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Original Paper

Caveolins as tumour markers in lung cancer detected by combined use of cDNA and tissue microarrays

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Abstract

To identify new potential diagnostic markers for lung cancer, the expression profiles of 37 lung tumours were analysed using cDNA arrays. Seven samples were from small-cell lung cancer (SCLC), two from large-cell neuroendocrine tumours (LCNEC), and 28 from other non-small-cell lung cancers (mainly squamous cell cancer and adenocarcinoma). Principal component analysis and the permutation test were used to detect differences in the gene expression profiles and a set of genes was found that distinguished high-grade neuroendocrine carcinomas (SCLC and LCNEC) from other lung cancers. In addition, several genes, such as *caveolin-1* (*CAV1*) and *caveolin-2* (*CAV2*), were constantly deregulated in all types of tumour sample, compared with normal tissue. The expression of these two genes was investigated further at the protein level on a tissue microarray containing tumours from 161 patients and normal tissues. Immunostaining for CAV1 was negative in 48% of tumours, whereas 28% of the tumours did not express CAV2. Lack of CAV1 protein expression was not caused by methylation or mutation. In stage I adenocarcinomas, CAV2 protein expression correlated with shorter survival. In conclusion, the present study was able to identify genes that have not previously been implicated in lung cancer by the combined use of two different array techniques. Some of these genes may provide novel diagnostic markers for lung cancer.

Keywords: lung cancer; high-grade neuroendocrine tumours; cDNA microarray; tissue microarray; caveolin

Introduction

Genetic changes underlying the development of lung cancer have been shown to be diverse and complex [1,2]. Although different types of lung cancer share common characteristics, each is thought to arise at least partially from a different set of mutations and gene expression changes. The four most common histological types of lung cancer are adenocarcinoma (AC), squamous cell carcinoma (SCC), large-cell lung cancer (LCLC), and small-cell lung cancer (SCLC), grouped clinically into non-small-cell lung cancer (NSCLC) and SCLC. The separation between NSCLC and SCLC is based on the different treatment strategies. However, large-cell neuroendocrine tumours (LCNEC), which belong to NSCLC, can also be grouped with SCLC to form a high-grade neuroendocrine carcinoma (HGNEC) group. Both of these tumour types show neuroendocrine properties and their origin is thus suggested to be different from the others. In addition, both have an aggressive clinical course with early widespread metastases [3].

In order to discover new possible candidate genes for lung cancer diagnosis and prognosis, we have recently described the gene expression profiles of AC and SCC, and the genes that are commonly and differentially expressed in these two types of NSCLC [4,5]. In the present study, we performed cDNA array screening to detect gene expression patterns in all major types of lung cancer and specifically to describe a set of genes unique to HGNEC. Furthermore, we studied the expression of two aberrantly expressed genes, *caveolin-1* (*CAV1*) and *caveolin-2* (*CAV2*), at the protein level by immunohistochemistry (IHC) on a tissue microarray (TMA) and screened for methylation status and mutations in *CAV1*.

Materials and methods

Lung specimens

Thirty-seven lung cancer tumours from Finnish patients were analysed using a cDNA array. The samples consisted of nine HGNECs (seven SCLCs and two LCNECs), one large-cell lung cancer (LCLC), and one adenosquamous carcinoma (AC-SCC), in addition to the 13 SCCs and 13 ACs described previously [4,5]. Two of the seven SCLCs were of combined type. All the tumours were classified according to the latest WHO classification. Four different histologically verified normal lung tissue specimens from patients with tuberculoma, intrabronchial granuloma, or lung cancer were used as reference. All lung cancer patients were followed up (range 72–162 months) and had a median survival time of 21.8 months (range 0.03–162.5 months). The mean age (56.8 years), smoking habits [47.6 pack years (PKY)], and gender distribution (33% females) of controls corresponded to those of the cancer cases (HGNEC/NSCLC: age 63.6/60.5 years; smoking 41.7/41.6 PKY; females 22%/18%).

All patients were personally interviewed and their consent to take part in the study and to use their tissue was obtained. The study protocol has been approved by the Ethical Review Board for Research in Occupational Health and Safety (75/E2/2001).

RNA extraction and cDNA array hybridization

RNA extraction and cDNA hybridization were performed as described previously [4]. The tumour samples contained a minimum 50% of cancer cells. Human Cancer Gene Filter 1.2 including 1176 tumour relevant genes was used for the cDNA array experiments (Clontech Laboratories, CA, USA). The expression levels of the raw images were determined using AtlasImage 2.0 software (Clontech).

Statistical analyses

In order to find genes that are differentially expressed in lung cancer compared with normal lung, the array results were analysed with two complementary statistical techniques, principal component analysis (PCA) and the permutation test (G-score), as described previously [4]. As the two statistical methods emphasize somewhat different features of the expression measurements, the combination of these methods will give a more extensive view of the deregulated genes than one method alone [4,5]. To detect specific gene expression patterns, HGNECs and NSCLCs were compared separately with the references and with each other, using two previously described statistical methods, PCA and G-score. Only those genes that appeared among the 25 most highly over- or under-expressed genes in both comparisons were included (Figure 1B).

