well known for their ability to regulate the chemotaxis of leukocytes or other types of cells, chemokines also play important roles in other fields, including promoting mitosis, the modulation of apoptosis, survival and angiogenesis. These functions could be beneficial for tumor growth. Previous reports, including ours, also showed that chemokines and their receptors might be important to mediate tumor metastasis.

In our on-going NSC project granted last year, we proposed to measure various types of chemokine receptors in skin cancers (BCC, SCC and melanoma) to see if there is differential expression of certain chemokine receptors in these skin cancers, which might explain for the variety of tumor behaviors. We have got some preliminary results revealing that CXCR4 is differentially expressed by BCC. The current results seem promising and clearly indicate that CXCR4/SDF-1 signaling pathway may be involved in the BCC tumorigenesis. In accordance with our results, several recent reports also showed that CXCR4/SDF-1 may also mediate tumor survival through exogenous challenges (apoptosis -resistance), in addition to their most well-known chemotactic function. Based on these results, we are going to pursue this project further and address whether CXCR4 expression may play an important role in BCC tumorigenesis. We herein propose to transfer CXCR4 gene to BCC cells using retroviral gene transduction method and then determine whether CXCR4 overexpression may alter the tumor behaviors/phenotypes of BCC, including tumor growth, proliferation and resistance to exogenous challenges which may lead to apoptosis. This project may help to further reveal the role-playing of chemokines and their receptors in skin cancer pathophysiology.

趨化激素乃是一群類似細胞激素的小分子蛋白. 雖然它們最為人熟知的功能乃是藉由趨化反應調控白血球的移行, 趨化激素在其它領域也扮演了十分重要的角色, 例如促進細胞的分裂, 調控細胞的凋亡以及血管新生作用; 這些廣汎的能力使得趨化激素可能在促進腫瘤生長方面扮演了某種角色. 事實上, 許多近來的研究也紛紛指出趨化激素及其受體對於腫瘤轉移現象有重要的影響

在我們目前正在執行的國科會計畫中(Chemokine Receptors and Skin Cancer Metastasis, NSC 91-2314-B-037-299),為了印證是否某些特定趨化激素受體在皮膚癌病程進展中會扮演特定的角色, 我們測定了不同種類的皮膚癌細胞中各種趨化激素受體的表現, 並得到一些初步的結果. 我們發現趨化激素受體 CXCR4 在基底細胞癌中會有明顯的表現. 此結果顯示趨化激素 SDF-1 以及其受體 (CXCR4) 可能在基底細胞癌的進展過程中扮演重要角色. 與我們的研究結果呼應的是最近有研究指出 SDF-1/CXCR4 可以調控腫瘤細胞對抗外來侵犯而存活. 基於這些結果, 我們提出此計畫繼續深入探討 SDF-1/CXCR4 在在基底細胞癌的病程進展過程所可能扮演的角色. 我們計畫以反轉錄病毒的基因轉介法使基底細胞癌過度表現 CXCR4 基因, 進而再研究是否 CXCR4 基因的過度表現會導至基底細胞癌細胞各種行為或表型的改變: 例如生長或分裂的行為以及受到外來因子侵犯後基底細胞癌細胞的抵抗能力. 我們預期此計劃將可使我們進一步了解趨化激素及其受體在皮膚腫瘤病態機轉中所扮演之角色.

二、緣由與目的

Skin cancers are traditionally divided into melanoma and non-melanoma types. The latter predominantly applies to squamous cell carcinoma (SCC) and basal cell carcinoma (BCC). Melanoma is the leading skin cancer causing mortality and metastases occur in 15-36% of patients. On the other hand, BCC is the most common skin cancer in many areas(Ramachandran, *et al* 2001). Although BCC grows slowly and invades the adjacent tissues, it rarely metastasizes, with the reported incidences ranging from 00028 to 0.1 percent(Berti and Sharata 1999). Metastases rate of the other cutaneous epithelial cancer - squamous cells carcinoma (SCC), by the way, sits in between (7.4~15.8%)(Dinehart and Pollack 1989). Although there might be some common predisposing factors (e.g. UV radiation exposure) for melanoma, SCC and BCC(Armstrong and Kricker 2001), it is obvious that these three most common epithelial skin cancers have strikingly different behaviors, including metastasis potential, but little is known about the molecular mechanisms sit behind.

. Chemokines are a superfamily of small, cytokine-like secreted proteins which now consists of over 40 members(Baggiolini 2001). By interacting with specific seven-transmembraned, G-protein coupled receptors, chemokines are capable of triggering various cellular events including cytoskeletal rearrangement, firm adhesion to endothelial cells, and directional migration and act in a coordinated fashion with cell-surface proteins (e.g. integrins) to direct the specific homing of various subsets of hematopoeitic cells, including phagocytes, lymphocytes, dendritic cells to specific anatomical sites(Baggiolini 1998). Although most well known for their ability to regulate the chemotaxis of leukocytes or other types of cells, chemokines also play important roles in other fields, including promoting mitosis and the modulation of apoptosis, survival and angiogenesis(Gerard and Rollins 2001). Conceivably, these functions could be beneficial for tumor growth.

