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Expression of CD44 Variant Isoforms in Normal and Neoplastic Cells of the Lung

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CD44 is a cell surface receptor that has been implicated in lymphocyte homing, hematopoiesis, cell migration and possibly also tumor metastasis. In the present study, expression of CD44 variant (CD44v) isoforms was analyzed in 23 lung cancer specimens together with corresponding normal lung tissues by Southern blot analysis coupled with reverse transcription-polymerase chain reaction amplification. We found that CD44v isoforms were expressed in all lung cancer specimens, suggesting a possible role in the establishment of metastases by these highly malignant tumors, but normal tissues were also positive. This is in marked contrast to the previous reports of essentially negligible expression of CD44v isoforms in normal colon and breast, and suggests a physiological function in the lung.

Key words: CD44 — Adhesion molecule — Lung cancer — Metastasis — Isoform

Tumor metastasis is the major cause of death for lung cancer patients.[1] Although the molecular mechanisms involved in this life-threatening process are not yet fully understood and are exceedingly complex, cell-substrate and cell-cell recognitions mediated by adhesion molecules appear to be critical for the multistep, tumor-host interaction.[2–4] We previously reported that expression of integrins, one of the most extensively studied families of adhesion molecules, is frequently altered in lung cancer, i.e., prominent loss of α_1 and aberrant upregulation of α_{RLC}, a newly identified integrin α subunit, occur.[5,6]

CD44 is a ubiquitous cell surface receptor which also serves as an adhesion molecule in cell-substrate and cell-cell interactions.[7–12] The existence of various CD44 isoforms resulting from alternative splicing of 10 variant exons was recently identified,[13–15] and certain of these CD44 variant (CD44v) isoforms were shown to be selectively expressed in a number of metastatic rat cell lines.[16] Furthermore, overexpression of a CD44v isoform carrying variant exons 4 through 7 was shown to confer full metastatic behavior upon a nonmetastatic rat cell line.[16] In man, selective expression of CD44v isoforms in neoplastic tissues has been reported for the colon and breast, with a possible relation to the progression, and especially metastasis, of these human tumors.[17–19] However, very little is known about *in vivo* expression of CD44v isoforms in normal and neoplastic cells of the lung.

In this study, we compared expression of CD44v isoforms in 23 lung cancer specimens and in the corresponding normal lung tissues. CD44v isoforms were found to be expressed in both in virtually all cases, suggesting that they may play a role not only in the malignant behavior of lung cancers but also in the physiological functioning of the lung.

MATERIALS AND METHODS

Tumor samples Tumor samples together with uninvolved lung parenchymal tissues were collected during surgery at Aichi Cancer Center from 18 patients diagnosed histologically as having lung cancer (3 small cell lung cancer (SCLC) and 15 non-small cell lung cancer (NSCLC)). In addition, multiple samples from primary and metastatic sites along with normal lung parenchymal tissues were obtained from 5 lung cancer patients at the time of necropsy (3 SCLC and 2 NSCLC). Two normal colonic tissues were also included in this study as controls for the specificity of hybridization. All tissues were quickly frozen in liquid nitrogen and stored at −80°C until analysis. RNA was isolated by extraction with guanidinium thiocyanate followed by ultracentrifugation in cesium chloride solution.

Southern blot analysis of reverse transcriptase (RT)-polymerase chain reaction (PCR) products PCR using random-primed cDNAs (100 ng total RNA equivalent) was performed for 30 two-step cycles (94°C for 1 min, 72°C for 4 min) with CD44-specific primers as described previously.[20] The sense and anti-sense primers used to

