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Identification of trophinin as an enhancer for cell invasion and a prognostic factor for early stage lung cancer

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ABSTRACT

The explosion of microarray studies has promised to shed light on the identification of disease markers. To find novel prognostic factors, we used the expression profile of a poor prognostic factor of lung cancer, survivin (*BIRC5*), as a template to search for and compare transcriptome expression profiles in a lung adenocarcinoma microarray dataset. Trophinin (*TRO*) was identified as one of the best-correlated genes. The trophinin expression in lung cancer specimens was examined by immunohistochemical staining. The role of trophinin in cancer metastasis was further investigated by approaches of overexpression and knock down with small interfering RNA (siRNA). For stage I lung adenocarcinoma, the patients without trophinin expression had a better overall and disease-free survival. Overexpression of trophinin increases cell invasion ability and knock down with siRNA inhibits cell invasion. Through a combination of data mining and biochemical assays, we identified trophinin, which could enhance cell invasion, as a novel prognostic factor for early stage lung cancer.

1. Introduction

Lung cancer is the leading cause of cancer mortality in most countries, including Taiwan.[1,2] Approximately 80% of cases are classified histologically as non-small cell lung cancer, with a 5-year survival rate of only 14%.[3–5] Surgical removal is often the choice of treatment in primary lung cancer, but widespread dissemination of the cancer often defeats this mode of treatment. Many pre-operative variables that affect the survival of patients with non-small cell lung cancer have been identified.[6–9] Tumour size, vascular invasion, poor differentiation, high tumour-proliferative index and several genetic alterations, including *p53*[9,10] and *K-ras*[11,12] mutations, have also been implicated with prognostic significance. Search for novel prognostic markers remains an important task for lung cancer diagnosis and treatment.

Genome-wide microarray technologies, which are widely used to monitor global gene expression in cancer, have identified numerous differentially expressed genes[13–16] suggesting that microarray has promised to shed light on the identification of disease markers for diagnosis. However, researchers are often faced with the difficulty of effective utilisation of

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the vast information source gathered through these microarray studies. The unmet needs include how to efficiently translate existing raw microarray data into a workable model to aid in the greater understanding of target prioritisation. The combination of *in silico* biology and empirical methods may circumvent the bottlenecks from microarray profiling to target identifications.

In this study, a methodology was first set up to extract useful information from microarray dataset for identification of new prognostic factor of lung cancer. We hypothesised those genes showing similar expression pattern in microarray database, which might belong to different categories of biological functions, may have the same impact on clinical outcomes. A clustering/classification algorithm was applied to search for genes that show similar expression profiles to a previously identified prognostic factor for non-small cell lung cancer, survivin,[17,18] from lung adenocarcinoma microarray dataset. Immunohistochemical staining of the selected gene product in lung cancer specimen was performed. The results were correlated with the clinical characteristics and outcomes. Ectopic expression of selected gene and inhibition by small interfering RNA (siRNA), which monitored with cell invasion abilities, were also attempted.

2. Materials and methods

2.1. Microarray analysis

A total of 58 samples were used for microarray analysis, including pairwise samples from 27 female patients, who underwent surgery for lung cancer at the Taipei Veterans General Hospital, and four lung cancer cell lines, $CL_{1\text{-}0}$, $CL_{1\text{-}1}$, $CL_{1\text{-}5}$, and $CL_{1\text{-}5}$-F4.[19] The quality of the total RNA for microarray analysis was determined using Spectra Max Plus (Molecular Devices) and had an OD260/OD280 ratio ranging from 1.9 to 2.1. Protocols and reagents for hybridisation, washing and staining followed the Affymetrix's instructions (http://www.affymetrix.com/support/technical/manuals.affx). Labeled cRNA was hybridised to the Affymetrix GeneChip Test 3 Array to verify the quality prior to hybridise to the Affymetrix Human Genome U133A Array. All statistical analyses were performed by the Statistical Program for Social Sciences program package (SPSS) version 13.0 (SPSS Inc., Chicago, IL), R 2.1.1, and GeneSpring 7.3 (Agilent Technologies). The normalisation methods, including MAS5 (Microarray Suite software 5.0) and RMA (Robust Multi-chip Average), were verified by GeneSpring.

2.2. Cell lines and culture conditions

$CL_{1\text{-}5}$, a highly invasive lung adenocarcinoma cell line, was grown in RPMI-1640 medium. Madin-Darby canine kidney (MDCK) cells, clone 3B5, were grown in DMEM medium (Life Technologies, Inc.) with 10% foetal bovine serum (FBS) (Life Technologies, Inc.) and 2 mM L-glutamine (Life Technologies, Inc.) at 37 °C in a humidified atmosphere of 5% CO_2–95% air.