It is now well-established that chemokines or chemokine receptors are expressed by many types of tumor cells. Recently, Müller and colleagues (Muller, *et al* 2001) demonstrated that the ability of breast cancer cells to metastasize to particular tissues was the result of the expression of a specific chemokine receptor, CXCR4, on breast cancer cells.

Coordinately, its ligand, stromal cell derived factor- (SDF-1α, also known as CXCL12) was expressed in target tissues(Muller, *et al* 2001). The study was based on the fact that both metastasis of cancer cells and normal migration of blood leukocytes involve passage through the blood and lymphatic circulation and movement across vascular barriers, indicating that metastatic cancer cells may simply co-opt signals that normally control leukocyte migration and homing (Murphy 2001). Therefore, chemokine is conceivably may be involved in both situation since it is already well known for its function to regulate leukocyte movements. SDF-1/CXCR4 pathway has also been implicated in the metastasis of prostate cancer cells to bone (Taichman, *et al* 2002) and several chemokines have been purified and characterized from other tumor sources(Zhou, *et al* 2002).

In our previous NSC project (**91-2314-B-037-299**, 吳孟澤醫師 as Co-PI), we hypothesized that the expression level of chemokine receptors might be different in BCC, SCC and malignant melanoma, based on the facts that: (1) these three epithelial skin cancers possess strikingly different clinical behaviors, including metastatic patterns, (2) chemokines/chemokine receptors may play critical roles in the metastasis of cancer cells, and (3) basal cell carcinoma cell line show no detectable chemokine receptor (CCR7) expression in our preliminary conventional RT-PCR study, we therefore proposed to measure various types of chemokine receptors in BCC, SCC and melanoma to see if there is differential expression of certain chemokine receptors in these three skin cancers, which might explain for the variety of tumor behaviors at least to some extent. In the past five months (Aug/2002-Dec/2002), we have got some preliminary results as follows: (1) Using both real-time and conventional RT-PCR, we found there was prominent expression of chemokine receptor CXCR4 in a BCC cell line compared to SCC and melanoma cell lines, (2) Immunohistochemical studies showed there were CXCR4 expression in parts of tumoral tissues from BCC patients, (3) Western blotting studies demonstrated CXCR4 expression in a BCC cell line, (4) Using RT-PCR amplification, we successfully cloned human CXCR4 cDNA from the total RNA of BCC cell line, which was further confirmed by DNA sequencing.

The current results seem promising and clearly indicate that CXCR4/SDF-1 signaling pathway may be involved in the BCC tumorigenesis. Actually, several recent reports had shown that CXCR4/SDF-1 may also mediate tumor survival through exogenous chanllenges (apoptosis –resistance), along with their well-known chemotactic function(Zhou, *et al* 2002). Based on these results, we herein propose to transfer CXCR4 gene to BCC cells and then determine whether CXCR4 overexpression may alter the tumor behaviors/phenotypes of BCC in vitro.

三、結果與討論

Functional transduction of BCC cells with CXCR4

To determine whether CXCR4 alone may play important roles in BCC tumorigenesis, we used retroviral vector (pLNCX2) to transfer CXCR4 gene into BCC-1/KMC cells (CXCR4-BCC). Magnet-bead positive selection was further used to increase CXCR4 expression, as ~91% CXCR4-BCC showed clear staining with anti-CXCR4 mAb (Figure 1A). Measured by qPCR as shown in Figure 1B, CXCR4 expression was significantly enhanced in CXCR4-BCC compared with wild-type BCC-1/KMC (~37-fold) and pLNCX2-BCC (~14-fold). Calcium flux assay was further used, as described previously (Wiley, *et al* 2001), to determine that CXCR4 was functional in CXCR4-BCC cells, as shown in Figure 1C.

