行政院國家科學委員會專題研究計畫成果報告

非小細胞肺癌之 CD44 表現
Expression of CD44 Variant Exons in Non-Small-Cell Lung Cancer

計畫編號：NSC 89-2314-B-002-080
執行期限：88 年 8 月 1 日至 89 年 7 月 31 日
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一、中文摘要

CD44 是細胞表面的醣蛋白受體，其功能包括與細胞外物質之黏附，細胞之移動，淋巴球之生成及回歸。CD44 基因由 19 個顯譯區構成，標準型（CD44s）是由顯譯區 1~5 及 16~18 構成，變異型（CD44v）則是在顯譯區 5 與 16 之間接合數目不等之顯譯區 v1-v10。人類腫瘤的 CD44 表現差異很大，有些呈過度表現，有些則不表現。正常肺組織與肺癌之 CD44 表現亦人言各殊。本計劃是以反轉錄一聚合酶鏈鎖反應（RT-PCR）-DNA 序列分析法來研究原發性肺癌與正常肺組織之 CD44v mRNA 之表現，使用之引子位於 CD44 顯譯區 5 與 16，可增量所有型式之 CD44v mRNA。結果顯示，56 個原發性肺癌之病人（37 例腺癌，15 例鱗狀細胞癌，2 例類癌瘤），其腫瘤與正常肺組織表現之 CD44v mRNA 最常見者為 CD44v10,v8-10，v6-10，v3-10 及 v2-10。其中 v2-10 過去從未被報告過。在正常肺組織，90% 以上均表現 v10，v8-10 及 v6-10，而 v3-10 之表現率為 79.6% ，v2-10 為 37% 。在類癌瘤，有一例完全不表現 CD44v，另一例則表現 v10 與 v8-10。在腺癌，90% 以上均表現 v10 與 v8-10，v6-10，v3-10，v2-10 之表現率為 81% ，54% ，10.8 % 。在鱗狀上皮癌，v10，v8-10，v6-10 及 v3-10 之表現均為 100% ，v2-10 則為 86.7% 。統計分析顯示 CD44v3-10 及 v2-10 之表現隨肺癌之細胞型不同而不同，但 CD44v 之表現並不受肺癌分期之影響。

關鍵詞：CD44，變異型，肺癌

Abstract

CD44 is a cell surface receptor with functions of extracellular matrix binding, cell migration and lymphocyte homing. The CD44 gene is made of 19 exons. The standard form (CD44s) consists of exons 1-5 and 16-18. The variant forms arise from alternative splicing of combinations of exons 6-15, designated v1-10, onto CD44s framework. CD44 expression in human tumors is variable. Previous studies on CD44v expressions in normal lung and primary lung cancer have also given variable results. So we studied CD44v expression in resected primary lung cancers and normal lung tissue by reverse-transcription-polymerase-chain-reacton (RT-PCR) and direct sequencing. We found that normal lung tissue expressed CD44v10, v8-10, v6-10, v3-10 and v2-10 (in decreasing frequencies). In two cases of carcinoid tumor, most of the CD44v transcripts were lost. In 37 cases of adenocarcinoma, the expression of v6-10, v3-10 and v2-10 were also diminished. However, in 15 cases of squamous cell carcinoma, the expression of the longer CD44v transcripts (v6-10, v3-10 and v2-10) were all increased. The staging of cancer did not affect the CD44v expression. We conclude that CD44v expressions in primary lung cancers were associated with histologic types.

Keywords: *CD44, variant form, lung cancer*

INTRODUCTION

CD44 is a cell surface glycoprotein receptor with functions of extracellular matrix binding, cell migration, lymphopoiesis and lymphocyte homing (1,2). It is a heterogene-ous group of cell surface glycoproteins. The heterogeneity can be generated by glycosylation (3), or by alternative splicing of ten variable exons (4). The CD44 gene is made of 19 exons. The standard form, CD44s, has coding sequence consisting of exons 1-5 and 16-18, terminating in either exon 18 or 19. The variant isoforms (CD44v), all larger in molecular weight, arise from alternative splicing of combinations of exons 6-15, designated v1-v10, onto the CD44s framework (4). Several CD44v isoforms have been associated with acquisition of metastatic potential by tumor cells in animal experiments (5). In human tumor cell lines, the pattern of CD44 expression is variable. Some cell lines expressed no CD44, some only the standard form, and others various isoforms (6). CD44 expression in human primary tumors is also variable. Both gain and loss of CD44 expression have been correlated with dissemination of human cancers (6). Previous studies on the CD44 expressions in normal lung and lung cancers have shown different results. Washimi et al (7) demonstrated that normal lung tissue and