2.3. Stable transfected clone selection

Plasmid encoding the full-length trophinin gene (a kind gift from Dr. Fukuda[20]) was constructed in HA-pcDNA3.1. Subconfluent MDCK cells were transfected with HA-tagged trophinin or vector with lipofectamine (Invitrogen), according to the manufacturer's instructions. MDCK cells stably expressing various constructs were selected in medium containing 800 μg/ml G418 (Calbiochem). Individual clone was picked up and lysed with RIPA buffer (150 mM NaCl, 0.1% SDS, 0.5% sodium deoxycholate, 1% NP-40, 50 mM Tris pH 8.0, and 10 μg/ml each of leupeptin, aprotinin, chymostatin, and pepstatin). Exogenous trophinin expression was examined by probing with 1:2000 dilution of anti-HA (3F10, Roche) antibody followed by incubation with secondary antibodies conjugated to horseradish peroxidase, and developed using the ECL system (Amersham Pharmacia Biotech). Beta-actin was used as the internal control.

2.4. Invasion assay

MDCK cells were subjected to invasion assay as described earlier.[21] Invasion assays of various MDCK stable clones were evaluated via 24 well Transwell (8 μm pore size polycarbonate membrane, Costar) chambers. For invasion assay, 117 μg Matrigel (BD Bioscience) were added to the upper surface of the membrane and allowed for gelling at 37 °C for overnight. 5×10^3 cells of various MDCK stable clones, including MDCK (vector control or vehicle) and two trophinin-transfected MDCK stable clones were suspended in 400 μl DMEM containing 10% FBS and were seeded to the upper chamber, whereas 600 μl DMEM containing 10% FBS were added to the outer side of the chamber. After being cultured in a 37 °C incubator for 16 h, cells on the upper surface of the membrane were removed by a cotton tip applicator and migratory cells on the lower membrane surface were fixed by methanol and stained with crystal violet. Cell invasion ability was determined by counting all invaded cells of each stable clone under a phase-contrast microscope on three independent membranes and then normalised with parental MDCK cells as relative ratio.

2.5. siRNA knock down assay

Three chemical synthesised siRNAs were purchased from Ambion. The siRNA sequences for trophinin (*TRO*, U04811) are: T1 (5′-CGAGACUAGCAAGAUGAAAtt-3′), T2 (5′-CCACAGAAGAGGACAGUGUtt-3′), and T3 (5′-GCCCAAAAUAACUUGGCAGtt-3′). These siRNAs were transiently transfected into $CL_{1\text{-}5}$ cells by using Lipofectamine. For optimising the siRNA knock down effect, cells were transfected twice with trophinin siRNAs at 24-h intervals. Seventy-two hours after the first transfection, cells were collected for invasion assay.

2.6. Quantitative reverse transcription-polymerase chain reaction (Q-RT-PCR)

Total RNA was isolated from cells with siRNA treatment using an RNeasy RNA extraction kit (Qiagen) plus DNase I (Promega). Five micrograms of total RNA from each sample was subjected to reverse transcription by a ThermoScript RT-PCR system (Invitrogen). The cDNA product diluted to 200× from each sample was used to perform Q-PCR analysis, using trophinin and GAPDH TaqMan probes, according to the

manufacturer's instruction (Applied Biosystems). The probe and primer sequences for trophinin, designed through a commercially available assay-on design (Applied Biosystems), were: probes (5′-AGCGGTATCTGAAACC-3′), forward primer (5′-TGGCTTCTCGTATGGCTAGTGA-3′) and reverse primer (5′-CATGGGAAGGACTACTGCAATTTAT-3′). The values from the quantitative RT-PCR were normalised to the housekeeping genes *GAPDH*. A $2^{\text{delta-CT(threshold cycle)}}$ method was used. Briefly, the CT value of the internal control gene was used to calculate normalised target gene expression, referred to as $2^{\text{delta-CT}}$, in order to correct differences between samples. Assays were performed in triplicate with Applied Biosystems model 7700 instruments.

2.7. Patients and specimens

A total of 141 patients, who underwent surgery for non-small-cell lung cancer at the National Taiwan University Hospital from 1991 to 2000 with available stored tissue blocks, were included as potential subjects in this study. The Institutional Review Board of National Taiwan University Hospital approved this study. One hundred and nineteen patients underwent lobectomy (84.4%), whereas the other 22 patients underwent pneumonectomy (15.6%). None of the patients had received neoadjuvant chemotherapy or radiation therapy before surgery. Specimens of lung cancer tissue and adjacent normal lung tissue obtained at surgery were immediately snap-frozen in liquid nitrogen and stored at −80 °C until use.

The World Health Organisation criteria of 1982[22] were used for histologic classification. Tumour size, local invasion, and lymph node metastasis were determined at pathologic examination. The final disease stage was determined by a combination of surgical and pathologic findings according to the current TNM system for lung cancer staging.[23] Relapse time was determined from the date of surgery to the date of local recurrence or systemic metastasis.