CAV1 methylation analyses

The *CAV1* promoter region and exon 1 were screened for possible methylation sites using direct sequencing of sodium bisulphite-treated DNA. Ten tumour samples with low CAV1 expression and four normal lung samples were analysed. A 275 bp fragment was amplified from the promoter region [6] and a 348 bp fragment was amplified from exon 1 (F: 5-AGAATTT-TggggATgTgTTTA-3; R: 5-AACTAAAACCAAAA-CAACAAT-3) (hot start at 95 °C for 15 min, followed by 40 cycles with 30 s at 95 °C, 30 s at 55 °C, and 90 s at 72 °C). The PCR products were purified with a QIAGEN gel extraction kit. Sequencing was performed on an ABI-prism 310 sequencer (Applied Biosystems, Warrington, UK) using ABI Prism BigDye™ terminator cycle sequencing Ready Reaction Kit 3.1 (Applied Biosystems).

CAV1 mutation screening

The 14 samples described above were also screened for mutations in exon 3 of the *CAV1* gene. A modified set of primers (F: 5-CTgTgCTCATgTTgTgTCAC-TTC-3; R: 5-gAACTTgAAATTggCACCAgg-3) was used to amplify a 446 bp PCR product [7]. The previously reported mutation at codon 132 (P132L) was detected according to refs 7 and 8. Exon 3 was further screened for novel mutations by direct sequencing of the purified PCR product.

Semi-quantitative real-time RT-PCR

RT-PCR quantification was used to verify the array data for three genes that were differentially expressed between HGNEC (eight cases) and other tumours (six AC and five SCC cases) and controls (three cases). These 22 samples were included in the cDNA array. Ten-microlitre PCR reactions were performed on a LightCycler (Roche Diagnostics GmbH, Mannheim, Germany) using the LightCycler FastStart DNA Master^{PLUS} SYBR Green I (Roche) and 0.5 mM of each primer (MCM2-F: TAgTggCAAgTgAgCAgT-C; MCM2-R: gCTgAAgAAATCTCTgAAAg; DLK1-F: AAATggATTCTgCgAgg; DLK1-R: TTCATAgA-ggCCATCgTC; CD9-F: AACTTCATCTTCTggCTT-g, CD9-R: CgATCAgAATATAgACTCCTgT). Relative concentrations were attained as described in ref 4.

Tissue microarrays

Tissue microarray (TMA) blocks were constructed using a tissue-arraying instrument (Beecher Instruments, MD, USA). Four replicates of tumour tissue 1.0 mm in diameter from at least two different paraffin wax blocks from each case were placed in TMA blocks in addition to two samples representing normal central bronchus and peripheral lung from 161 patients. Lymph node metastases were obtained from 43 patients and two replicates were included in the

same TMAs. Seventy-two tumour samples were classified as SCC, 56 as AC, five as AC-SCC, 11 as LCLC, nine as SCLC (one combined SCLC, both types represented on arrays), four as LCNEC (one combined, both types represented on arrays), and four as pleomorphic carcinomas. Two samples presented CIS (carcinoma *in situ*). The 38 cases used in the cDNA array were also included in the TMA.

Caveolin-1 and *-2* immunohistochemistry (IHC)

Sections (4 μm) were cut from the TMA, dewaxed, and rehydrated in a series of graded alcohols. The sections were pretreated for antigen retrieval in a microwave oven (4 × 5 min) in Tris–EDTA buffer (pH 9.0). The antibody staining was performed in a TechMate Horizon immunostainer (DAKO ChemMate, Denmark). The primary mouse monoclonal anti-caveolin-1 (clone 2297) and anti-caveolin-2 (clone 65) antibodies (BD Transduction Laboratories, CA, USA) were incubated at room temperature for 25 min at 1 : 50 (CAV2) and 1 : 150 (CAV1) dilutions. Negative controls were performed by replacing the primary antibody with buffer. Blood vessels and other mesenchymal tissue, which highly express caveolins, served as positive controls on TMA slides.

Scoring and statistical analyses of IHC results

Each sample on the TMA blocks was examined by two independent investigators (HW and KS). The percentage of positively stained tumour cells and the intensity of staining were recorded for each sample. An arbitrary scale of 0, 1, and 2 was used. A value of zero was given when less than 10% of the cells were positive, 1 when there was weak homogeneous staining, and 2 when intense (high) staining corresponding to the staining of mesenchymal and peripheral lung tissue was present (Table 1).

Survival analyses were performed using the Kaplan–Meier method for survival curves and survival differences were analysed using the log-rank-test. The chi-square or Fisher's exact test was used to analyse the correlation between caveolin expression status and patients' clinical parameters (Table 2). Multivariate analyses were performed using the Cox proportional regression model to control other possibly confounding factors using SAS version 8 software (SAS Institute Inc, NC, USA).