non-small-cell lung cancer both expressed CD44 v4, v5, v6 or v7. But Miyoshi et al (8) showed that only non-small-cell lung cancer cells, but not normal lung tissue, expressed CD44v6, and the expression of CD44v6 is associated with lymph node metastasis. In order to identify all the CD44v transcription products of normal lung and lung cancers, we began our study on surgically resected primary lung cancers and their normal counterpart lung tissue, using reverse-transcription-polymerase-chain-reaction (RT-PCR) with primers flanking the CD44 alternative splicing sites so as to amplify all the transcripts of CD44v. We then directly sequenced the amplified transcripts to identify the CD44v exons contained in each transcript, and correlated the findings with histology and staging of lung cancers.

MATERIALS AND METHODS

(1) Patients and Samples

Fresh tumor samples, along with uninvolved lung tissue, were obtained from 56 patients at the time of resection. All patients had preoperative diagnosis of primary lung cancer. After resection, tumor samples and the uninvolved lung tissue were immediately frozen in liquid nitrogen and stored at –70oC until processing.

(2) Extraction of RNA, RT-PCR and Sequencing

Total RNA was extracted, using the single-step method by acid guanidinium thiocyanate. First-strand cDNA was synthesized from total cellular RNA (2 ug), using random hexanucleotide primers and Moloney Murine Leukemia virus (MmuLV) transcriptase. Subsequent PCR was performed using one-fifth of the cDNA and the primers P1 and P2:

P1 (sense): 5'CAC AGA CAG AAT CCC TGC TAC CA 3'

P2 (antisense): 5'GTG GAA TGT GTC TTG GTC TC 3'

The P1 primer is located at the end of exon 5, and the P2 at the start of exon 16. The PCR protocol was: 94oC (1 sec), 55oC (1 sec), 72oC (40 sec), for 35 cycles, followed by a 10-min extension at 72oC. The amplified products of CD44v were electrophoresed in 0.8% agarose gel and extracted using a gel exraction kit (Viogene, USA). Direct sequencing of the purified DNA was performed using an automatic DNA sequencer, a sequencing kit (BigDye Terminator Cycle Sequencing, PE Applied Biosystems, USA) and primers P1 and P2.

RESULTS

Tumor samples from 54 patients with primary lung cancer were studied. Thirty-five (64.8%) were male, nineteen (35.2%) were female. Their ages ranged from 30 to 81 years (M±SD: 60.3±11.7). Thirty seven (68.5%) had adenocarcinoma, 15 (27.8%) squamous cell carcinoma, and two (3.7%) carcinoid tumor. Eleven (20.4%) were at stage I, eight (14.8%) stage II, thirteen (24.1%) stage IIIa, ten (18.5%) stage IIIb and ten stage IV.

PCR products of size 120, 300, 570, 930 and 1050 base pairs (bp) were detected on agarose gel. These products were amplified from cDNA of tumor as well as normal lung tissue, with varying frequencies and amounts. Sequence analysis showed that the 120 bp product encoded CD44v10, the 300 bp product v8-10, the 570 bp product v6-10, the 930 bp product v3-10 and the 1050 bp product v2-10. All these variant isoforms, except for CD44v2-10, have been identified previosly in other types of cells (6).

Expression of CD44v in normal lung tissue CD44v10, v8-10 and v6-10 were present in nearly all of the normal lung samples. The longer transcripts, v3-10 and v2-10, were detected in 79.6% and 37% of normal lung samples, respectively.

Expression of CD44v in carcinoid tumor In one case the tumor expressed no CD44v at al. In the other case CD44v10 and v8-10 transcripts were detected, but other three longer transcripts (v6-10, v3-10 and v2-10) were absent.

