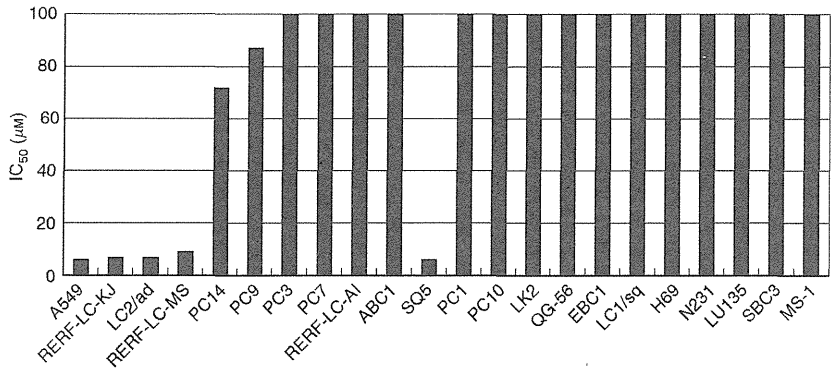

Figure 1 IC_{50} values for 22 lung cancer cell lines responding to enzastaurin treatment by MTS assay. According to sensitivity to enzastaurin, these 22 cell lines were classified as sensitive (IC_{50} of $\leqslant 10\,\mu M$) or resistant (IC_{50} of $>50\,\mu M$).

Table 1 Unique genes correlated with sensitivity to enzastaurin

Gene symbol	Gene title	F-statistic	*P*-value	Correlation coefficients	Eight-gene predictor
DUSP1	Dual specificity phosphatase 1	49.2	8.39E−07	−0.69	*
ILF3	Interleukin enhancer binding factor 3, 90 kDa	48.5	1.10E−06	0.67	*
LITAF	Lipopolysaccharide-induced TNF factor	36.0	9.75E−06	−0.70	*
JAK1	Janus kinase 1 (a protein tyrosine kinase)	27.1	6.36E−05	−0.65	*
COPS7B	COP9 constitutive photomorphogenic homologue subunit 7B (Arabidopsis)	19.3	5.48E−04	0.66	*
RAD23A	RAD23 homologue A (*S. cerevisiae*)	23.0	9.10E−04	0.74	*
TNFAIP1	Tumour necrosis factor, α-induced protein 1 (endothelial)	19.5	0.002	−0.65	*
MIRN21///TMEM49	Transmembrane protein 49///microRNA 21	14.1	0.003	−0.66	*
PSEN1	Presenilin 1 (Alzheimer disease 3)	9.5	0.012	−0.65	
PPAP2A	Phosphatidic acid phosphatase type 2A	11.3	0.014	−0.75	
IGF1R	Insulin-like growth factor 1 receptor	10.6	0.019	−0.66	
SART3	Squamous cell carcinoma antigen recognised by T cells 3	9.4	0.019	0.65	
NDFIP1	Nedd4 family interacting protein 1	6.0	0.029	−0.66	
MLPH	Melanophilin	8.2	0.034	−0.65	
SEMA3C	Sema domain, immunoglobulin domain (Ig), short basic domain, secreted (semaphorin) 3C	5.9	0.056	−0.67	
UGDH	UDP-glucose dehydrogenase	5.9	0.062	−0.68	

Abbreviations: ANOVA = analysis of variance; TNF = tumour necrosis factor. Note: F-statistic and *P*-values were calculated by ANOVA. *Genes used as eight-gene predictor are shown.

resistant. Five cell lines (A549, RERF-LC-KJ, LC2/ad, RERF-LC-MS and SQ5) were sensitive (IC_{50} of $\leqslant 10\,\mu M$), and the remaining 17 cell lines were resistant to enzastaurin (IC_{50} of $>50\,\mu M$). The five cell lines sensitive to enzastaurin consisted of four AC (4/10, 40%) and one SCC (1/7, 14%) cell line; no SCLC (0/5) cell lines were enzastaurin sensitive. These results suggest that enzastaurin has anti-tumour activity against NSCLC.

Gene expression-drug sensitivity correlation

We have previously performed gene expression profile analysis of the same set of 22 lung cell lines by Affymetrix GeneChip (Gemma *et al*, 2006). First, we used the MTS results for enzastaurin for the development of a molecular model of sensitivity to enzastaurin. Twenty-three genes were significantly correlated with sensitivity to enzastaurin (correlation coefficients of >0.65). Next, pathway analysis was performed using the 23 genes to provide a viewpoint of the biological function of the genes, as previously described (Miyanaga *et al*, 2008). Pathway analysis removed the incorporated genes out of the imported 23 genes. Sixteen genes, associated with sensitivity to enzastaurin, were identified based on the biological functions of altered/associated genes (Table 1; Figure 2A). Pathway analysis revealed that JAK1 was the final target gene for the sensitivity to enzastaurin in lung cancer cells (Figure 2A). We next identified the optimal number of genes whose expression could accurately distinguish the sensitive cells from the resistant ones. Analysis of variance (ANOVA) was done to remove the genes with variance. The top eight genes (DUSP1, ILF3, LITAF, JAK1, COPS7B, RAD23A, TNFAIP1 and MIRN21/TMEM49) according to the ANOVA were subsequently found to be the minimum number necessary for prediction of drug response (Figure 2B; Table 1). We used the eight most strongly correlated genes to build an SVM algorithm model by which the five sensitive cells were distinguished from the 17 resistant cells. Overall, the SVM classification based on the above-mentioned eight genes, correctly classified the sensitivity to enzastaurin of all of the 22 cells (data not shown). Next, we examined the robustness of the eight-gene predictor, for classifying cells into the enzastaurin-sensitive group, in an independent set of NSCLC cells, and found that the eight-gene predictor correctly classified all five resistant cells (Table 2). Thus, we had ultimately identified an eight-gene signature that was validated for its ability to predict the sensitivity to enzastaurin in an independent set of lung cancer cells.

RTKs phosphorylation and miRNA expression-drug sensitivity correlation

Pathway analysis revealed that JAK1 was an important gene for the sensitivity to enzastaurin in lung cancer cells. JAK1 and its downstream STAT3 gene expression levels of sensitive cells were

