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行政院國家科學委員會補助專題研究計畫成果報告

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※ 生長因子及其受體與黏著蛋白 ※

※ 在具有神經侵犯攝護腺癌上之表現 ※

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計畫主持人：鄭建睿

共同主持人：吳建志

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□赴國外出差或研習心得報告一份

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□出席國際學術會議心得報告及發表之論文各一份

□國際合作研究計畫國外研究報告書一份

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行政院國家科學委員會專題研究計畫成果報告

生長因子及其受體與黏著蛋白在具有神經侵犯攝護腺癌上之表現

Expression of growth factors, receptors and adhesion molecules in the prostatic carcinoma with perineural invasion

計畫編號：NSC 89-2314-B-038-003

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I、中文摘要

攝護腺癌神經周邊侵犯可以於 80 至 100 % 的攝護腺癌中見到。我們將著重在生長因子及其受體與黏著蛋白在具有神經周邊侵犯攝護腺癌上的表現，以了解攝護腺癌神經周邊侵犯的可能機轉。我們在具有神經周邊侵犯的攝護腺癌上對上述蛋白進行免疫組織化學染色，以了解這些蛋白在不同腫瘤分化時，於神經周邊與非神經周邊腫瘤細胞上的表現。結果顯示僅有 EGFR 於兩者之間有意義。FGF-2, TGF-β1 and FGFR-1 儘管於分化不良的病例中，有較高的表現。但於神經周邊並無明顯過度表現的現象。CD44 與 NCAM 於我們的病例中僅有 9.3 和 20.3 % 表現。ICAM-1 可見於 82 % 的分化良好，83% 中度分化與 75% of 分化不良病例中表現。於神經周邊與非神經周邊腫瘤細胞上的表現，並無明顯統計學上的差異。

關鍵詞：攝護腺癌，神經周邊侵犯，生長因子，生長因子受體，黏著分子

I Abstract:

Perineural invasion is frequently observed in prostatic carcinoma, prevalent in 85 to 100 % of cases. The possible mechanisms or mediators underlying this propensity for perineural invasion are approached, especially on the growth factors, their receptors and adhesion molecules. Immunohistochemistry studies of these proteins are performed at paraffine-embedded sections of prostate cancer with perineural invasion. Expressions of these factors between the areas of perineural and non-perineural invasion were compared at cases of low, intermediate and high grade. Only EGFR has statistically significant difference between them. FGF-2, TGF-β1 and FGFR-1 reveal more intense staining at cases of high grade, but no difference between the perineural and non-perineural invasion areas. CD44 and NCAM expression are low in our cases, 9.3 and 20.3 %, respectively. ICAM-1 expression is found in 82 % of low grade, 83% of intermediate grade and 75% of high grade cases without significant difference between the perineural and non-perineural area.

Keywords: perineural invasion, prostate carcinoma, growth factor, growth factor receptors, adhesion molecule

II、Introduction:

Perineural invasion is frequently observed in routine pathological practice diagnosis of prostatic carcinoma, prevalent in 85 to 100 % of cases. The mechanisms or mediators underlying this propensity for perineural invasion are not understood. Some previous studies suggest that the neural elements within the tumor may facilitate tumor cells growth by inhibiting apoptosis, possibly through a paracrine mechanism. Intimate relationship between the nerve and prostatic carcinoma attracts us to approach

the mechanism of growth advantage and physical adhesion between the nerve and prostatic carcinoma cells.

Ⅲ. Materials and Methods:

The cases of prostatic carcinoma diagnosed at Taipei Medical Collage Hospital and Wan-Fang Hospital during 1995~1997 were included in this study. The tissue was obtained from surgical specimen resected by the transurethral prostatectomy and radical prostatectomy. Tissue sample was fixed in 10 % buffered formalin, then dehydration and embedded within paraffine. The routine H & E stained slides were reviewed. We select 54 prostatic adenocarcinoma (11 cases of low grade (combined Gleason grade 2, 3, 4), 23 cases of intermediate grade (combined Gleason grade 5, 6, 7) and 20 cases of high grade (combined Gleason grade 8, 9, 10)) with perineural invasion. There are areas of perineural and non-perineural invasion in same case for comparison. Some non-neoplastic area are also included.