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amplify CD44 cDNAs carrying various isoforms were: S1, 5′-CAGACCTGCCCAATGCCTTTGATGGACC and AS1, 5′-GTGAGTGTCCATCTGATTCAG, respectively (Fig. 1). The quality of the cDNAs used in the present study was checked in advance by PCR reaction using actin-specific primers (data not shown). The PCR products amplified using S1 and AS1 primers were electrophoretically separated on 1.4% agarose gels and then transferred onto Hybond N^+ nylon membranes (Amersham, Buckinghamshire, UK) for repeated hybridizaions with probe S and probe M. Probe S, which hybridizes to invariant regions adjacent to the insertion site of variant exons, was used to detect all CD44 isoforms, including the CD44 standard isoforms as well as various CD44v isoforms. It was prepared from PCR products of peripheral blood leukocyte cDNA using S1 and AS1 primers. CD44v isoforms carrying the region corresponding to that of the rat metastatic variant (exons 4 through 7) were detected with probe M, which contains CD44v exons 4 through 7. Random-primed cDNA of Calu3, an NSCLC cell line known to express whole CD44v exons,[21] was used to generate probe M by the RT/PCR technique. The PCR primers used to prepare probe M were: MS1, 5′-AAAACAGAACCAGGACTGGAC and MAS1, 5′-GGTGTGAGATTGGGTTGAAGA (Fig. 1).

RESULTS

Expression of CD44 isoforms in primary lung cancer and metastases in comparison with that in the corresponding normal lung tissues We examined expression patterns of CD44 isoforms by RT-PCR (Fig. 1) in 5 cases in which complete sets of RNAs extracted from primary and metastatic deposits as well as from the corresponding normal lung were available for analysis (Fig. 2). Four of 5 cases exhibited similar expression patterns of CD44 isoforms among normal, primary tumor and metastatic deposit RNAs, while the remaining single case (Case 1) showed much higher expression of CD44v isoforms in a metastatic deposit compared with normal lung and primary tumor levels. All normal lung specimens including Case 1 expressed readily detectable CD44v isoforms.

Expression of CD44 isoforms in paired specimens of normal lung and lung cancer We also analyzed expression patterns of CD44v isoforms in 18 paired sets of normal lung and lung cancer specimens which were obtained at the time of surgical resection (Fig. 3). Although 2 cases (Cases 7 and 10) were found to express CD44v isoforms more abundantly in lung cancer than in normal lung, most of the cases (16 of 18) expressed

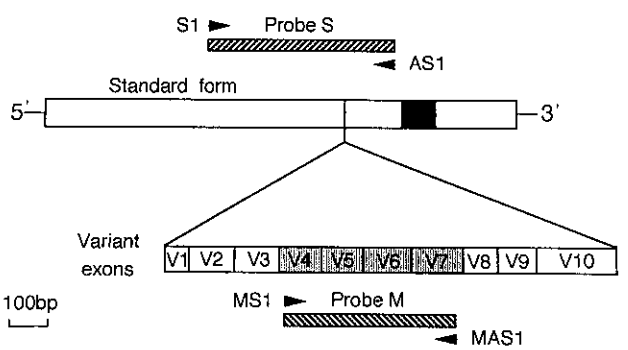

Fig. 1. Schematic representation of the standard and variant CD44 isoforms as well as of the probes used in the present study. Boxed region, coding region; solid box, the transmembrane domain; hatched bars, probes S and M; S1, AS1, MS1 and MAS1, PCR primers. Probe S detects both the standard and variant CD44 isoforms, while probe M hybridizes mRNA species containing variant exons 4 through 7 (shaded box) which correspond to rat metastatic variant mRNA.