Table 1. CAV1 and CAV2 protein expression in lung cancer detected by immunohistochemistry

		Caveolin-1		Caveolin-2	
Histology	**Expression***	***n***	**%**	***n***	**%**
All types	0	77	48.4	45	28.3
	1	42	26.4	46	28.9
	2	40	25.2	68	42.8
SCC	0	35	48.6	15	20.8
	1	18	25.0	19	26.4
	2	19	26.4	38	52.8
AC	0	25	44.6	16	28.6
	1	17	30.4	19	33.9
	2	14	25.0	21	37.5
AC-SCC	0	2	40.0	1	20.0
	1	1	20.0	2	40.0
	2	2	40.0	2	40.0
LCLC	0	6	54.5	5	45.4
	1	4	36.4	4	36.4
	2	1	9.1	2	18.2
LCNEC	0	2	50.0	2	50.0
	1	1	25.0	1	25.0
	2	1	25.0	1	25.0
SCLC	0	7	77.8	7	77.8
	1	2	22.2	1	11.1
	2	0	0	1	11.1
Pleomorph	0	0	0	0	0
	1	0	0	0	0
	2	4	100.0	4	100.0

* 0 =< 10% of cells positive; 2 = weak homogenous staining; 3 = high staining equivalent to the staining of mesenchymal and peripheral lung tissue.

Results

Gene expression patterns in HGNEC compared with controls and NSCLC

Figure 1A shows a three-dimensional representation of the gene expression profiles of 37 lung tumours, demonstrating a clear separation of most HGNECs from other carcinomas. Figure 1B shows those genes that were differentially expressed in HGNEC versus other lung tumours and normal lung. Seven genes were down-regulated in neuroendocrine tumours and 12 were up-regulated. Four of the seven genes down-regulated in HGNEC were also down-regulated in NSCLC compared with normal lung, but this was significantly less than in HGNEC. Three genes were down-regulated only in NSCLC and six genes were up-regulated only in NSCLC. The up-regulation of two and the down-regulation of one gene in HGNEC were verified by RT-PCR. *CD9* was down-regulated in all except one HGNEC (mean 11.9-fold change, range 0.9–24.1), whereas it was down-regulated in only two NSCLCs (3.4; 0.6–16.8). *MCM4* was up-regulated in all HGNECs (12.6; 6.4–20.4) compared with the normal lung. *DLK1* was expressed in seven of nine HGNECs, but in no NSCLCs or normal lung controls (Figure 2). The results correlated with those seen in the cDNA array study.

Commonly deregulated genes in all types of lung cancer

When all 37 patients were compared with the controls, 77 genes were found in the top 25 most up- and the 25 most down-regulated genes, calculated using PCA and/or the permutation test (Figure 3). Of these genes, 69% appeared in the top 25 in NSCLC and

Table 2. Association between CAV1 and CAV2 protein expression and clinical parameters*

	All				SCC				AC			
	CAV1		CAV2		CAV1		CAV2		CAV1		CAV2	
	Neg† $n = 77$	Pos† $n = 82$	Neg $n = 45$	Pos† $n = 114$	Neg $n = 35$	Pos† $n = 37$	Neg $n = 15$	Pos† $n = 57$	Neg $n = 25$	Pos† $n = 31$	Neg $n = 16$	Pos† $n = 40$
Age, years												
<65.1	40	38	26	52‡	17	15	9	23	16	15	12	19§
≥65.2	35	44	17	62	18	22	6	34	8	16	3	21
Stage												
I	34	40	18	56	14	16	4	26	12	18	6	24
II	18	19	10	27	12	10	6	16	4	4	3	5
III	20	16	13	23	9	11	5	15	7	4	5	6
IV	3	7	2	8	0	0	0	0	1	5	1	5
Lymph node												
Negative	36	46	18	64‡	16	19	4	31‡	12	19	6	25‡
Positive	40	36	26	50	19	18	11	26	13	12	10	15
Metastasis												
Negative	72	75	41	106	35	37	15	57	23	26	14	35
Positive	3	7	2	8	0	0	0	0	1	5	1	5
Smoking‖												
Yes	42	49	27	64	17	20	7	31	15	21	11	25
No/ex	28	25	13	40	14	10	5	19	7	9	3	13
Asbestos												
No	45	45	24	66	20	15	6	29	14	20	9	25
Exposed	28	31	17	42	12	17	6	23	10	11	6	15
Fibres												
<1 mio	47	50	29	68	22	21	10	33	12	18	9	21
≥1 mio	21	22	10	33	9	9	2	16	9	11	5	15

* Clinical parameters were missing as follows: age, stage, lymph node status and metastasis for two; smoking for 15; asbestos exposure for 10; and fibre count for 19 patients.
† Immunostaining for CAV1 and CAV2; neg = negative; pos = weak (value 1) or high (2) staining.
‡ $p < 0.1$.
§ $p = 0.03$.
‖ Ex-smokers = quit smoking more than 6 months before surgery.

70% in HGNEC (data not shown). Thirty-one genes appeared in both NSCLC and HGNEC top/bottom 25 lists. Both *caveolin-1* (*CAV1*) and *caveolin-2* (*CAV2*) were among the commonly down-regulated genes.