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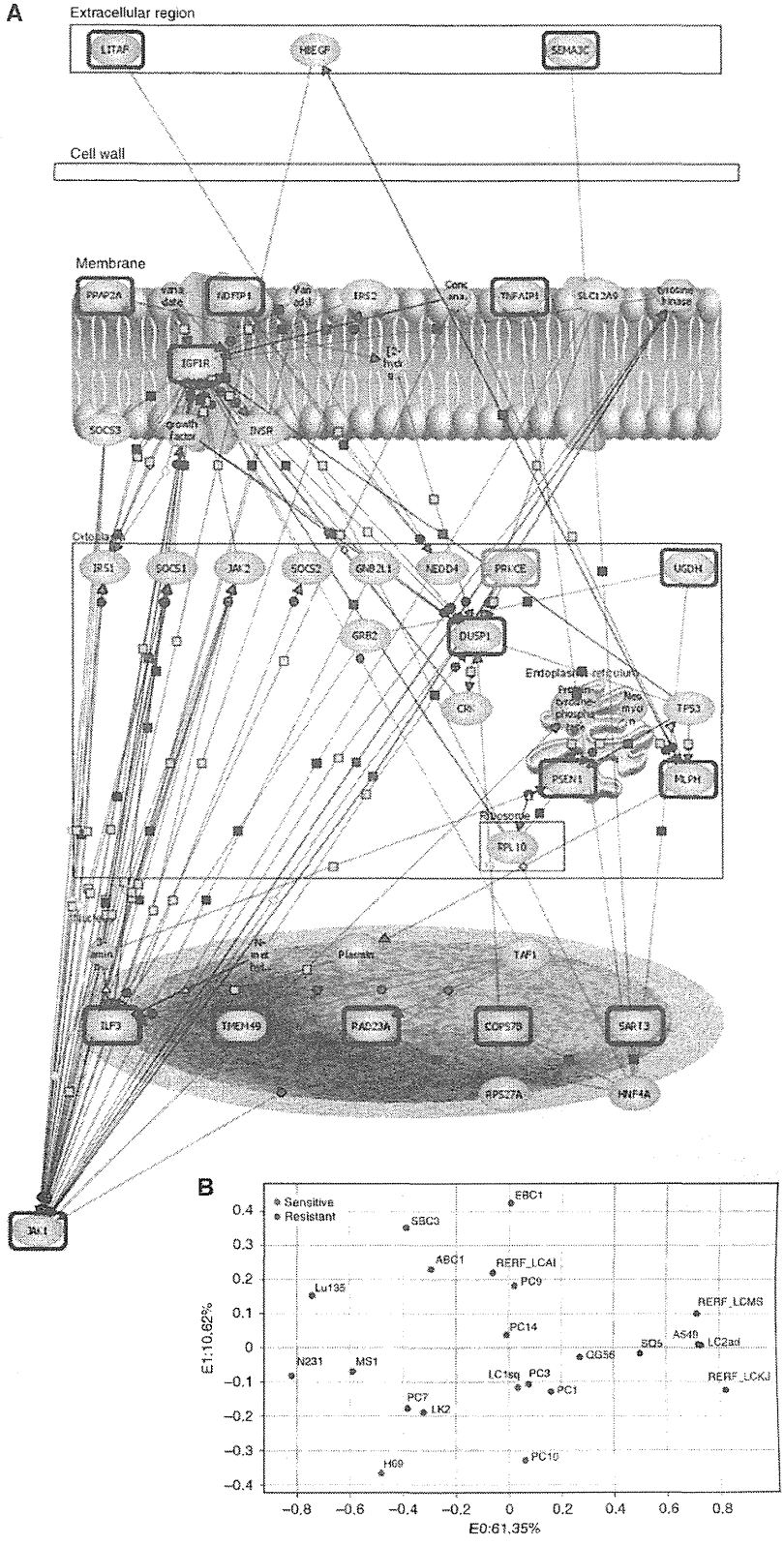

Figure 2 Sixteen genes associated with enzastaurin response were established by pathway analyses and prediction of drug response using an eight-gene signature. (**A**) Sixteen genes (blue circle) associated with enzastaurin response and PKC (red circle) belonged to the same signal pathway. (**B**) Principal component analysis based on the eight-gene profile correctly distinguished the sensitive cells from the resistant ones. The colour reproduction of this figure is available at the *British Journal of Cancer* online.

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significantly higher than those of resistant cells (Figures 3A and B). To further clarify the signalling mechanism correlated with the sensitivity to enzastaurin, we also examined RTKs phosphorylation expression profiles of the same set of 22 lung cancer cells. The top 10 RTKs phosphorylation associated with enzastaurin sensitivity are shown in Table 3 (correlation coefficients of >0.50). Pathway analysis using the 23 genes and 10 RTKs phosphorylation associated with sensitivity to enzastaurin also revealed that JAK/STAT signal pathway was mainly involved in the drug response (data not shown). Among the 10 RTKs phosphorylation, the expression of two RTKs mainly associated with angiogenesis and lymphangiogenesis (VEGFR2 and VEGFR3) was significantly elevated in sensitive cells compared with in resistant cells (Figures 3C and D).

Table 2 Validation of the eight-gene predictor by examining the SVM value in an independent set of five NSCLC cell lines

		Histology	IC_{50} (μM)	Predicted class*
1	H1650	AC	>100	Resistant
2	H1975	AC	>100	Resistant
3	RERF-LC-OK	AC	>100	Resistant
4	VMRC-LCD	AC	>100	Resistant
5	LC-1F	SCC	>100	Resistant

Abbreviations: AC = adenocarcinoma; SVM = support vector machine; NSCLC = non-small-cell lung cancer. Note: *Cell lines were classified as sensitive (IC_{50} of $\leqslant 10\,\mu$M) and resistant (IC_{50} of $>50\,\mu$M) to enzastaurin.

Table 3 Kinase and miRNA correlated with the sensitivity to enzastaurin

	Kinase	F-statistic	*P*-value	Correlation coefficients
(a)				
1	M-CSFR	11.51	0.02	−0.82
2	VEGFR2	9.17	0.03	−0.68
3	FER	9.00	0.02	−0.60
4	EphA1	7.58	0.02	−0.61
5	VEGFR3	6.76	0.05	−0.58
6	TNK1	4.45	0.09	−0.71
7	NGFR	3.73	0.11	−0.68
8	MATK	2.95	0.15	−0.52
9	Hck	2.26	0.20	−0.53
10	SYK	1.82	0.23	−0.58
	miRNA	**F-statistic**	***P*-value**	**Correlation coefficients**
(b)				
1	hsa-miR-15a*	18.56	0.0004	0.51
2	hsa-miR-454*	16.65	0.0006	0.53
3	hsa-miR-92a	15.96	0.0007	0.52
4	hsa-miR-301b	12.49	0.0021	0.54
5	hsa-miR-130b	11.85	0.0026	0.54
6	hsa-miR-106b*	11.42	0.0032	0.52
7	hsa-miR-345	9.25	0.01	0.54
8	hsa-miR-31	7.25	0.05	−0.76
9	hsa-let-7a	4.04	0.09	0.54
10	hsa-miR-193b	2.76	0.14	−0.64
11	hsa-miR-193b*	2.76	0.15	−0.61
12	hsa-miR-21	2.24	0.18	−0.53
13	hsa-miR-30c-2*	1.93	0.24	−0.52

Abbreviations: ANOVA = analysis of variance; miRNA = microRNA. Note: F-statistic and *P*-values were calculated by ANOVA. *The miRNA name used in TaqMan microRNA array analysis.