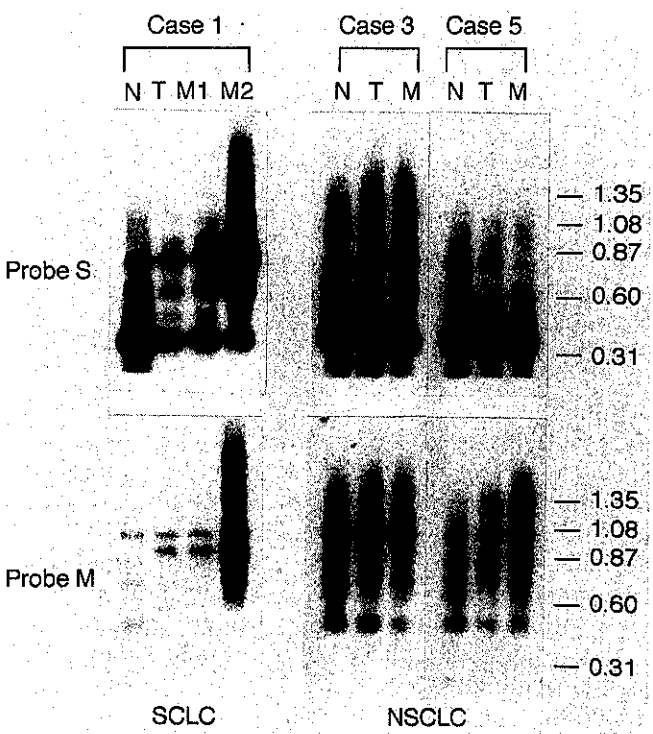

Fig. 2. Expression of CD44 isoforms in primary and metastatic sites of lung cancers in comparison with corresponding normal lung tissues. Cases 1 and 5 show higher expression of CD44v isoforms in metastatic deposits than in normal lung and the primary tumors, while Case 3 expresses both standard and variant CD44 isoforms in normal lung tissues at levels similar to those in the corresponding primary tumor and metastatic deposits. N, normal lung; T, primary tumor; M, metastatic deposit. Case 1, small cell carcinoma; Cases 3 and 5, squamous cell carcinomas.

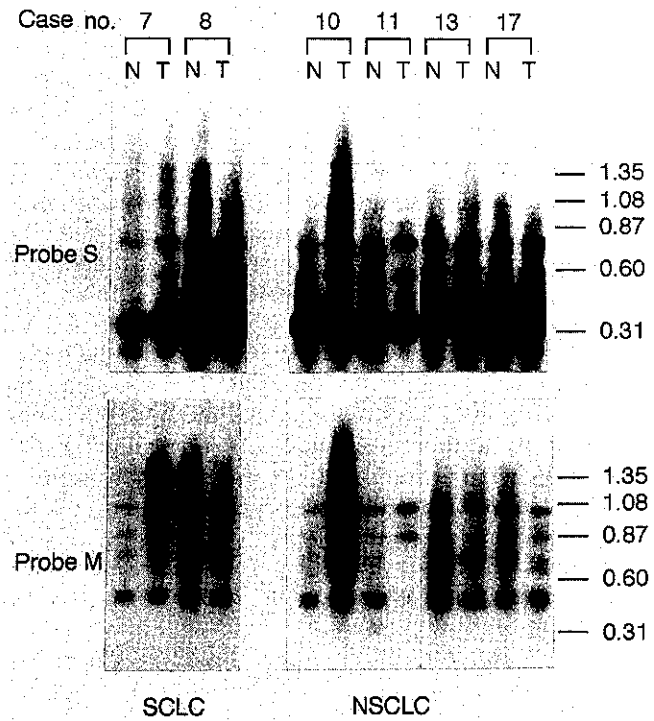

Fig. 3. Expression of standard and variant CD44 isoforms in lung cancers in comparison with corresponding normal lung tissues. Cases 8, 11, 13 and 17 show expression of CD44v isoforms at similar levels in both normal lung (N) and lung cancer (T). In contrast, CD44v isoforms appear more abundant in lung cancers than in the corresponding normal lung samples in Cases 7 and 10. Cases 7 and 8, small cell carcinomas; Cases 10, 11 and 13, adenocarcinomas; Case 17, squamous cell carcinoma.

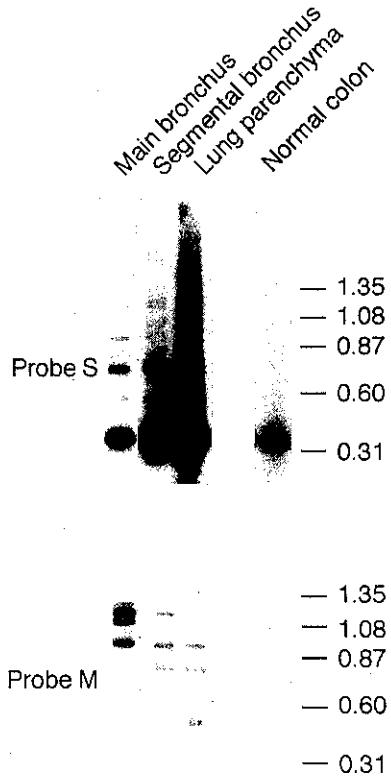

Fig. 4. Expression of standard and variant CD44 isoforms in various regions of normal lung tissues as well as in normal colon tissues. Clear expression of both standard and variant CD44 isoforms is evident in both central and peripheral lung samples, whereas expression of the CD44v isoforms is almost negligible in a normal colon specimen.