Immunohistochemistry:

The primary antibodies of TGF-β1 (*sc*-146, Santa Cruz, California, 1:50); FGF-2 (basic fibroblast growth factor, *sc*-79, Santa Cruz, California, 1:50), EGFR (H11, Dako, Denmark, 1:50), FGFR-1 (Flg, *sc*-276, Santa Cruz, California, 1:50), NCAM (CD56, 1B6, Novocastra, UK, 1:250), ICAM –1 (CD54, Santa Cruz, California, 1:200), CD44 (DF 1485, Dako, Denmark, 1:40) and S-100 (Dako, Denmark, 1:200) were used. Appropriately positive and negative controls were obtained for evaluation the final results. The ABC method was used for immunohisto-chemistry. The final reaction product was visualized with DAB. The slides was sub-sequently submitted for another immunohisto-chemistry studies for S-100 (for identify the nerve) as previously described method. The horseradish streptavidin conjugates with alkaline phosphatase. Result reaction product was visualized with new fushion. The slides were counterstained with hematoxyline and dehydrated. The slides were mounted for microscopy observation.

Quantitation of the expression of the stroma and carcinoma cells between areas of the perineural invasion and non-perineural invasion

Measurements were performed in the carcinoma foci adjacent to the nerve with perineural invasion, which represented the carcinoma cells with perineural invasion (PN). The carcinoma cells measured as that of non-perineural invasion (NPN) were performed only in the carcinoma focus within microscopic field (200X, Olympus, BH-2) without any nerve core found. Total 1000 carcinoma cells were counted randomly within the PN and NPN carcinoma focus. The intensity of stroma staining was scored as 0, 1+, 2+, 3+ and was carried out by two independent observers.

Statistical Analysis:

The numbers of positively staining carcinoma cells and intensity of stroma staining are recorded separately for PN and NPN area in low, intermediate and high grade cases. The t-test and $\chi 2$ test are used to determine the significance of differences.

Ⅳ: Results:

(1) Basic Fibroblast Growth factors (bFGF, FGF-2):

The immunohistochemistry study of FGF-2 reveals positive staining at stroma in all cases. The intensity staining at stroma is stronger in high graded cases than in the low graded cases. But no difference is seen between the PN and NPN area within the carcinoma of low, intermediate and high graded cases.

(2) Transforming Growth Factor-β 1

The immunohistochemistry study of TGF-β 1 reveals positive staining at luminal secretory cell, basal cell and regional stroma in all cases. The intensity is stronger in carcinoma area than in the non-neoplastic area. More intense staining is noted at high graded cases. But no difference is seen between the PN and NPN area within the

carcinoma of low, intermediate and high graded cases.

(3) Epidermal Growth Factor Receptors (EGFR)

The immunohistochemistry study of EGFR reveals focal positive membranous staining at 31 out of 54 cases, 6 in low grade, 13 in intermediate grade and 12 in high grade cases. There are marked increased staining noted on the tumor cells of PN area compared to the NPN area. ($p < 0.01$)

(4) Fibroblast Growth Factor Receptor-1 (FGFR-1)

The immunohistochemistry study of FGFR-1 reveals focal positive membranous staining at 26 out of 54 cases, 6 in low grade, 8 in intermediate grade and 12 in high grade cases. There are marked increased staining noted on the tumor cells located peripheral area of tumor nodules, just adjacent to the stroma, which often reveals positive staining for FGF-2. No significantly increased number of positively staining cells is found within PN area compared to the NPN area.

(5) CD44

The immunostaining results of CD44 reveals focal positive membranous staining on the cell membrane of tumor cells in only 5 out of 54 cases, one in low grade cases, one in intermediate grade and 3 in high grade. In these positively staining cases, There are marked increased staining signals noted at tumor cells of PN area compared to the NPN area. But the case number is too small to make significant conclusion.

(6) Neural Cell Adhesion Molecule (NCAM)

The immunohistochemistry study of NCAM reveals focal positive membranous staining at 11 out of 54 cases, no case in low grade, 1 in intermediate grade and 10 in high grade cases. No difference is noted between the areas of PN and NPN in all 11 cases.