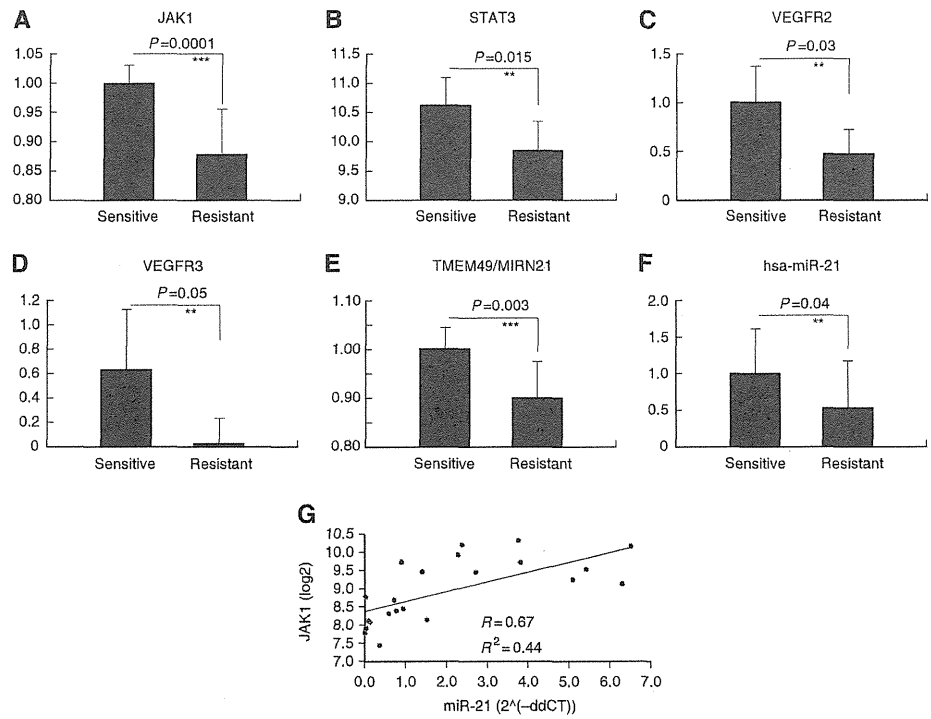

Figure 3 JAK1, VEGFR2, VEGFR3 and miR-21 were correlated with drug response. (**A** and **B**) JAK1 and STAT3 gene expression levels were significantly higher in the sensitive cell group than in the resistant cell group. (**C** and **D**) Elevated levels of VEGFR2 and VEGFR3 expression were observed in sensitive cells. (**E**) Expression of MIRN21/TMEM49 was significantly higher in sensitive cells than in resistant cells, by gene-chip analysis. (**F**) Mature miR-21 expression was significantly higher in sensitive cells than in resistant cells by quantitative RT–PCR analysis. (**G**) Quantitative comparison of miR-21 and JAK1 showed a significant positive correlation between these two molecules. $^{**}P<0.05$ when compared with the resistant cells. $^{***}P<0.01$ when compared with the resistant cells.

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In order to investigate post-transcriptional regulation, miRNA microarray analysis of the 22 cells was also performed. We identified 13 miRNAs correlated with enzastaurin sensitivity (correlation coefficients of >0.50) (Table 3). Interestingly, MIRN21/TMEM49, a host gene of miR-21, was included among the eight genes associated with enzastaurin sensitivity, and was expressed at significantly higher levels in sensitive cells compared with in resistant cells (Figure 3E). In addition, a correlation between miR-21 and enzastaurin sensitivity was found in miRNA array analysis (correlation coefficients -0.53) (Table 3). Recent reports demonstrated that miR-21 is a major miRNA that may play an oncogenic role in lung carcinogenesis (Volinia *et al*, 2006; Yanaihara *et al*, 2006; Seike *et al*, 2009). The expression levels of miR-21 were examined by real-time quantitative RT-PCR. miR-21 expression was significantly higher in sensitive cells than in resistant cells ($P<0.05$, paired *t*-test) (Figure 3F). The quantitative comparison of miR-21 and JAK1 showed a significant positive correlation between these two (Pearson's correlation, $r=0.67$, $P<0.05$) (Figure 3G). We ultimately recognised JAK1, VEGFR2, VEGFR3 and miR-21 as factors concerned with sensitivity to enzastaurin. In particular, JAK1 is the most significant molecule involved in drug response.

JAK1 expression effect on drug sensitivity in A549 cells

To investigate further the effect of JAK1 on sensitivity to enzastaurin, JAK1 protein expression of 11 NSCLC cells was evaluated by western blot analysis. Elevated JAK1 protein was observed in enzastaurin-sensitive NSCLC cells (Figure 4A). Next, we inhibited JAK1 protein using JAK1 inhibitor in enzastaurin-sensitive A549 and RERF-LC-KJ cells. After the treatment of JAK inhibitor (1 μM), JAK1 and its downstream p-STAT3 expression was completely diminished until 72 h in A549 cells (Figure 4B). We examined the effect of enzastaurin and JAK inhibitor combination therapy on cell growth. Concurrent JAK inhibitor and enzastaurin therapy significantly decreased the growth-inhibitory effect of enzastaurin, compared with enzastaurin monotherapy in enzastaurin-sensitive A549 cells (Figure 4C). Enzastaurin therapy after JAK inhibitor 1 μM treatment also diminished the growth-inhibitory effect of enzastaurin, compared with enzastaurin monotherapy in A549 cells (Figure 4D). The IC_{50} values of concurrent enzastaurin with JAK inhibitor and enzastaurin therapy after JAK inhibitor were 76 and 83, respectively, whereas that of enzastaurin monotherapy was 5.8 (Figures 4C and D). In addition, RERF-LC-KJ cells, which are also sensitive to