Immunostaining for CAV1 and CAV2

Immunostaining for CAV1 was negative in 48% of tumours, whereas 28% of the tumours did not express CAV2. The expression did not differ noticeably between the different NSCLC types, whereas the SCLCs had a slightly different expression profile, with almost 80% of cases showing no positivity. All four pleomorphic carcinomas showed high expression of both CAV1 and CAV2 (Table 2). In the cases with intense staining, both the cell membranes and the cytoplasm were stained. No clear membrane expression could be detected in cases with weak immunostaining. Stromal cells expressed caveolins at an intense or moderate level. The expression of CAV1 and CAV2 in lymph node metastases from 43 cases was very similar to that of the corresponding tumour tissue. Normal bronchial epithelium was completely negative for CAV1 and only faintly positive in the basal layer for CAV2. In peripheral lung, CAV1 expression was detected in type I pneumocytes but not in type II pneumocytes. CAV2 stained type I pneumocytes intensely, whereas only some of the type II pneumocytes expressed CAV2. Smooth muscle cells, adipocytes, and blood vessels stained intensively for both CAV1 and CAV2, as has been described previously (Figure 4).

A strong correlation between CAV1 and CAV2 immunostaining was found. CAV2 expression was at the same level (97 of 161 cases) as or stronger (56/161) than CAV1 expression.

Table 2 shows the relationship between CAV1 and CAV2 expression and different clinical parameters in univariate analyses. CAV1 and CAV2 expression did not differ between the different stages or other clinical parameters, except for CAV2 between different age groups among adenocarcinoma patients ($p = 0.03$). CAV1 and CAV2 showed no correlation with disease outcome in the Kaplan–Meier analysis when all histological types were analysed as one group or separately. However, in stage I adenocarcinoma, only one of six patients with no CAV2 immunostaining