2.8. Immunohistochemical study

Paraffin sections (thickness, 5 μm) of tumour tissue from the patients were analysed immunohistologically using an anti-mouse monoclonal antibody against trophinin (1:10) kindly provided by Dr. Fukuda,[20] which was followed by biotinylated second antibodies. In brief, sections were deparaffinised with xylene, then rehydrated. The sections were incubated in PBS-T (0.2% Tween 20/PBS) for 20 min and were quenched with 3% H_2O_2 in distilled water for 30 min, followed by blocking with 10% goat serum for another 20 min (Kit, Histostain-sp bulk, Zymed, CA). The sections were incubated with primary antibodies at 4 °C overnight. After rinsing in PBS, sections were incubated with biotinylated secondary antibody (1:100) for 1 h and with the streptavidin peroxidase conjugate for another hour (Kit, Histostain-sp bulk, Zymed, CA). The reaction product was demonstrated by a DAB substrate kit (Zymed, CA). The counterstaining was performed using haematoxylin.

Because trophinin expression was normally detected in the chorionic villi of the placenta from the 6th to 10th week of pregnancy,[24] the staining of human placental tissue (7th week) selected from the pathology profiles of the Department of Gynaecology and Obstetrics, National Taiwan University Hospital, was used as a positive control. The trophinin protein expression was expected to be present in the cytoplasm.[24] The negative control staining was done without the addition of primary antibody. Positive trophinin expression was defined as more than 10% of cancer cells in five microscopic fields (200×) positively stained.

2.9. Statistical analysis

Where appropriate, the data were presented as the mean ± standard deviation. The statistical analyses were performed with the SPSS, version 10.0 (SPSS Inc., Chicago, IL). Differences in the clinicopathological characteristics between patient groups with and without trophinin expression were analysed by the chi-square test or by Fisher's exact test, when necessary. The survival of lung cancer patients was also analysed with respect to trophinin expression by the Kaplan–Meier method, and the difference in survival was analysed with the log-rank test. Multivariate analysis was performed by applying a Cox proportional hazard model to calculate the hazard ratio and its 95% confidence interval for gender, age, different cell types, stages, type of surgery and trophinin expression. All of the statistical tests were two-sided. A p value of less than 0.05 was considered to be statistically significant.

3. Results

3.1. Trophinin as one of the best genes correlated with survivin in lung adenocarcinoma microarray database

Microarray profiling of lung adenocarcinoma was performed on Affymetrix Human Genome U133A microarray chips. Since survivin (*BIRC5*) is a poor prognostic factor for lung cancer, we hypothesised those genes exhibiting similar expression profiling to survivin might be of clinical importance in prognosis of lung cancer. Therefore, the expression profile of survivin, which was overexpressed in lung adenocarcinoma (Fig. 1), was used as a template to search for and compare transcriptome expression profiles in 54 lung adenocarcinoma microarray datasets from 27 pair-wised tissue specimens. The analysis was normalised by RMA (Robust Multichip Average) method. A total of 691 transcripts were identified with a Pearson's correlation coefficient > 0.6.

To reduce the number of targets, microarray datasets generated from four progressively increasing invasion ability cell lines ($CL_{1\text{-}0}$, $CL_{1\text{-}1}$, $CL_{1\text{-}5}$, and $CL_{1\text{-}5}$-F4) were subjected to Spearman's correlation coefficient >0.6 analysis, resulting in the identification of 5273 transcripts. Intersection of the two revealed 277 genes with similar expression pattern with survivin. Because different microarray normalisation methods will result in different gene selections, we also used MAS5 as an alternative normalisation method and performed the same correlation analysis as described earlier. Intersection of the two analyses of two normalisation methods (RMA and MAS5) identified 46 genes.

Among the 46 best-correlated genes, trophinin (*TRO*) (Fig. 1, Pearson's correlation coefficient = 0.602, $p = 0.001$) was selected for further investigation because its expression is high in the human embryonic tissue as well as lung cancer