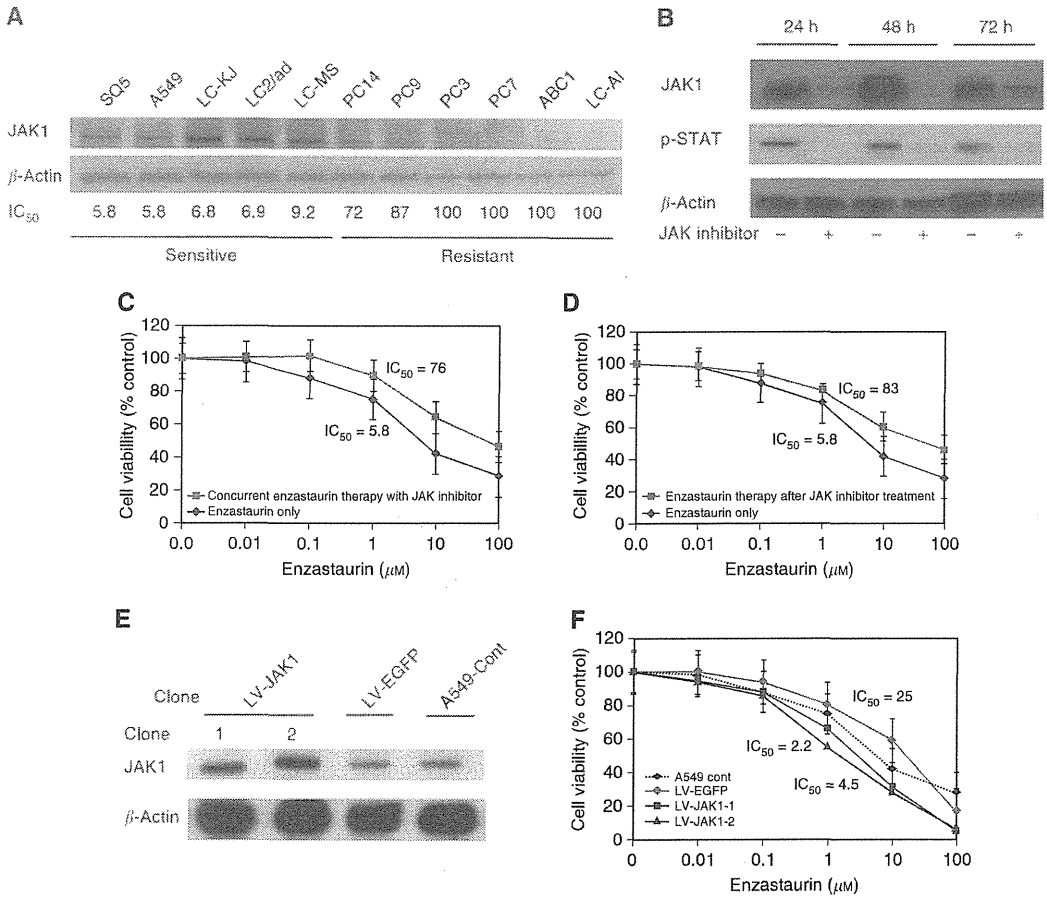

Figure 4 Effect of combination therapy with enzastaurin and JAK1 expression on cell growth in lung cancer cells. (**A**) JAK1 expression levels were significantly higher in the sensitive cell group than in the resistant cell group, by western blotting. (**B**) Completed inhibition of JAK1/STAT signalling by JAK1 inhibitor in A549 cells. P-STAT3 was completely inhibited until 72 h after the treatment of 1 μM JAK inhibitor. (**C**) Enzastaurin treatment with JAK inhibitor for 72 h was examined in A549 cells. Each result is expressed as cell viability in treated samples compared with the untreated sample (100%) for enzastaurin alone and concurrent therapy with the 1 μM JAK inhibitor treatment. (**D**) The effect of JAK inhibitor treatment (1 μM) for 24 h followed by enzastaurin treatment for 72 h was examined in A549 cells. (**E**) Lentiviral-mediated production of JAK1 in A549 cells. Western blotting showed that JAK1 expression levels were significantly higher in two LV-JAK1 clones than in the control clones. (**F**) Enzastaurin treatment for 72 h was examined in LV-JAK1-A549 cells. Each result is expressed as cell viability in the treated samples compared with the untreated sample (100%) for enzastaurin therapy.

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enzastaurin, showed resistance after JAK inhibitor therapy in combination with enzastaurin (data not shown). In RERF-LC-KJ cells, both IC_{50} values of concurrent enzastaurin with JAK inhibitor and enzastaurin therapy after JAK inhibitor were over 100, whereas that of enzastaurin monotherapy was 6.8. To confirm further the ability of JAK1 to indicate drug sensitivity to enzastaurin, we developed a lentiviral vector for the expression of JAK1 and established stable JAK1-overexpressing A549 cells (LV-JAK1-A549 cells). Western blot analysis showed the overexpression of JAK1 in LV-JAK1-A549 cells (Figure 4E). The growth-inhibitory effect of enzastaurin on LV-JAK1-A549 cells was assessed by MTS assay. The drug sensitivities of two LV-JAK1-A549 cells were greater than those in the control cells (Figure 4F). The IC_{50} values of two LV-EGFP A549 cells were 2.2 and 4.5, respectively, whereas that of LV-EGFP A549 cells was 25 (Figure 4F). These results indicate that JAK1 expression contributed to the drug sensitivity and could be used as a drug-sensitive marker to enzastaurin in lung cancer cells.

JAK/STAT3 pathway directly activates miR-21

A significant correlation between JAK1 and miR-21 was found in our set of NSCLC cells (Figure 3G). STAT3 is a transcription factor activated by JAK1, and its binding to the target sites in miR-21 promoter upon IL-6 induction has been reported previously (Löffler *et al*, 2007; Iliopoulos *et al*, 2010). To verify the association between JAK1 and miR-21, miR-21 expression was quantified after the stimulation of IL-6 by qRT–PCR analysis. Upon IL-6 exposure, p-STAT3 expression was significantly upregulated, resulting in the overexpression of miR-21 at 24 h in A549 cells (Figures 5A and B). We also evaluated the miR-21 expression in LV-LAK1 A549 cells. In the JAK1-overexpressing cells, miR-21 expression was significantly higher than in parent cells (Figure 5C). These results supported the concept that miR-21 is directly induced by JAK/STAT signalling in NSCLC cells.

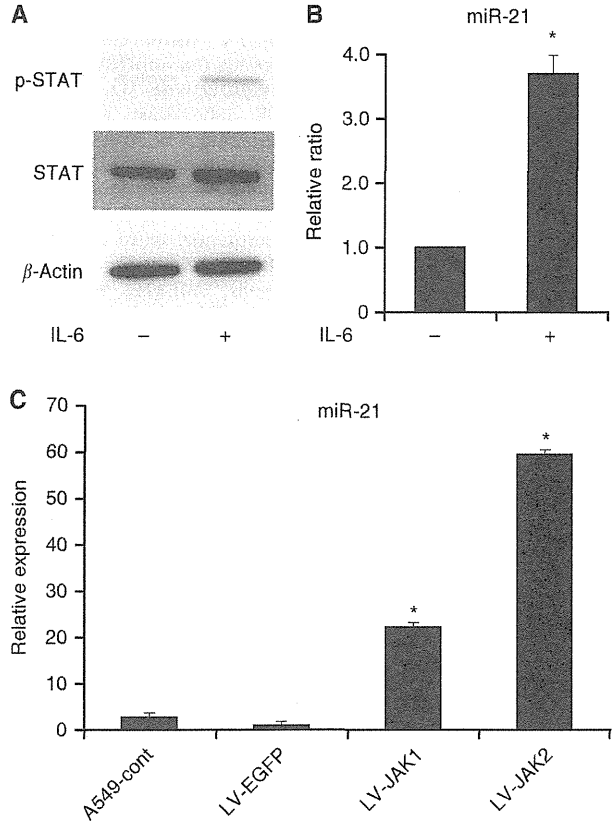

Figure 5 Association between JAK1 and miR-21 expression. (**A**) p-STAT3 was overexpressed after IL-6 stimulation of A549 cells for 24 h. (**B**) After IL-6 stimulation, miR-21 expression was significantly increased, as measured by qRT–PCR analysis. (**C**) MiR-21 expression of two LV-JAK1 cells was significantly higher than in the control cells, as measured by qRT–PCR analysis. Data were mean ± s.d. from three independent experiments. $^*P<0.05$ when compared with the respective parent cells.

DISCUSSION

Enzastaurin has recently been evaluated as second- or third-line therapy of NSCLC in a phase II study (Oh *et al*, 2008; Chiappori *et al*, 2010). Synergistic effects of the combination of enzastaurin and cytotoxic drugs including cisplatin, gemcitabine and pemetrexed have been found in NSCLC cells in an *in vitro* study (Rademaker-Lakhai *et al*, 2007; Morgillo *et al*, 2008; Tekle *et al*, 2008). A recent study showed that enzastaurin inhibited *in vivo* metastasis of NSCLC cells (Körner *et al*, 2010). It is known that PKCs mediate the regulation of the cell cycle; enzastaurin is also able to inhibit several proteins involved in cell-cycle regulation, for example, E2F-1 associated with G1/S checkpoint and Cdc25C resulting in G2/M checkpoint (Tekle *et al*, 2008). These checkpoint arrests provide the tumour cells with the opportunity to repair their DNA, which has been damaged by cytotoxic drugs. Reduction of E2F-1 expression and phosphorylated Cdc25C by enzastaurin might explain the abrogation of the checkpoint arrest and could facilitate cytotoxic drug-damaged cells to undergo apoptosis. Furthermore, a recent study demonstrated that enzastaurin had a cooperative effect with gefitinib and was able to revert gefitinib resistance in cancer cells through the inhibition of Akt and VEGF pathways (Gelardi *et al*, 2008). These studies suggest that enzastaurin might be a promising novel agent in NSCLC patients.