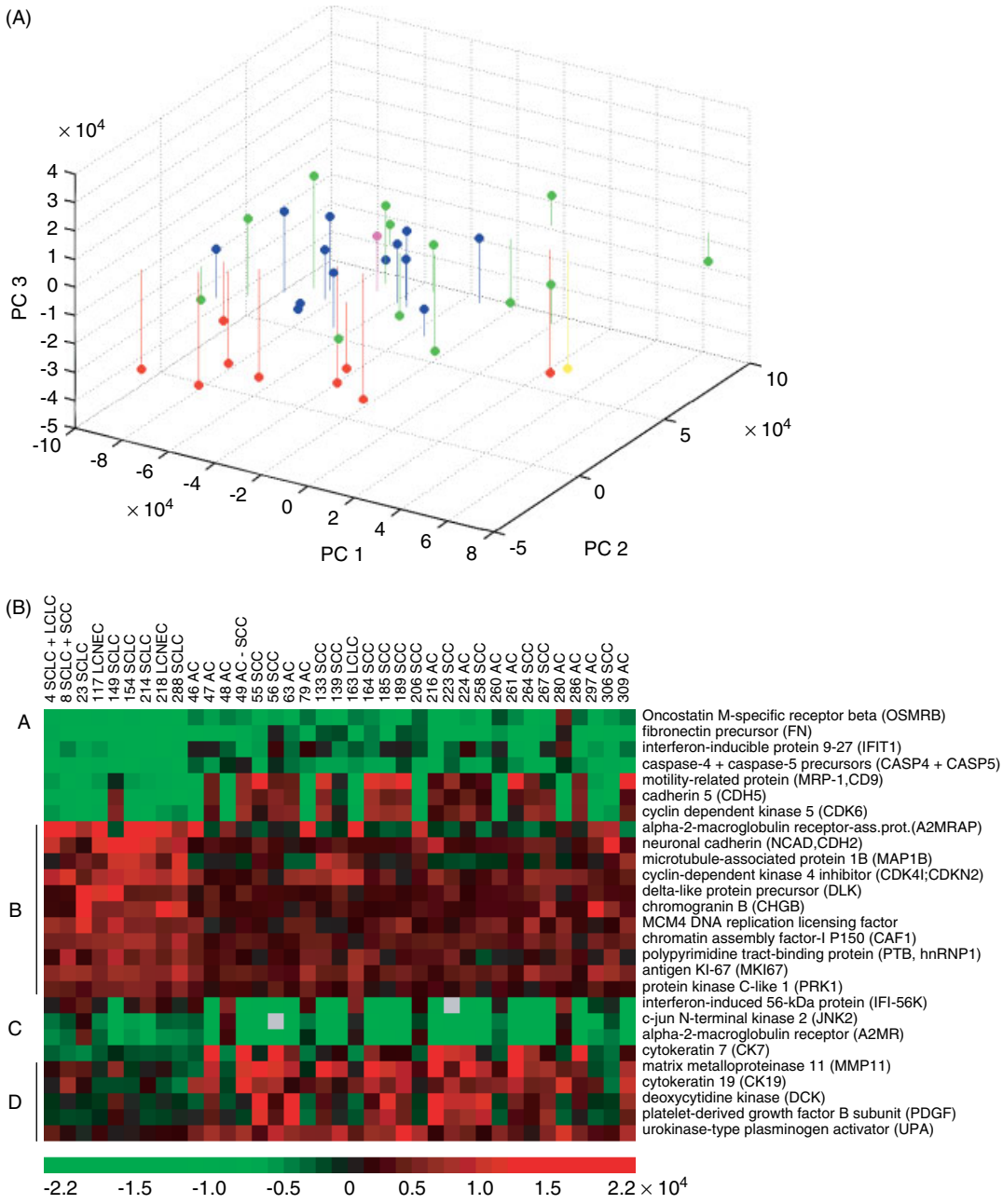

Figure 1. (A) Projection of the expression data onto the first three principal components (PC). The plot was calculated by PCA for all genes. To facilitate viewing of the three-dimensional image, each projection point (marked by a filled circle) is connected by a line to the plane where PC 3 has value zero. Red dots represent HGNEC cases, green AC, blue SCC, yellow LCLC, and lilac AC-SCC. (B) Thirty genes that were differentially expressed in HGNEC and in other lung tumours. The study samples are listed at the top, with the first nine samples being HGNEC. The genes are clustered as follows: panel A: down-regulated in HGNEC; panel B: up-regulated only in HGNEC; panel C: down-regulated only in NSCLC; and panel D: up-regulated only in NSCLC. The brightness of colour correlates with the degree of 'normalized expression difference' as shown at the bottom of image. The normalized expression difference was obtained by subtracting from each cDNA spot (1) the background value, (2) the adjusted intensity of four controls (mean), and finally (3) the mean intensity of the array

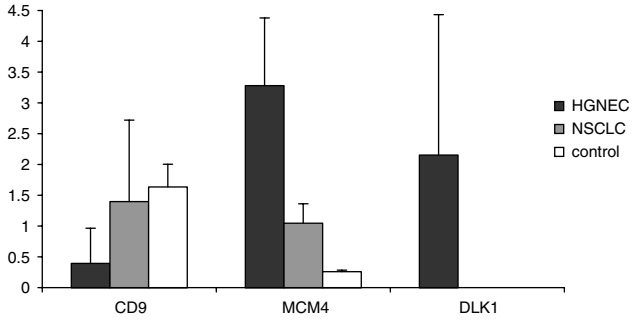

Figure 2. Gene expression results obtained by RT-PCR of three genes in eight HGNEC cases, 11 NSCLC cases, and three controls. The mean relative gene expression levels (fold change marked on *y*-bar) were obtained after normalization to a housekeeping gene (*PL2A*). No *DLK1* expression could be detected for normal lung and NSCLC samples