(7) Intercellular Adhesion Molecule-1 (ICAM-1)

The immunohistochemistry study of ICAM-1 reveals variable positive membranous staining at 43 out of 54 cases. The ICAM-1 staining could be found on the basal cell layer of regional non-neoplastic prostatic glands. The strong staining on the regional histiocytes and lymphoid cells could be seen. Only 11 cases (including 2 cases in low grade, 4 cases in intermediate grade and 5 cases in high grade cases) reveal no any staining in all tumor cells. No difference is noted between the areas of PN and NPN area in all 43 positively staining cases.

V: Discussion:

FGF-2 down-regulated androgen receptor protein in a dose-dependent manner (1). FGF-2 is significantly increased in stroma of prostate cancers when compared with uninvolved prostate. Although FGF-2 is over-expressed in high graded prostate cancer, our experiments showed that the difference of FGF-2 expression was not sufficient to explain the mechanism of perineural invasion.

TGF-β1 may act as both paracrine and autocrine factors to influence prostate function and the stromal-epithelial cell interaction. The overexpression of TGF-β1 proteins and underexpression of TGF-β receptors in prostate cancer, especially at high grade cases, was described (2). Increased expression of TGF-β is usually accompanied by a loss in the growth inhibitory response to TGF-β. The regulation of stroma cells by TGF-β1 was also noted within the in vitro study.(3)

EGFR immunostaining was focal and located in the basal cells in normal prostates and labelling localized, but wider in basal cells in benign prostatic hyperplasia (BPH). In the prostatic carcinoma, both basal and columnar cells appeared stained and the number of immunolabelled stromal cells was higher than in BPH. In prostatic carcinoma, the acquisition of EGFR by the secretory-like neoplastic cells develops an autocrine regulation.The acquired EGFR may be significant in tumorgenesis(4). The tumor cells around the PN area expressed more EGFR than that of NPN area. The results

suggested that EGFR expression in prostatic carcinoma was prone to involve the perineural space.

Overexpression of FGFR-1 in the prostate cancer was correlated with poor diffe- rentiation. Both increase in FGF2 concentra-tion and increased expression of FGFR-1 in our cases of prostate cancers could explain the a potential paracrine stimulation of prostate cancer cells by the surrounding stromal cells, which may play an important role in prostate cancer progression. No significant difference between the PN and NPN area found in our study suggested that FGFR-1 didn't play any role in perineural invasion.

The CD44 antigen could play a role in the regulating of cell and cell-substrate interaction as well as cell migration (5). In one study, the CD44 expression was strongly reduced in prostate cancer metastases as well as in the corresponding primary tumor (6). Our results also revealed low positive rate in prostate cancer. The low expression of CD 44 in prostatie cancer was closely associated with methylation of CD44 gene (7). The low expression of CD44 in our cases was hard to conclude the significance of this antigen

NCAM were found at 93% of adenoid cystic carcinoma of salivary gland with perineural invasion (8). In cases of prostate cancer in this study, NCAM were found in only 20.3% cases and most cases were high graded cases. Even marked difference was seen between the PN and NPN area in five cases. But no significant difference was found after statistical analysis.

No difference of ICAM-1 expression was observed between histological grades in our cases. But no difference was found between PN and NPN area in our study.

VI: 計畫成果自評:

1.研究內容與原計畫相當相符，除部份 Primary antibodies 染色效果不佳，難以判讀改以其它抗體取代外，其餘大致相符。

2.研究結果與當初預期有些差異，原因可能所研究之 growth factors，receptors 與 adhesion molecules 可能出現於其它 cancer 但不出現於 prostate cancer。或因病例數不足，無法達到統計上的意義。本人將繼續收集病例，繼續觀察這些蛋白於攝護腺癌上的變化，尤其是與神經侵犯之相關性。

3.對於 EGFR 與神經侵犯的相關性，是值得學術期刊發表，其它因子假如經由收集病例後，觀察在攝護腺癌與神經侵犯有相關性，也值得進一步發表。

VII.Reference:

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