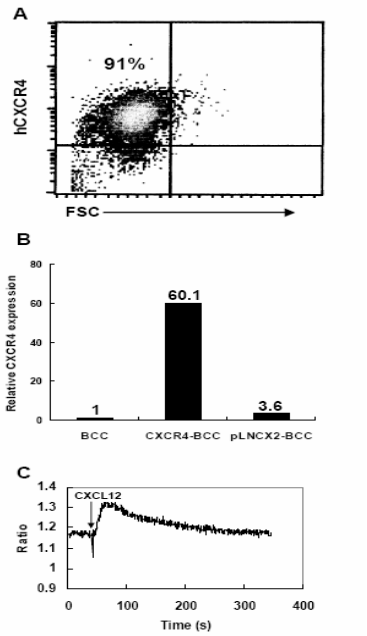

Fig. 1. Transduction of BCC cell line (BCC-1/KMC) with CXCR4. **(a)** CXCR4-transduced BCC cells were stained with FITC-labeled anti-human CXCR4 (hCXCR4) mAb after magnetic bead enrichment. *FSC*, forward scatter. **(b)** Relative expression levels of CXCR4 in BCC-1/KMC (BCC), CXCR4-BCC and pLNCX2-BCC by qPCR. **(c)** Calcium flux assay. CXCR4-BCC cells were labeled with fura-2-acetoxymethyl ester and exposed to 500 ng/ml CXCL12 in a calcium-containing buffer as described previously (Tiffany, *et al* 1997).

CXCL12 enhances CXCR4-BCC proliferation under low serum in vitro

To address whether expression of CXCR4 may enhance BCC tumor growth, pLNCX2-BCC and CXCR4-BCC cells were cultured with normal serum (10% FCS) or low serum (0.5% FCS) in the presence or absence of CXCL12. CXCR4-BCC and pLNCX2-BCC cells showed similar proliferation rates in normal serum, either with or without CXCL12 (data not shown). By contrast, CXCL12 treatments resulted in significant increase of CXCR4-BCC cell proliferation compared with PBS treatments (24 h, ~1.2-fold, P=0.027; 48 h, ~2.2-fold, P=0.004) (Figure 2). The enhanced growth with the treatments of

CXCL12 could be neutralized by anti-CXCR4 mAb (300 μg/ml), but not by the isotype control mouse IgG_{2B} (Figure 2). CXCL12 did not significantly enhance the proliferation of pLNCX2-BCC cells in low serum (*P*=0.4, data not shown). Thus, CXCL12 may enhance BCC tumor growth by CXCR4 expression under nutrient deprivation (low serum).

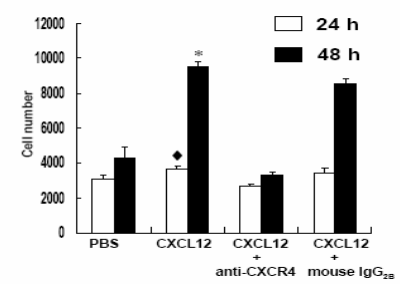

Fig. 2. CXCL12 enhances proliferation of CXCR4-BCC cells in low serum culture *in vitro*. CXCR4-BCC cells (3×10^4 cells/well in triplicate) were cultured in 0.5% FCS and then treated with CXCL12 (500 ng/ml) in the presence of anti-CXCR4 mAb or isotype control mAb. Numbers of viable cells were counted by WST-1 assay at indicated time points. At 24 h: ♦, *P*= 0.027; at 48 h: ∗, *P*= 0.004, versus PBS-treated cells.

CXCL12/CXCR4 interactions protect CXCR4-BCC from apoptosis by UVB irradiation in vitro

To determine whether CXCR4 signaling may render apoptosis resistance for BCC cells, UVB irradiation was used to induce apoptosis in pLNCX2-BCC and CXCR4-BCC cells. As shown in Figure 3, with CXCL12 pre-treatment, there was ~38% (*P*=0.04) decrease of apoptosis in CXCR4-BCC cells (using caspase-3 activity as a marker). This protective effect could be negated by co-treatment with CXCR4-blocking T22 peptide. The presence of either CXCL12 or CXCL12 plus T22 did not significantly affect the UVB-induced apoptosis in pLNCX2-BCC cells (Figure 3). Thus, CXCL12/CXCR4 interactions might provide BCC cells with significant resistance to apoptosis *in vitro*.

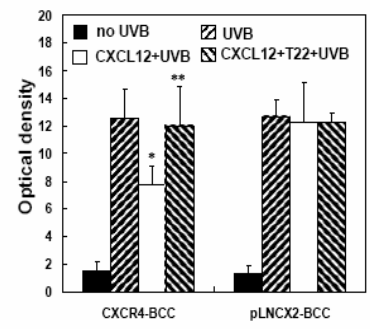

Fig. 3. CXCR4/CXCL12 interactions provide apoptosis-resistance for CXCR4-BCC cells in *vitro*. CXCR4-BCC and pLNCX2-BCC cells were exposed to CXCL12 (500 ng/ml) in the presence or absence of T22 (1μg/ml) as indicated, and subject to UVB irradiation afterwards. Caspase-3 activity, as as an indicator for apoptosis, was then quantified by optical density (460 nm) of fluorescent dye (AMC). In CXCR4-BCC group: *, *P*=0.04; **, *P*=0.86, versus UVB-irradiated cells without CXCL12 and T22.

四. 計畫成果自評

We have faithfully executed this granted project and are now preparing the manuscript for submission to scientific journal.

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