Enzastaurin inhibited the downstream PKCβ signalling, PI3K/AKT pathway and the phosphorylation of glycogen synthase kinase-3β (Keyes *et al*, 2002; Graff *et al*, 2005). Anti-tumour and anti-angiogenic activity of enzastaurin was also demonstrated in tumour xenograft models, including NSCLC, and was confirmed using a standardised clonogenic assay in patient-derived tumour explants (Keyes *et al*, 2004). Significant reduction of VEGF protein levels following enzastaurin treatment, together with a significant decrease in intratumoural vessel density, has been demonstrated *in vivo* (Keyes *et al*, 2004). In the current study using a RTKs phosphorylation antibody array, we found elevated levels of VEGFR2 and VEGFR3 in the enzastaurin-sensitive cells. Our results are in agreement with previous data concerning enzastaurin and anti-angiogenic activity. These findings demonstrated that lung cancer cases with activated angiogenic activity should respond to enzastaurin treatment.

In this study, using gene-chip and pathway analysis, we identified 16 genes that correlated with sensitivity to enzastaurin. Pathway analysis also revealed that JAK1 was the most important molecule affected by enzastaurin treatment of NSCLC. The JAK is a non-RTK and can activate STAT3 transcriptional factor. The STAT3 is also persistently activated in about half of NSCLC tumours and is involved in tumour invasion, metastasis and angiogenesis through differential gene regulation (Haura *et al*, 2005; Song *et al*, 2011). Increased levels of JAK1 and STAT3 were observed in the sensitive cells in this study. Knockdown of JAK resulting in p-STAT3 also diminished the growth-inhibitory effect of enzastaurin in the sensitive cells. In contrast, overexpression of JAK1 by lentiviral-mediated production enhanced the drug sensitivity to enzastaurin in the sensitive cells. These results suggest that JAK expression levels can be used as predictive markers of enzastaurin sensitivity. Non-small-cell lung cancer patients with an activated JAK/STAT3 pathway are suitable cases for enzastaurin treatment.

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MicroRNAs are small non-coding RNA molecules of about 20 nucleotides that are frequently located at chromosomal regions deleted or amplified in cancers, suggesting that miRNAs are a new class of genes involved in human tumourigenesis (Lu *et al*, 2005; Volinia *et al*, 2006; Yanaihara *et al*, 2006; Seike *et al*, 2009). Recently, miRNAs have been demonstrated as diagnostic and prognostic markers in lung cancer (Yanaihara *et al*, 2006; Seike *et al*, 2009). We previously reported that the inhibition of miR-21, whose upregulation is associated with EGFR mutations, can be a therapeutic strategy, either as a monotherapy or in combination with EGFR-TKI treatment (Seike *et al*, 2009). In this study, expression of miR-21 and its host gene, TMEM49, were significantly higher in enzastaurin-sensitive cells than in enzastaurin-resistant cells. In addition, a significant positive correlation was observed between miR-21 and JAK1. The STAT3 reportedly signals IL-6-induced upregulation of miR-21 in multiple myeloma cells (Löffler *et al*, 2007). We confirmed that JAK1 and its downstream target STAT3, containing three binding sites of miR-21 promoter, directly activated miR-21 in NSCLC cells. These results suggest that, in lung cancer, miR-21 affects the response to enzastaurin through the JAK/STAT signalling pathway.

In conclusion, we have identified unique molecules; genes, RTKs and miRNAs that are correlated with sensitivity to enzastaurin and have constructed an eight-gene signature to distinguish the sensitive cells from the resistant cells. Furthermore, we demonstrate that JAK1 is the most significant factor concerned in response to enzastaurin. Patient selection based on the JAK expression might be useful for future clinical development of enzastaurin therapy in NSCLC.

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Conflict of interest

The authors declare no conflict of interest.

Translational Therapeutics

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A decrease in lung cancer mortality following the introduction of low-dose chest CT screening in Hitachi, Japan

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ABSTRACT

Recent US clinical trial demonstrated that CT screening prevents lung cancer death among high risk individuals. However, it remains unclear whether wide implementation of low-dose CT screening for lung cancer can decrease mortality in the community. Among residents in Hitachi City (Japan), where nearly 40% of inhabitants aged 50–69 years were estimated to have participated in the screening at least once from 1998 through 2009, the trend of lung cancer mortality was described in relation to the timing of implementation of the CT screening. Cancer mortality data were obtained from regional cancer registry and standardized mortality ratio (SMR) of lung cancer was calculated for each 5-year period during 1995–2009. In both men and women aged 60 years or older, age-specific lung cancer mortality rates were generally lower during 2005–2009 as compared with those during 1995–2004. For combined men and women aged 50–79 years, SMR was nearly unity prior to or during introductory phase of CT screening and during early period of implementation; however, it was significantly decreased during 2005–2009, well after the implementation of CT screening, with SMR (95% confidence interval) being 0.76 (0.67–0.86). Results suggest that wide implementation of low-dose chest CT screening may decrease lung cancer mortality in the community 4–8 years after introduction of the screening.

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1. Introduction

Screening for lung cancer using low dose computed tomography (CT) can detect smaller size of lung nodule than can chest radiography and thus has been expected to decrease lung cancer mortality. In June 2011, the National Lung Screening Trial (NLST) study group reported a 20% reduction in lung cancer mortality among persons randomly allocated to low dose CT screening compared with controls [1]. Based on a systematic review including the NLST study, the American College of Chest Physicians (ACCP) and the American Society of Clinical Oncology (ASCO) have released a clinical practice guideline, in which CT screening for lung cancer is recommended for high risk individuals [2]. In Japan, where CT screening for lung cancer was initiated first in the world [3] and has been implemented at community and workplace settings [4], evidence on the effectiveness of the CT screening is sparse and thus research is required to elucidate whether CT screening for lung cancer can decrease mortality.

Hitachi (Ibaraki prefecture), where low dose CT screening for lung cancer was initiated in the workplace in 1998 [5,6], is among areas with the largest number of the screening performed in Japan. Using data from a follow-up survey of 210 patients with lung cancer, 85% of which were on stage IA, detected on CT screening at Hitachi Medical Center and Hitachi Health Care Center, we reported an excellent survival (5-year survival of 90%) of these patients [7]. As of March 2006, nearly 30% of Hitachi citizens aged 50–69 is estimated to have received at least one CT lung cancer screening at either of the two medical facilities [7]. It would thus be of interest

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Table 1
The number of participants in low-dose chest CT screening in the two facilities[a] in Hitachi, Japan (as of March 2009).