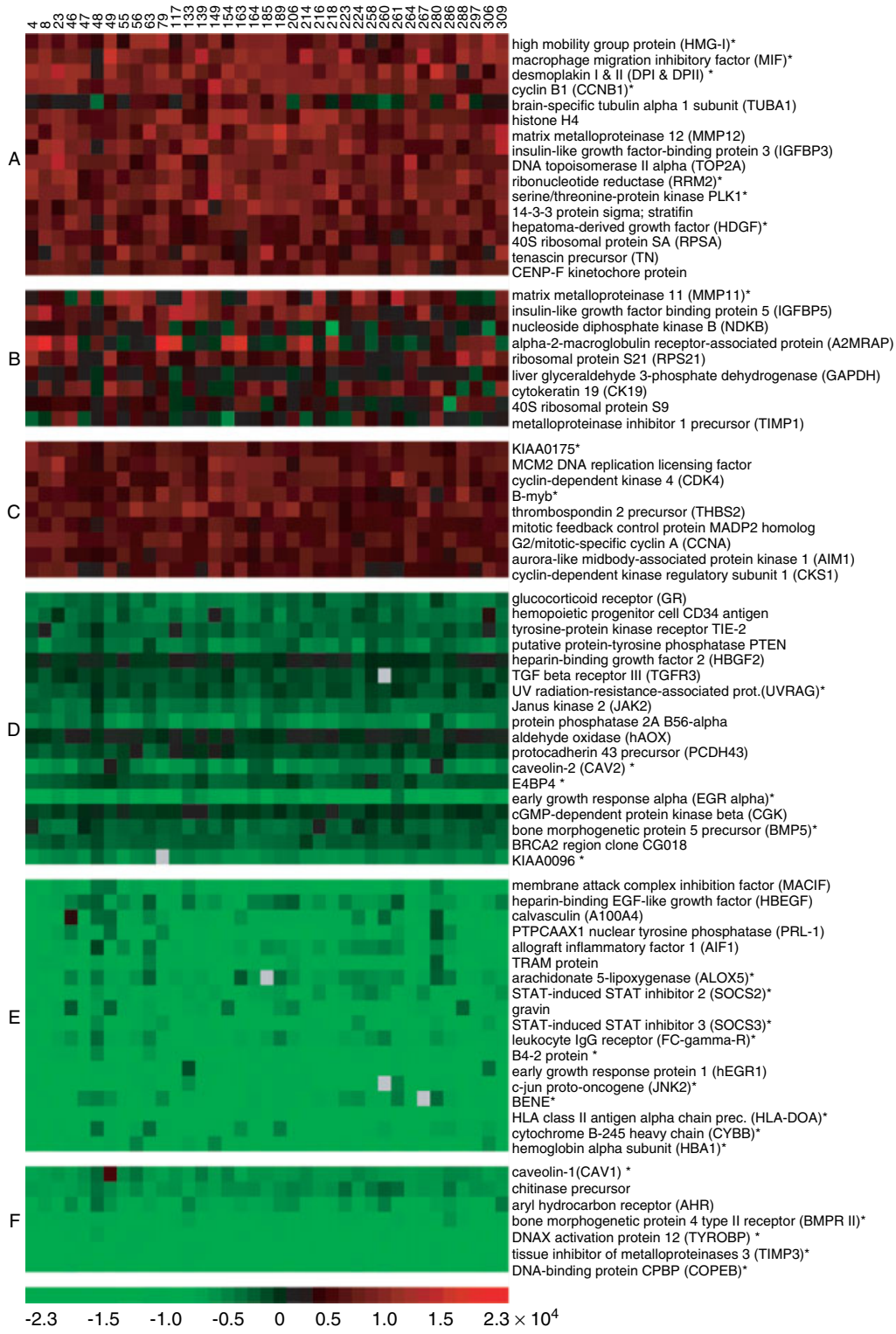

Figure 3. The gene expression profile of the 25 most over- and under-expressed genes in 37 lung tumours compared with four normal lung samples, calculated using both PCA analysis and the permutation test. Panels A and F: genes that are significantly deregulated (A: up-; F: down-regulated) according to both methods; panels B and E: genes significantly up- (B) or down- (E) regulated according to PCA only; panels C and D: genes significantly up- (C) or down- (D) regulated according to the permutation test only. Genes that also appear in the lists of 25 most up- and down-regulated genes in NSCLC and HGNEC are marked with an asterisk

died during the follow-up period, whereas 12 of 24 patients with CAV2 expression died of lung cancer and five of other reasons ($p = 0.088$) (Figure 5). The 5-year survival rate for patients with no CAV2 protein was 100%, compared with 53.5% in patients with CAV2 protein immunostaining. Multivariate analyses taking age and asbestos fibre content into account indicated that CAV2 positivity might be an independent unfavourable prognostic factor (hazard ratio 4.29, but $p = 0.17$).