CD44v isoforms at similar levels in both normal and cancer tissues of the lung. We observed no correlation between expression patterns of CD44v isoforms and patho-clinical data such as histological type, nodal involvement and disease stage. Southern blot analysis using an oligonucleotide probe specific for variant exon 6 also yielded similar results, indicating that most of the CD44v isoforms detected with probe M contained the variant exon 6 (data not shown).

Expression of CD44 isoforms in various regions of normal lung tissues Since considerable heterogeneity of cell types exists in the lung, we also compared expression of CD44v isoforms in central and peripheral regions (Fig. 4). Bronchial epithelial cells were collected by gentle scraping of the inner surface of the main bronchi and the segmental bronchi in two cases. Peripheral lung parenchyma samples of the same cases were also taken. Southern blot analysis using probes S and M revealed that CD44v isoforms were expressed in both the central and the peripheral parts of the lung, whereas preferential expression of the CD44 standard isoforms was observed in normal colon (Fig. 4).

DISCUSSION

CD44 is known to exist as a number of isoforms generated by alternative splicing of 10 variant exons. Expression of CD44v isoforms in normal colon and breast was previously reported to be essentially negligible, but it is significantly upregulated in colon and breast cancers, suggesting its potential use in early diagnosis as a marker for these neoplasms.[17–19] In the present study, investigation of the expression of various CD44 isoforms in 23 lung cancer specimens and in the corresponding normal lung tissues revealed a clear distinction between normal cells of the lung and normal colon and breast cells. In almost all our cases, CD44v isoforms were expressed at similar levels in both lung cancer and normal

lung samples. Consequently, expression of CD44v isoforms does not appear to have any potential as a marker for the detection of lung cancer cells, as was suggested for colon and breast cancers. These results also suggest that expression of CD44v isoforms is not acquired in the process of malignant transformation of bronchial epithelial cells, although they do not preclude the possibility that certain types of normal lung cells expressing CD44v isoforms may preferentially undergo malignant transformation.

CD44v isoforms are thought to participate in lymphocyte emigration from the blood stream at the sites of high endothelial venules of lymph nodes.[8, 22] In addition, among the several steps involved in the process of metastasis, CD44v isoforms have been suggested to play a role in arrest of tumor cells at secondary sites, leading to colonization and proliferation of metastatic deposits. Tumor cells that overexpress CD44v isoforms exhibit enhanced metastatic potential in certain animal models, while metastasis to draining lymph nodes and subsequent further spread in such animal models have been shown to be blocked by antibodies specific to CD44v exons.[16, 23, 24] Abundant expression of CD44v isoforms in certain types of human tumors such as colon and breast cancers has been suggested to have a possible relation to the metastatic potential of these cancers.[17–19] The present study demonstrates that CD44v isoforms are also expressed in virtually all lung cancer specimens. It is therefore possible that lung cancer cells may utilize CD44v isoforms, which are maintained during malignant transformation of bronchial epithelial cells, as part of their machinery to achieve high metastatic potential.

Expression of a number of adhesion receptors has been identified in the lung and this has been suggested to play important roles not only in pulmonary disease but also in development and maintenance of the normal structure and function of the lung.[25] It is therefore conceivable that CD44v isoforms, which are readily detectable in normal lung, may also play physiological roles in this organ, perhaps mediating interaction and migration of epithelial cells. This area awaits further studies using antibodies specific to CD44v exons. Experiments to determine the *in situ* localization of CD44v isoforms are clearly warranted to gain insight into functional roles of CD44v isoforms in the various cell types of the adult normal lung as well as at different periods of fetal lung development.

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