Age (year)	Men	Women	Total
50–54	10,108	5131	15,239
60–64	6223	6363	12,586
70–74	1775	1826	3601
80 or older	167	146	313
Total	18,273	13,466	31,739

History of smoking: men 74.1%, women 8.3%; total 46.2%.
[a] Hitachi Health Care Center and Hitachi Medical Center.

whether CT screening for lung cancer had any impact on mortality at community level. Here, we report the trend of lung cancer mortality among Hitachi citizens in relation to the timing of screening implementation.

2. Materials and methods

In Hitachi Health Care Center (Hitachi Ltd., Hitachi), chest CT screening for lung cancer has been conducted for ages 50–69 years for employees, retired persons, and their spouse since in 1998. In Hitachi Medical Center (Hitachi) also initiated chest CT screening for lung cancer for community dwellers aged 50 years or older in 2001. Details of the screening procedure, the numbers of participants and patients with lung cancer, and clinical features of screen-detected cases in each facility have been described elsewhere [5–7].

Table 1 shows the number of participants of the chest CT screening by sex and age as of March 2009. A total of 31,739 participants received the screening at least once (total 83,342 screenings, mean 2.6 times) at Hitachi Medical Center or Hitachi Health Care Center. The majority of the participants (men 89%, women 85%) were in ages 50–69, with the mean age of all participants being 57 years. Because we did not obtain information on address of screening participants, we assumed that the proportion of Hitachi residents among total screening participants was the same as that among participants with screen-detected lung cancer (76%). Based on this assumption, 36% Hitachi residents aged 50–79 (men 43%, women 30%) and 40% Hitachi residents aged 50–69 (men 47%, women 33%) were estimated to have received the screening program as of March 2009. Of these, 14,661 were current or past smokers, and more than a half (54%) had never smoked cigarette in their lifetime.

First, we examined time trend of lung cancer mortality and incidence in Hitachi. For this purpose, we obtained from the Ibaraki Cancer Registry data on lung cancer death and incidence by sex- and 5-year age-group for each Hitachi City and Ibaraki prefecture each year from 1995 to 2009 (1995 to 2007 for incidence). We also collected sex- and age-specific population statistics, which were derived from residential registry, in Hitachi City during the same period. Using these data, we calculated lung cancer mortality rate and its exact 95% confidence interval, based on the binomial distribution, by sex and 10-year age group in Hitachi City for three 5-year periods (1995–1999, 2000–2004, 2005–2009), each representing "stage of no or the beginning of CT screening program", "early stage of implementation", and "later stage of implementation".

Next, we compared the lung cancer mortality in Hitachi City with that for overall Japan. We first calculated the expected number of death from lung cancer by multiplying the sex- and age (5 years interval)-specific number of residents in Hitachi City by their corresponding mortality rate of lung cancer in Japan, and then summed. We then calculated standardized mortality ratio (SMR) as the observed number of death divided by the expected number of death as well as its exact 95% confidence interval based on the Poisson distribution. We repeated this analysis by using sex- and age-specific mortality data of Ibaraki prefecture. We also calculated age-standardized incidence rate of lung cancer in Hitachi for each year from 1995 to 2007 (standard population, the 1985 model population of Japan). All analyses were done with Stata version 10.1 (StataCorp, College Station, TX).

3. Results

Table 2 shows lung cancer mortality rate for each 5-year period by sex and age group. In men between ages 60 and 74, lung cancer mortality was slightly lower during the second period of 2000–2004 (early stage of CT screening), whereas it showed a large reduction during the third period of 2005–2009 (stable stage of CT screening) as compared with that during 1995–1999 (no CT screening or its introductory stage). Men aged 75–79 also showed a large reduction in mortality in the third period. Similarly, women aged 60 or older showed a decreasing trend of lung cancer mortality during the course of time.

Fig. 1 shows the time trend of age-standardized lung cancer mortality ratio (ages 50–79 year) for men and women combined in Hitachi using sex- and 5-year age group-specific lung cancer mortality statistics of whole population of Japan for calculation, together with that of age-standardized lung cancer incidence rate (ages 50–79 year). This analysis was limited to those aged 50–79 years, the majority (>99%) of CT screening participants in Hitachi. During the period of 1995–1999, when CT screening was not provided or has just introduced in Hitachi area, lung cancer mortality was comparable to that of national and prefectural levels; SMR (95% CI) was 0.95 (0.83, 1.08). There was also no material difference in mortality during the second period representing early stage of CT screening implementation; SMR (95% CI) was 0.97 (0.86, 1.09). During the third period (2005–2009), which corresponds to 4–8 years after the implementation of CT screening in both medical facilities in Hitachi, we observed a statistically significant, 24% reduction in lung cancer mortality; SMR (95% CI) was 0.76 (0.67, 0.86). Similar results were obtained when sex- and age-specific lung cancer mortality data of Ibaraki prefecture were used in calculating the expected number of lung cancer death in Hitachi; SMR (95% CI) was 1.04 (0.91, 1.17), 1.04 (0.92, 1.16), and 0.79 (0.69, 0.89) for 1995–1999, 2000–2004, and 2005–2009, respectively. In contrast, age-standardized incidence rate of lung cancer in Hitachi appears to increase after the introduction of CT screening in Hitachi Health Care Center (1998) and Hitachi Medical Center (2001).

To further examine which sex- and age-group showed reduction in lung cancer mortality, we repeated the above analysis for each sex- and/or age (10-year interval)-group using sex- and age (5-year interval)-specific lung cancer mortality of overall Japan (Table 3). For all the three age groups combined, a statistically significant decrease in SMR during the third period of 2005–2009 was observed in both men and women (men 24%, women 26%). A statistically significant decrease in lung cancer mortality was observed during the third period in men in 60s (32%), men in 70s (24%), and women in 70s (33%). Women in 60s also showed a decrease (25%), albeit statistically non-significant, in lung cancer mortality during that period.

4. Discussion

In the present study, we examined chronological changes of lung cancer mortality among residents of Hitachi City, where chest CT screening has been widely implemented as a community preventive service. As a result, we found a significant reduction in lung cancer mortality among target age groups 4–8 years after introduction of CT screening.

Table 2
Lung cancer mortality rate by sex, age, and period in Hitachi, Japan.

Age (year)	Period	Men			Women		
		Lung cancer death	Total population[a]	Lung cancer mortality rate (95% CI), per 100,000	Lung cancer death	Total population	Lung cancer mortality rate (95% CI), per 100,000
50–79	1995–1999	194	155,034	125 (108, 142)	57	166,975	34 (26, 44)
	2000–2004	228	169,910	134 (117, 153)	57	179,002	32 (24, 41)
	2005–2009	203	187,636	108 (94, 124)	53	194,686	27 (20, 36)
50–59	1995–1999	24	75,647	32 (20, 47)	11	74,608	15 (7, 26)
	2000–2004	31	71,952	43 (29, 61)	9	74,167	12 (6, 23)
	2005–2009	29	70,589	41 (28, 59)	10	71,128	14 (7, 26)
60–69	1995–1999	75	53,376	140 (111, 176)	18	53,451	34 (20, 53)
	2000–2004	80	66,153	121 (96, 150)	19	63,347	30 (18, 47)
	2005–2009	61	72,528	84 (64, 108)	18	73,492	24 (15, 39)
70–79	1995–1999	95	26,011	365 (296, 446)	28	38,916	72 (48, 104)
	2000–2004	117	31,805	368 (304, 441)	29	41,488	70 (47, 100)
	2005–2009	113	44,519	254 (209, 305)	25	50,066	50 (32, 74)

CI, confidence interval.
[a] The sum of the population each year.