Expression of CD44v in adenocarci-

noma More than 90% of the 37 adenocarcinomas expressed the two shortest CD44v transcripts. However, those longer transcripts containing v6 were expresssed less frequently: v6-10: 81.1%; v3-10: 54.1%; v2-10: 10.8%.

Expression of CD44v in squamous cell carcinoma In contrast to carcinoid tumor and adenocarcinoma, all of the 15 squamous cell carcinomas expressed CD44v10, v8-10, v6-10 and v3-10 transcripts, and 13 of 15 (86.7%) expressed the longest transcript v2-10. The expression frequency of v3-10 and that of v2-10 were significantly higher in squamous cell carcinoma than in adenocarcinoma or carcinoid.

Correlation with clinical, histological, or staging parameters The patients with expression of CD44v3-10 or v2-10 in their lung tumors did not differ from patients without expressions in mean age or sex distribution. The frequency of v2-10 expression seemed to be lower in stage I patients. This figure, however, might have been distorted due to the large proportion of adenocarcinoma in stage I patients (9/10). None of the CD44v transcripts were associated with metastatic (stage IV) disease. Neither tumor nor nodal stage affected the percentage of CD44v expression.

DISCUSSION

Our study showed that the principal CD44v mRNA transcripts in normal lung tissue were CD44v10, v8-10, v6-10, v3-10 and v2-10. The former three transcripts were present in almost all the normal lung samples, while the latter two longer transcripts occurred less commonly. Our study was the first to show the entire sequence of the many different CD44v transcripts in normal and neoplastic lung tissues. Compared with previous studies that used immunhistochemical method or RT-PCR-southern-hybridization to show individual variant exons (7-10), our study drew a clearer outline of the extracellular CD44v transcripts. We also came to know that all CD44v transcripts of normal lung and lung tumors started with v10, and that the most universal CD44v transcripts were short ones such as v10, v8-10 and v6-10. Our results also revealed that while CD44v mRNA may vary in length, they never skipped any variant exons before finally being spliced to CD44s. Without sequencing analysis, one might attribute certain cellular biological behavior (such as metastasis) to the presence of a single exon (such as v6). Once the sequence of the entire CD44v transcripts became known, the importance of the entire mRNA transcript became surfaced. The association between biological phenomenon and gene structure can be further clarified.

Previous studies using immunohistochemical method and RT-PCR-southern-hybridization showed that CD44v6 was associated with lymph node metastasis in non-small-cell lung cancer (8). Our study showed that CD44v6 was associated with histologic type of lung cancer. CD44v6-10 (or any longer CD44v mRNA containing v6) was absent in both carcinoid tumors, but was present in over 80% of the adenocarcinomas and all of the squamous cell carcinomas. The finding is similar to the results of previous

studies, which showed that CD44v isoforms were rarely expressed in another neuroendocrine tumor – small cell lung cancer (9,10). As for non-small-cell lung cancer, the CD44v transcripts v10 and v8-10 were present in both adenocarcinoma and squamous cell cainoma. When the length of CD44v transcripts increased, the expression rate in adenocarcinoma decreased, being 81%, 54% and 10.8% for v6-10, v3-10 and v2-10, respectively. But the expression rates of these transcripts in squamous cell carcinomas remained high, and were significantly higher than adenocarcinomas. These findings suggested that CD44v expression may be associated with histogenesis of the lung cancers. The neuroendocrine tumors lost the expression of v6-10 and longer transcripts; the adenocarcinomas lost v3-10 and v2-10, while squamous cell carcinomas overexpressed v3-10 and v2-10. The presence of CD44 v6-10 in non-small-cell lung cancers but not in neuroendocrine-origined lung tumors may be associated with the fact that small cell lung cancer and carcinoids are usually not or less infiltrated by inflammatory cells, in contrast to squamous cell carcinomas and adenocarcinomas. Wittig et al (11) have shown that CD44v7 (which were present in the transcript v6-10) encoded a region which was essential for the survival of infiltrating lymphocytes and the persistence of inflammation in murine experimantal colitis.

CONCLUSION

CD44v transcripts were overexpressed in squamous cell carcinoma, but were underexpressed in adenocarcinoma, and mostly lost in carcinoid tumor of the lung.

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