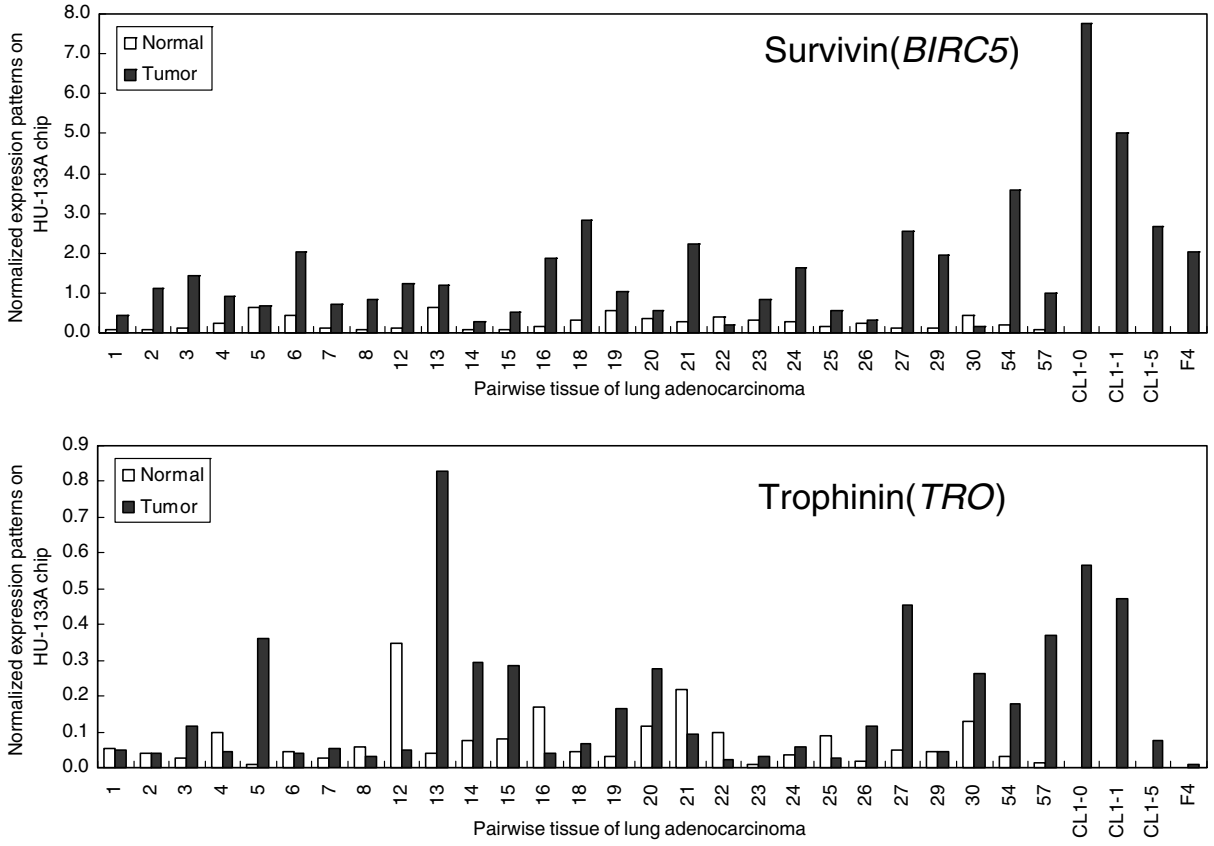

Fig. 1 – The gene expression patterns of survivin (*BIRC5*) and trophinin (*TRO*) from Affymetrix chips. Normalisation was performed by per chip and per gene by using GeneSpring® 7.2 software (Silicon Genetics, Redwood City, CA). The similarity between survivin and trophinin was presented by Pearson's correlation coefficients (0.602) with $p = 0.001$.

and undetectable in normal adult tissue, which is similar to the expression of survivin, and is not seen in the other 45 genes.

3.2. The correlation between immunohistochemical staining of trophinin and clinical characteristics of patient with lung cancer

To test our hypothesis that trophinin might serve as a prognostic marker for lung cancer, we examined trophinin expression using a trophinin-specific monoclonal antibody in lung cancer tissues from 141 patients undergone surgical intervention. The relationships between the trophinin expression and the clinicopathological characteristics, post-operative relapse, and survival were also examined.

Paraffin sections of lung cancer tissue from the 141 patients (100 male and 41 female) were subjected to immunohistological assessment. The trophinin staining in adenocarcinoma and squamous cell carcinoma are shown in Fig. 2a and b, respectively. The trophinin protein expression was mostly present in the cytoplasm, but not in the nucleus. The positivity is mainly found everywhere in the tumour and the distribution is heterogeneous.

The proportion of positive trophinin expression was significantly higher for female patients ($p = 0.003$) and patients with an age less than 65 years ($p = 0.027$) (Table 1). No statistically significant correlations were found between trophinin expression and histological type, pathological staging, or lymph node metastasis. For patients with lung adenocarcinoma, the proportion of positive trophinin expression was also significantly higher for female patients ($p = 0.028$) and patients with T1–2 disease ($p = 0.021$). For patients with squamous cell carcinoma, no statistically significant correlations were found between trophinin expression and age, gender, pathological staging, tumour invasion, or lymph node metastasis.