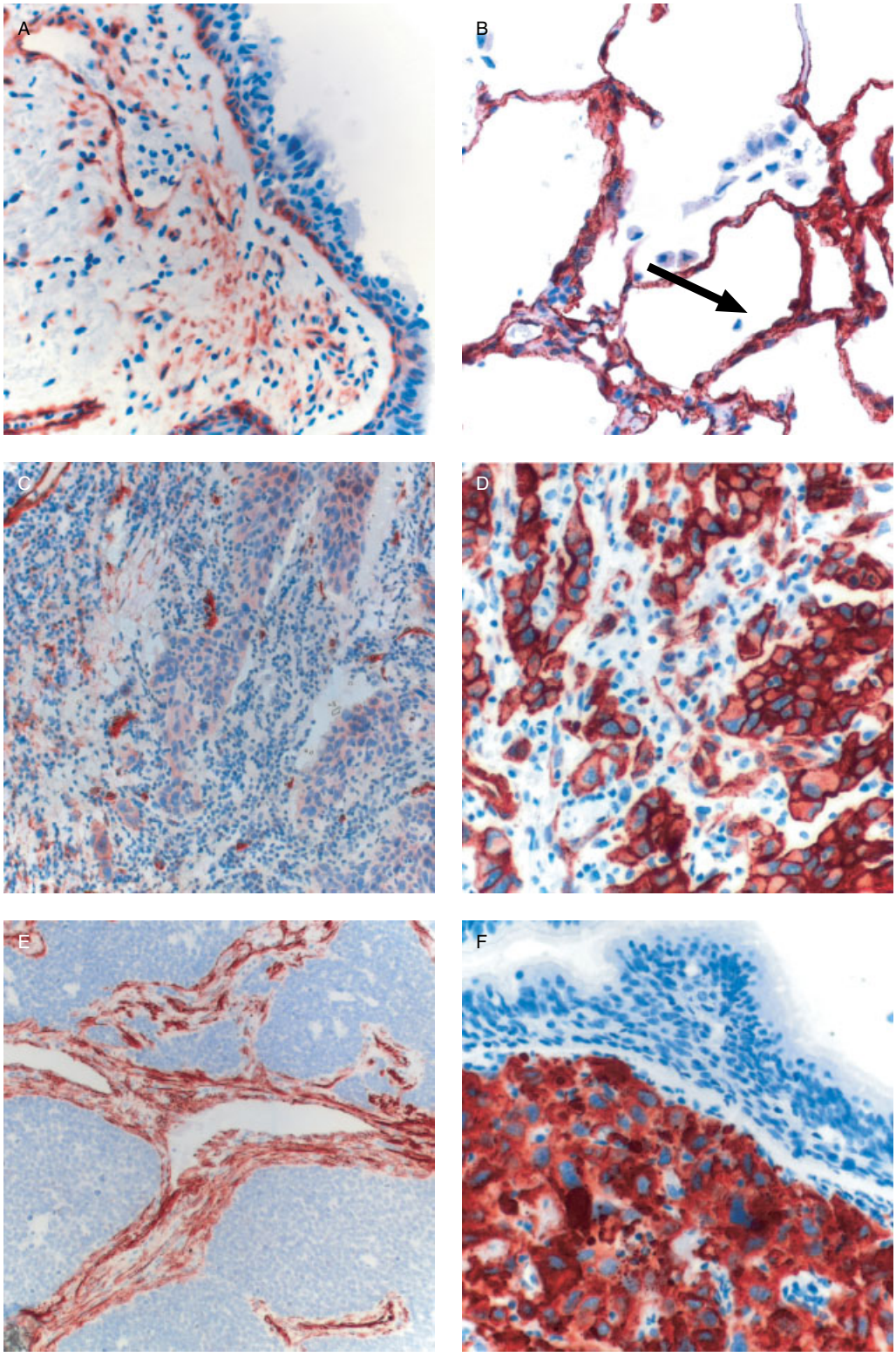

Figure 4. Immunohistochemical localization of CAV1 and CAV2 protein in lung tumours. (A) CAV2 immunostaining in bronchial tissue present only in the basal layer. (B) Peripheral lung with intense CAV2 staining of type I pneumocytes and endothelium, and some positivity in type II pneumocytes (arrow). (C) Weak homogenous CAV2 staining in SCC tumour cells and strong staining in blood vessels. (D) Strong CAV2 staining in AC tumour cells, especially on the cell membranes. (E) Negative staining of CAV1 in SCLC tumour cells but strong staining of stroma. (F) Adenosquamous carcinoma shows strong CAV1 expression in tumour cells but no protein expression in normal bronchial epithelium

Mutation and methylation analyses of CAV1

Ten tumour samples with low CAV1 expression and four normal lung samples were screened for methylation of CpG islands in the promoter region and exon 1. No methylation was found in the promoter region or in the CpG-rich exon 1; neither did the same samples harbour any mutation in exon 3.

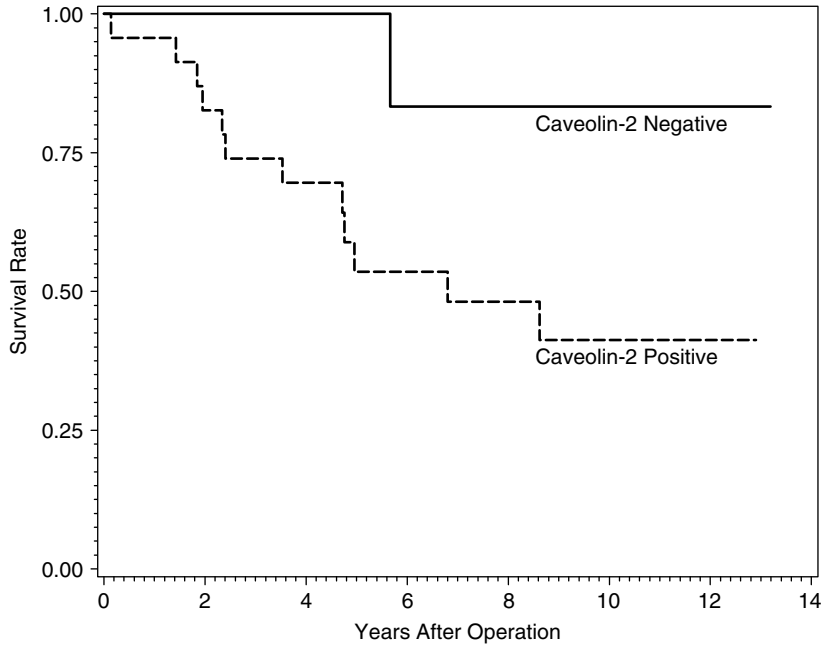

Figure 5. Survival among stage I adenocarcinoma patients. Patients with CAV2 expression ($n = 24$) showed a trend towards poorer prognosis compared with patients without CAV2 protein expression ($n = 6$) calculated using the Kaplan–Meier method

Discussion

Our cDNA array results show that HGNECs form a separate group among lung carcinomas according to their general expression profile. Furthermore, a set of genes was identified that was characteristic of HGNEC cases in comparison with normal lung controls and other lung cancer cases. Some of the up-regulated genes in the HGNEC group have previously been associated with neuroendocrine differentiation or SCLC [eg delta-like protein (DLK1) and chromogranin B] [9–11], but several novel genes were also found. Neural cadherin (NCAD) and microtubule associated protein 1 (MAP1B) are known to be expressed in neuron-derived tissue [12,13], but their expression has not, to our knowledge, been studied before in HGNEC. Other potentially interesting genes included *hnRNP1* and *MCM4*. Both of these genes have close relatives, *hnRNP A2/B1* and *MCM2*, both well-studied, potential early lung cancer detection markers [14,15]. Among the down-regulated genes in HGNEC was *motility related protein* (*MRP-1*, also known as *CD9*), known to be associated with neuroendocrine differentiation [16]. It has been suggested that a low CD9 level could be one factor contributing to the high metastatic phenotype of SCLC [17].