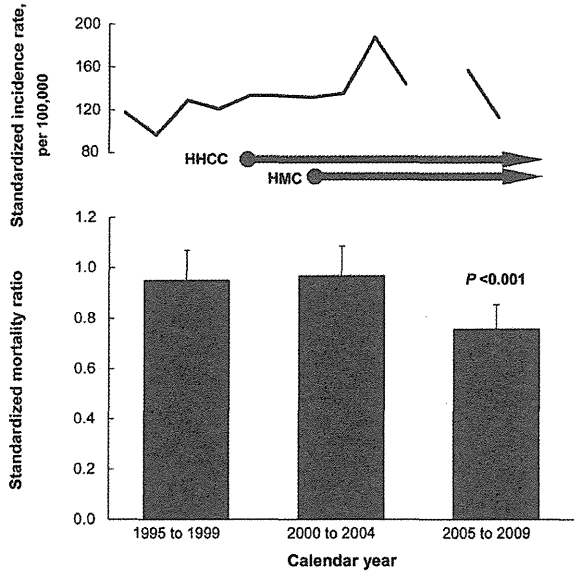

Fig. 1. Time trend of standardized mortality ratio (based on sex- and age (5-year interval)-specific lung cancer mortality in Japan) and standardized incidence rate (standard population is the 1985 model population of Japan. Data for 2005 is not shown due to a flaw in registration system) of lung cancer among residents aged 50–79 years in Hitachi, Japan. HHCC, Hitachi Health Care Center; HMC, Hitachi Medical Center. Arrows indicate implementation period of CT screening. Error bar indicates 95% confidence interval.

A recent finding from NLST provided strong evidence supporting the effect of low-dose CT screening in decreasing lung cancer mortality among those with a history of smoking [1]. Protective effect of the CT screening on lung cancer mortality was also supported from a prognostic investigation of patients with lung cancer detected on CT screening in the US [8]. Similarly, we previously reported an excellent long-term survival of patients with lung cancer detected on the CT screening in Hitachi area [7]. Lung cancer mortality in a cohort of smokers who received CT screening for lung cancer in New York State was compared with two unscreened cohorts (CPS-II and CARET), with adjustment for age, sex, and smoking history [9]. The authors found a significant reduction of deaths from lung cancer (36% and 64% for CPS-II and CARET, respectively). Our present finding is in line with these results and provides further evidence to support that community-wide implementation of CT screening can contribute to the reduction of lung cancer mortality in the community.

In the present observation among residents of Hitachi City, a significant reduction of lung cancer mortality was observed during 2005–2009, which corresponds to 4–8 years after introduction of chest CT screening in both facilities. The timing of reduction in lung cancer mortality observed in the present study agrees well with that in the New York State cohort [9], in which the two rounds of screening provided a mortality reduction starting in the 6th–8th year after enrollment.

It remains unclear whether chest CT screening for lung cancer can decrease mortality among nonsmokers. However, given that more than a half of participants in CT screening for lung cancer at the two facilities in Hitachi City were nonsmokers and that 60% of lung cancer patients detected on that screening (as of March 2006) were nonsmokers, it may be reasonable to infer that observed reduction in lung cancer mortality among Hitachi residents may be due, at least in part, to the effect of CT screening on lung cancer mortality among nonsmokers. In fact, we also observed a significant reduction in lung cancer mortality for women, majority of whom are never smokers, during the third period (2005–2009), suggesting that CT screening is also effective in preventing lung cancer death among nonsmokers. Moreover, data from an ongoing cohort study of CT-screening participants including nonsmokers in Japan showed a reduction in lung cancer mortality [10]. The ACCP and ASCO guideline stated, based on available evidence, that CT screening for lung cancer should not be performed for low risk individuals [2]. However, given that nearly one-fourth of lung cancer in the world are unrelated to smoking [11] and in Japan, 31% and 80% of lung cancer deaths for men and women, respectively, are probably not related to smoking [12], the effectiveness of CT screening for lung cancer among nonsmokers should be evaluated in future studies.

The limitations of the present study warrant mention. The present analysis based on community level data, compared with cohort or randomized control studies, is more likely to suffer from bias. As a major concern, decreasing trend in lung cancer mortality observed in this population might be ascribed to factors other than chest CT screening. Of such factors, smoking is potentially important. Although long-term, representative data are not available on smoking prevalence in Hitachi, recent statistics on health indicators showed no material difference in smoking prevalence between Hitachi City and Ibaraki Prefecture [13]. In addition, no reduction

Table 3
Standardized mortality ratio[a] and its 95% confidence interval by sex, age, and period.

Age (year)	Men			Women		
	1995–1999	2000–2004	2005–2009	1995–1999	2000–2004	2005–2009
Total (50–79)						
O/E	194/201.4	228/227.6	203/267.1	57/61.8	57/65.2	53/71.3
SMR	0.96 (0.83, 1.11)	1.00 (0.88, 1.14)	0.76 (0.66, 0.87)	0.92 (0.70, 1.19)	0.87 (0.66, 1.13)	0.74 (0.56, 0.97)
50–59						
O/E	24/28.9	31/28.5	29/29.1	11/10.7	9/11	10/10.2
SMR	0.83 (0.53, 1.23)	1.09 (0.74, 1.54)	1.00 (0.67, 1.43)	1.03 (0.52, 1.85)	0.82 (0.38, 1.56)	0.98 (0.47, 1.81)
60–69						
O/E	75/76.2	80/82.5	61/89.2	18/18.2	19/21.1	18/24.0
SMR	0.98 (0.77, 1.23)	0.97 (0.77, 1.21)	0.68 (0.52, 0.88)	0.99 (0.59, 1.56)	0.90 (0.54, 1.40)	0.75 (0.45, 1.19)
70–79						
O/E	95/96.3	117/116.6	113/148.8	28/33	29/33.1	25/37.2
SMR	0.99 (0.80, 1.21)	1.00 (0.83, 1.20)	0.76 (0.63, 0.91)	0.85 (0.56, 1.23)	0.88 (0.59, 1.26)	0.67 (0.44, 0.99)

O, observed number of death; E, expected number of death; SMR, standardized mortality ratio.
[a] The expected number of lung cancer death was estimated using mortality data for whole population of Japan.

in SMR for cardiac disease, another smoking related disease, was observed during 2005–2009 in Hitachi (1.15 and 1.11 for men and women, respectively [13]). These data argue against smoking cessation as a plausible explanation for the reduction of lung cancer mortality observed among Hitachi residents. An increase of the incidence of lung cancer after the implementation of CT screening in the two medical facilities in Hitachi provides an additional support for the specific effect of CT screening, rather than other factors, in decreasing lung cancer mortality. Finally, we are uncertain whether the present finding in Hitachi could be generalized to other communities in Japan or other countries, which have different background in terms of risk factors for lung cancer as well as availability of medical service for patients with lung cancer detected on the screening.

5. Conclusion

In Hitachi City, a significant reduction in lung cancer mortality was observed 4–8 years after introduction of low-dose CT screening for lung cancer, suggesting that wide implementation of CT screening can decrease lung cancer mortality at community level. To enhance the benefit of CT screening and minimize its harm, future studies should be designed to address issues including clinical work-up of in-determined nodules, cost-effectiveness, and integration of smoking cessation practices [14].