3.3. Trophinin is a poor prognostic factor for patients with stage I lung cancer

The median survival times for patients with different stages were: stage I: 63 months; stage II: 24 months; stage III: 39 months; stage IV: 22 months. The median disease-free periods for patients with different stages were: stage I: 48 months; stage II: 14 months; stage III: 16 months; stage IV: 9 months.

The median survival time for patients without trophinin expression was longer than for patients expressing trophinin, although the difference was not statistically significant (61.8 versus 45.8 months, $p = 0.256$). As we adjusted the factor of stage, we found that among patients with stage I disease, the survival period of individuals without trophinin expression was significantly longer (80.4 versus 50.2 months, $p = 0.042$). A multivariate analysis, using Cox forward regression model, was conducted for gender, age, histology, type

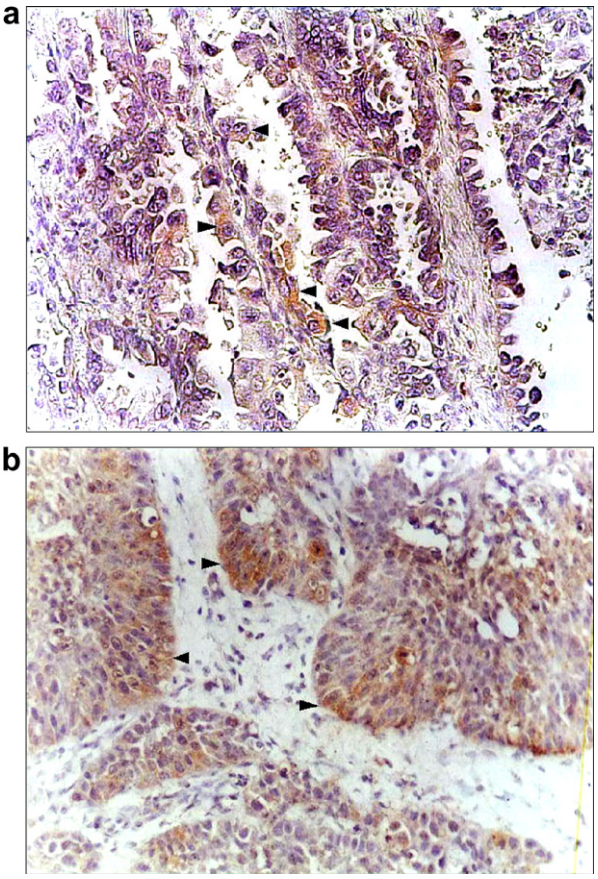

Fig. 2 – Immunohistochemical staining of trophinin in adenocarcinoma (a) and squamous cell carcinoma of lung (b). The arrowheads indicate trophinin protein expression, which was mostly present in the cytoplasm. (Avidin–biotin peroxidase complex method, 200× magnification).

of surgery, together with trophinin expression for patients with stage I disease. The patients with positive trophinin expression had a poor survival after adjusting other prognostic variables (Hazard risk: 2.28, 95% C.I: 1.00–5.20, $p = 0.049$).

As for patients with different histology, the difference of survival between patients with and without trophinin expression was statistically significant for adenocarcinoma. The difference for overall survival and disease-free survival period between patients with and without trophinin expression was even more significant in stage I lung adenocarcinoma (overall survival: 78.8 versus 39.6 months; $p = 0.006$, Fig. 3, disease-free survival period: 69.8 versus 34.5 months; $p = 0.03$, Fig. 4). For squamous cell carcinoma, there was no difference between patients with and without trophinin expression.

3.4. *Trophinin is an enhancer of cell invasion*

To provide a mechanistic basis for trophinin as a novel poor prognostic factor and to delineate the biological effects of trophinin in cellular invasion, we took two approaches. Firstly, we used MDCK renal epithelial cells as our model system, which is a suitable model for assaying cell invasion process,[20] to establish various trophinin stable clones. The expression levels of exogenous HA-tagged trophinin were verified via Western blotting (Fig. 5, lower panel), and these stable clones were used in the invasion assay. Overexpression of trophinin in two different MDCK cell clones resulted in a two-fold increase in invasion ability compared with those of vector control (vehicle) or parental MDCK cells (Fig. 5, upper panel).

Secondly, we employed three different chemical synthesised siRNA respectively to knock down the expression of trophinin in a highly invasive lung adenocarcinoma cell line, $CL_{1\text{-}5}$. The gene expression level of trophinin is low in $CL_{1\text{-}5}$ cells (Fig. 1) as confirmed by Q-RT-PCR (data not shown) and

Table 1 – Trophinin expression and clinicopathological data for 141 lung cancer patients

	Trophinin negative *n* (%)	Trophinin positive *n* (%)	*p* value[a]
Gender			
Female	14 (34.1)	27 (65.9)	
Male	62 (62.0)	38 (38.0)	0.003
Age			
<65	28 (43.7)	36 (56.3)	
⩾65	48 (62.3)	29 (37.7)	0.027
Histology			
Adenocarcinoma	38 (50.3)	37 (49.7)	
Squamous cell	38 (57.6)	28 (42.4)	0.412
Stage			
I–II	50 (51.5)	47 (48.5)	
III–IV	26 (59.1)	18 (40.9)	0.408
Tumour status			
T1–2	58 (50.9)	56 (49.1)	
T3–4	18 (66.7)	9 (33.3)	0.139
Lymph node status			
N0	40 (49.4)	41 (50.6)	
N1–3	36 (60.0)	24 (40.0)	0.211

a The differences were analysed by the chi-square test.