The expression profiles of the different types of lung cancer shared many common characteristics. Among the 34 overexpressed genes in all lung cancer types, 21 encode for proteins involved either in the cell cycle (13 genes) or in extracellular matrix and cell-to-cell communication and maintenance (eight genes). Other genes that were overexpressed in the tumour samples included those encoding growth factors (three genes), transcription regulators (three genes), and ribosomal proteins (two genes). The commonly underexpressed genes in our tumour samples encode for a far more heterogeneous group of proteins. Functional classes that were down-regulated in the tumour samples included those encoding for proteins related to transcriptional regulation (ten genes), stress response and immune defence (five genes), growth controllers (five genes), cell adhesion receptors and cell surface antigens (four genes), oncogenes and tumour suppressors (three genes), and xenobiotic metabolizing enzymes (two genes). Many of these genes have been discussed in more detail previously [4,5].

As both *CAV1* and *CAV2* were shown to be deregulated in all types of lung cancer, we decided to study these genes in more detail at the protein level. Caveolins form the major integral membrane components of caveolae, with CAV1 and CAV2 appearing as hetero- or homo-dimers [18]. The *CAV1* gene may possess both tumour suppressor activities and the ability to promote metastasis (reviewed in ref 19). Introduction of *CAV1* into some tumour cell lines can inhibit tumour growth and reduce tumourigenicity [20,21]. However, it has also been shown that *CAV1* overexpression can mediate metastasis and tumour growth by inhibition of c-Myc-induced apoptosis or by inducing the formation of filipodia [22,23]. Accordingly, CAV1 protein expression has been associated with both positive and negative prognosis in many different types of tumour, including the lung [24–27]. CAV2 has traditionally been considered a dispensable partner of CAV1. However, recent results of *Cav2* knockout mice have indicated a selective and important role for Cav2 in the lung [28]. Accordingly, CAV2 has not previously been implicated to play any major role in other types of cancer, but it has not been studied in primary lung tumours.

In our study, about half of the tumours did not express CAV1 protein and one-third showed no expression of CAV2. Two studies on CAV1 expression in primary lung tumours and lung cancer cell lines have been published to date, both showing expression patterns similar to ours [23,29]. Caveolins exist both as membrane proteins and as soluble proteins in multiple cellular compartments [19], as was also seen in the immunostaining of our samples. The expression of CAV1 and CAV2 was not associated with survival except for the trend found in stage I adenocarcinomas. In this group, 71% of the patients with CAV2 expression died, compared with 17% of the CAV2-negative patients. The death rates among CAV2-positive patients were much higher than expected among stage I patients [30]. The results did not reach statistical significance but the outcome was most likely hampered by the relatively low number of patients and a large group of censored observations (death due to other causes). The finding by Ho *et al* [23] of a correlation between lymph node positivity and CAV1 expression in lung adenocarcinomas with poor prognosis could not be confirmed in our study. However, only 12 of our samples were adenocarcinomas with lymph node metastases. Taking all the data together, both caveolins may play a role in the later stages of tumourigenesis, particularly the metastatic behaviour of tumours.

In agreement with other studies, we showed that CAV1 is highly expressed in type I, but not in type II, pneumocytes [31,32]. We could not detect CAV1 expression in bronchial epithelium, which is in contrast to the findings of Racine *et al*, who reported CAV1 and CAV2 expression in bronchial cell lines [29]. This discrepancy could result from studying tissues versus cell cultures. It has been shown that in cell cultures of primary type II pneumocytes, CAV1 is initially absent but appears after culturing [32]. As the proposed originating cells of most NSCLCs, ie bronchial epithelial cells (SCC) and type II pneumocytes (AC), did not express CAV1, CAV1 can be assumed to be highly up-regulated in 5–35% of tumours, depending on the type.

The absence of methylation and somatic mutations in *CAV1* in our study further supports up-regulation of *CAV1* in a proportion of lung tumours. Promoter region methylation and mutations in exon 3 have been described in prostate and breast tumours [6,8], but the originating cells in both of these tumours seem to express CAV1. The situation for CAV2 could be reversed, as we detected faint staining of bronchial basal cells and type II pneumocytes. The initial finding of *caveolin* down-regulation by cDNA array resulted from massive expression of caveolins in the pulmonary capillary endothelium and type I pneumocytes in the control samples. Our findings demonstrate the importance of the validation of the initial findings using different methods, especially at the cellular level.

In this study, we have shown that the expression profiles of different types of lung cancer share many characteristics, yet each type has unique properties. Among the genes distinctly deregulated in HGNEC were *CD9, DLK1, NCAD, MAP1B, hnRNP1*, and *MCM4*. Some of the properties of these genes might partly cause the aggressive phenotype of these tumours. Furthermore, we have shown that both *CAV1* and *CAV2* are deregulated, at both RNA and protein levels, in a large proportion of all types of lung cancer. Survival analyses indicated that CAV2 positivity might be an independent unfavourable prognostic factor in adenocarcinoma patients.

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