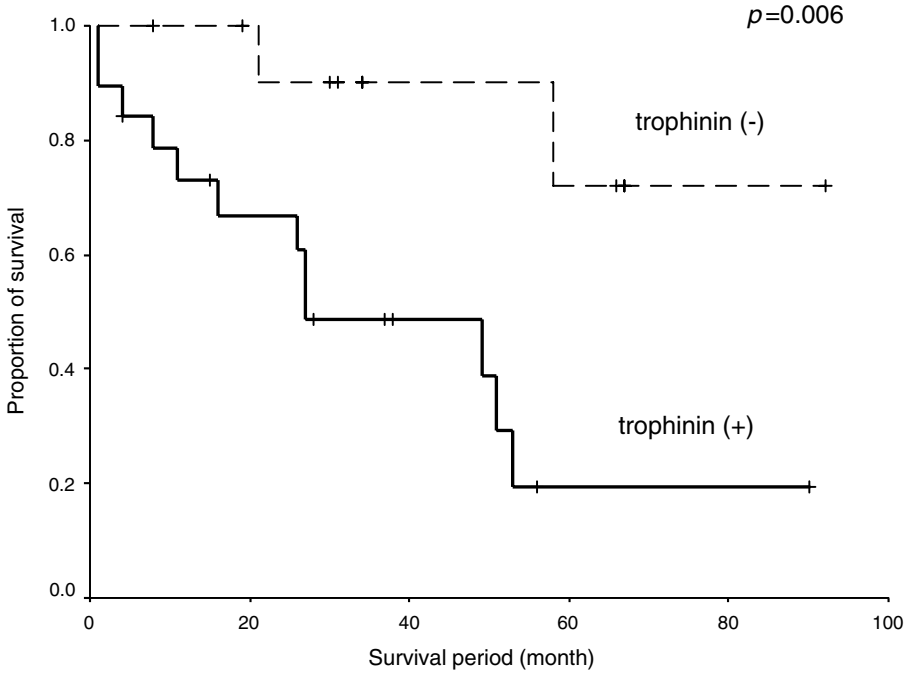

Fig. 3 – Survival analysis for stage I lung adenocarcinoma patients (*n* = 31), with (solid line) and without (dotted line) trophinin expression. The divergence between the curves representing the two groups of patients is evident, with survival time for the trophinin-expression group significantly shorter as determined by a log rank test (*p* = 0.006).

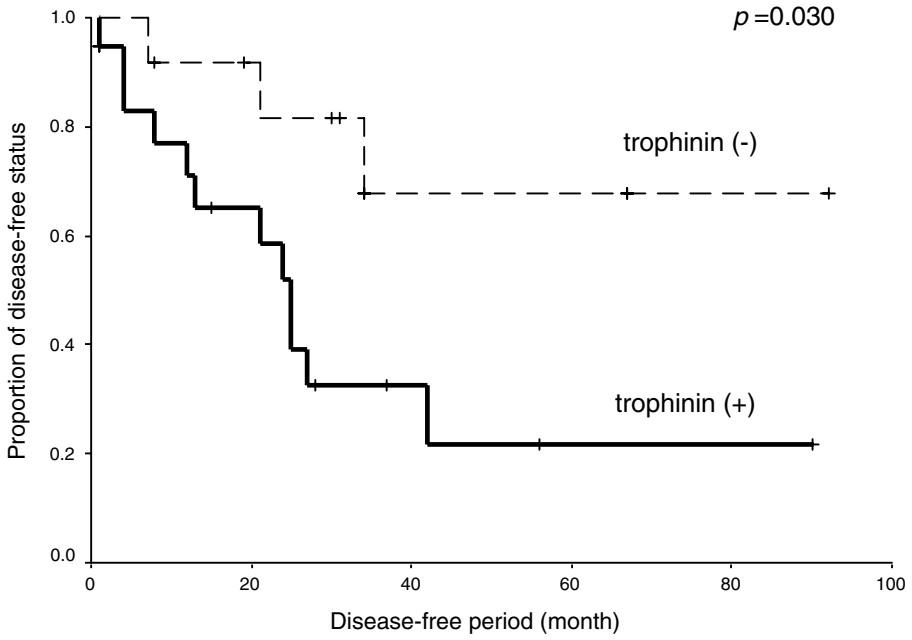

Fig. 4 – Recurrence analysis for stage I lung adenocarcinoma patients (*n* = 31), with (solid line) and without (dotted line) trophinin expression. The divergence between the curves representing the two groups of patients is evident, with survival time for the trophinin-expression group significantly shorter as determined by a log rank test (*p* = 0.030).

the change of protein expression level of trophinin cannot easily be detected in $CL_{1\text{-}5}$ cells (data not shown). Therefore, we monitored the trophinin knock down effect by using Q-RT-PCR. To optimise the knock down effect of trophinin siRNA, $CL_{1\text{-}5}$ cells were transfected twice with siRNA as described in the Materials and methods section. Endogenous trophinin RNA was knocked down by ~80%–90% (Fig. 6, lower panel) in all three trophinin siRNAs. Knock down of endogenous trophinin in $CL_{1\text{-}5}$ led to a decrease in invasion ability as compared to the vehicle transfected parental $CL_{1\text{-}5}$ cells (Fig. 6, upper panel). Together, these data are in agreement with the idea that trophinin may promote cell invasion.

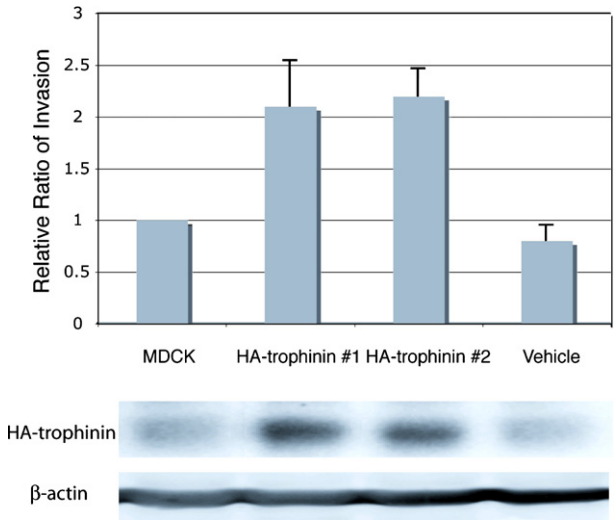

Fig. 5 – Invasion ability of trophinin-transfected MDCK stable clones. Four MDCK generated cell lines: MDCK (parental cell line), two different HA-trophinin-transfected MDCK stable clones (#1 and #2), and one vector transfection control (vehicle) are used in the invasion assay. Overexpression of trophinin in two different MDCK clones resulted in a two-fold increase in invasion ability compared with those of vector control (vehicle) or parental MDCK cells.

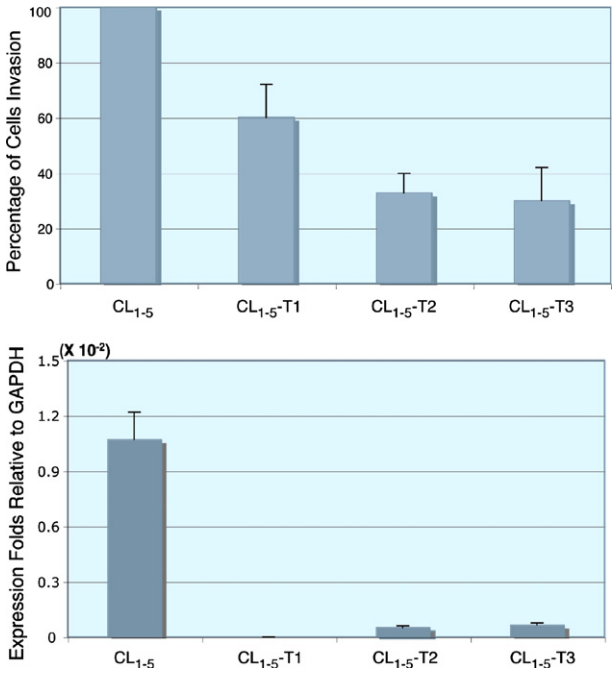

Fig. 6 – Trophinin knockdown using siRNA monitored with its effect on reduction of invasion ability of highly invasive CL_{1-5} cell lines. CL_{1-5} cells were double transfected with three different siRNAs as indicated. Knock down of endogenous trophinin in CL_{1-5} led to a decrease in invasion ability as compared to the parental CL_{1-5} cells (upper panel). Each sample was triplicated. Endogenous trophinin RNA was knocked down by about 80–90% (lower panel) as shown in Q-RT-PCR.

4. Discussion

In the post-genomic era, the search for novel prognostic and therapeutic targets for lung cancer is at an unprecedented rate. Using the traditional biochemical approaches is far from adequate in efficiently extracting useful information from various high throughput studies. This data-mining approach may be one of the easiest available means of gaining insight into the functional concordance of co-expressed genes, particularly when studying novel, poorly characterised genes.[13] Trophinin (*TRO*) was identified as one of the best-correlated genes with survivin by applying a clustering/classification algorithm with lung adenocarcinoma dataset. Trophinin was also demonstrated as a poor prognostic factor for lung cancer as survivin by using immunohistochemical study. Taken together, these findings support the methodology of data-mining from microarray database using a known prognostic factor to find novel molecule of prognostic value.

In this study, we demonstrated that trophinin, which was considered as an important adhesion molecule during embryo implantation, might influence the patient survival. The patients with trophinin expression were more likely to have a post-operative recurrence and a significantly shorter survival in stage I lung adenocarcinoma. These findings suggested that trophinin might be important in predicting the prognosis of early stage lung cancer, especially for adenocarcinoma. There is no previous study concerning the impact of trophinin on prognosis of cancer. Our study illustrated for the first time that lung cancer patients with positive trophinin expression had a worse outcome.

Our data showed that the percentage of positive trophinin expression was higher for patients with less advanced tumour invasion (T1–2) and for patients without nodal involvement ($N = 0$). These results support the hypothesis that trophinin expression was likely to occur in the early stage of cancer invasion/metastasis and decrease in the late stage, as the expression of trophinin is noted during the early phase of embryo implantation, but not late phase. The expression pattern of trophinin might explain why the lung cancer patients of stage I with trophinin expression have poor prognosis, whereas no significant survival difference could be demonstrated in patients with stage II, III, and IV.

Numerous studies have demonstrated that the cancer cells will damage the neighbouring tissue or migrate and affect the distant organ. Tumour cells first adhere to neighbouring cells, secrete the digestive enzyme and then invade the boundary of normal tissue. This process is reminiscent of the process of embryonic implantation, which is first the release of the unfertilised egg from the ovary, transportation of the embryo through the oviduct and uterus, and then invasion of the blastocyst to the endometrium.[25–28] Among the proteins involved in embryonic implantation, trophinin is a membrane protein that potentially mediates the initial adhesion between human embryo and uterine epithelial cells.[20,24] Strong expression of trophinin within a restricted area of human endometrium can be detected only at early secretory phase or time of implantation window, but not during proliferative and ovulation phases.[20,24] Considering its role in embryonic implantation while blastocyst invades the endometrium,

trophinin may also mediate the invasion process of cancer cells. We demonstrated that ectopic expression of trophinin could enhance cell invasion. Knock down of endogenous trophinin by siRNA in lung cancer cell lines led to a decrease in invasion ability. These findings are in agreement with a recent study that trophinin enhances invasiveness of the cells and promotes metastasis of testicular germ cell tumour.[29]

Trophinin is not expressed in normal tissues,[20] except in the endometrium and blastocyst during the implantation of the human embryo. However, trophinin expression was found in the lung cancer specimen. The mechanism of regulation of trophinin expression remains unknown. Survivin mRNA is selectively and abundantly expressed in embryonic and foetal tissues,[30] but undetectable or found at very low levels in normal differentiated tissue.[31,32] Re-expression of survivin in non-small cell lung cancer is also suggestive of poor prognosis.[17,18] Such expression pattern is similar to that of trophinin. Besides, survivin and trophinin have similar expression patterns in lung adenocarcinoma microarray dataset, which suggests trophinin and survivin might have some similar characteristics of expression regulation in common. Survivin is an anti- apoptosis gene; however, whether trophinin has a role in anti-apoptosis remains to be determined. As such, further investigation is warranted to clarify the regulation of trophinin and compare with the findings of studies about regulation of survivin expression.

It is noteworthy that the frequency of positive trophinin expression was significantly higher for female than for male ones. It may be due to the fact that its cytogenetic band is located on the X-chromosome and to the possible ability of escaping X-chromosome inactivation. The survival benefit of trophinin absence did not disappear if corrected for gender. We considered that the prognostic significance of trophinin might be present in both genders. However, our patient number might be too small to draw a conclusion. Since adenocarcinoma is currently the predominant histological subtype of non-small cell lung cancer[33] which has been more apparent in females in recent studies.[34,35] Thus, for patients with lung adenocarcinoma, the impact on the prognosis of by trophinin expression might be greater in female than in male patients. This needs to be confirmed by further studies with larger sample sizes.

In conclusion, using a bioinformatics approach to search for new prognostic markers, we identified trophinin and demonstrated that trophinin is a poor prognostic factor for early stage lung cancer. Moreover, trophinin exhibits the ability to enhance cancer invasion. These findings provide novel insight towards the process of cancer metastasis and the potential target for treatment.

Conflict of interest statement

None declared.

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