Conflicts of interest

There are no conflicts of interest to disclose.

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Long-term prognosis of patients with lung cancer detected on low-dose chest computed tomography screening

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ABSTRACT

The effectiveness of lung cancer screening using low-dose chest computed tomography (CT) remains elusive. The present study examined the prognosis of patients with lung cancer detected on CT screening in Japanese men and women. Subjects were 210 patients with primary lung cancer identified on CT screening at two medical facilities in Hitachi, Japan, where a total of 61,914 CT screenings were performed among 25,385 screenees between 1998 and 2006. Prognostic status of these patients was sought by examining medical records at local hospitals, supplemented by vital status information from local government. The 5-year survival rate was estimated according to the characteristics of patients and lung nodule. A total of 203 (97%) patients underwent surgery. During a 5.7-year mean follow-up period, 19 patients died from lung cancer and 6 died from other causes. The estimated 5-year survival rate for all patients and for those on stage IA was 90% and 97%, respectively. Besides cancer stage, smoking and nodule appearance were independent predictors of a poor survival; multivariable-adjusted hazard ratio (95% confidence interval) was 4.7 (1.3, 16.5) for current and past smokers versus nonsmokers and 4.6 (1.6, 13.9) for solid nodule versus others. Even patients with solid shadow had a 5-year survival of 82% if the lesion was 20 mm or less in size. Results suggest that lung cancers detected on CT screening are mostly curative. The impact of CT screening on mortality at community level needs to be clarified by monitoring lung cancer deaths.

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1. Introduction

Much attention has been paid to the effectiveness of low-dose chest computed tomography (CT) screening for lung cancer [1,2], which has poor prognosis. Screening with chest CT has been shown to have higher detection rate of lung cancer and, of cases identified, have higher curative resection rate than does screening with conventional chest X-ray [1–3]. However, data for the prognosis of lung cancer cases identified on CT screening are limited [4–6] and the effect of this screening procedure on mortality remains inconclusive. While several randomized controlled trials are ongoing [7–10], results of previous analyses [11–13] have not supported an effect of CT-based screening in lowering lung cancer mortality. Recently, however, the National Lung Screening Trial (NLST), a randomized trial targeted for current and former heavy smokers, found a 20% reduction in lung cancer death among participants screened with low-dose helical CT compared to participants screened with chest X-ray [14]. So far, screening lung cancer using CT has not been recommended in any set of guidelines except for research purpose [15,16].

In Hitachi Medical Area, a large-scale chest CT screening program for lung cancer has been introduced in two medical facilities since 1998 and 2001, respectively. We previously reported the characteristics of cancers detected on the CT screening [3,17]. In the present study, we followed 210 patients with lung cancer detected on the CT screening in collaboration with local hospitals and administrative office. The objectives of the present study were: (1) to estimate survival of prognosis of patients with lung cancer detected on CT screening and (2) to examine the prognosis of lung cancer patients according to a history of CT screening, patient characteristics, and the size and density of lung shadow on CT.

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2. Materials and methods

2.1. Study design and patients

In Hitachi Health Care Center (Hitachi Ltd, Hitachi), chest CT screening for lung cancer has been conducted for ages 50–69 years for employees, retired persons, and their spouse since in 1998. In Hitachi Medical Center (Hitachi) also initiated chest CT screening for lung cancer for community dwellers aged 50 years or older in 2001. Informed consent was obtained from each participant in both facilities prior to the examination. The protocol of the follow-up survey of patients whose cancer was detected on the CT screening has been approved by the ethics committee of Hitachi Health Care Center (1998–2001).

Detail of the screening procedure, the numbers of participants and patients with lung cancer, and clinical features of screen-detected cases in each facility has been described elsewhere [3,17]. In short, two readers independently interpreted the CT images. If they could not reach a consensus, the final decision was made at a reading conference. When we detected noncalcified solitary pulmonary nodules (SPNs) ≥8 mm (Hitachi Health Care Center) or those ≥5 mm (Hitachi Medical Center), a detailed CT scan was carried out 1 month later. For SPNs ≥11 mm in size, we recommended biopsy, thoracoscopy, thoracotomy, fine-needle aspiration, or a combination of these methods according to the standards of care at that time. For SPNs of 8–10 mm (Hitachi Health Care Center) or 5–10 mm (Hitachi Medical Center), detailed CT scans were performed at 3 months and 6 months. Further follow-up of SPNs was performed at referred hospitals according to the "Low-dose CT lung cancer screening guidelines for pulmonary nodules management" [18]. If there was a sign of growth in either scan, patients were recommended to receive confirmatory diagnostic tests as noted above. Among participants who underwent invasive diagnostic test, the number of false-positive cases was 14 and 5 for Hitachi Health Care Center and Hitachi Medical Center, respectively. Participants were given an advice on quitting smoking orally or using a leaflet at the time of screening if they were current smokers.

As of March 2006, a total of 61,914 CT screenings were performed among 25,385 screenees (Table 1). The characteristics of screening participants were different between the two facilities. For instance, screenees at Hitachi Health Care Center were on average younger than those at Hitachi Medical Center (57 years old versus 64 years old). Moreover, the proportion of those with a history of smoking was much higher in Hitachi Health Care Center than that in Hitachi Medical Center, reflecting a higher male-to-female ratio in Hitachi Health Care Center than in Hitachi Medical Center. Of all the screening participants, 169 cases of lung cancer were identified at the initial screening and 41 cases at repeat screenings. Mean (SD) diameter of the tumors identified was 17.5 mm (9.3 mm) and 178 (85%) were on stage IA. The observed difference in detection rate between the two facilities is probably ascribed to the differences of characteristics, especially age distribution, of screening participants as mentioned above. Of all the 210 lung cancer patients, 159 (76%) were residents of Hitachi city and 202 (96%) were referred to either Hitachi General Hospital (Hitachi Co Ltd) or Ibarakihigashi National Hospital.

Table 2 shows epidemiologic and clinical features of lung cancer cases detected on chest CT screening. Compared with patients whose cancer was detected at repeat screening, those whose cancer was detected at initial screening were more likely to be female and a nonsmoker, and tended to have a larger lesion in size. As regards histology, 195 (93%) were adenocarcinoma. Of all patients, 178 (85%) had stage IA cancer, 145 (81%) of which had a nodule of 20 mm or less in diameter. A total of 203 patients (97%) underwent surgery, 6 had unresectable lesion, and one refused any medical treatment. Mean time period between CT screening and initiation of medical therapy was 161 days.

2.2. Follow-up

We made a follow-up survey to determine prognostic status of the 210 patients with primary lung cancer detected on chest CT screening by examining medical records and log of screening participation, supplemented by vital status information from local government. We used two definitions of outcome: one for death from all causes and another for death from lung cancer. Censoring was made at either the date of death from causes other than lung cancer (if the outcome is death from lung cancer), the date of last contact, or 28 February 2010 (end of follow-up period), whichever came first. Follow-up period was calculated for each patient as time period from the date of initiation of medical therapy and the date of the occurrence of either outcome or censoring. We estimated

Table 1
Summary and results of thoracic CT screening in Hitachi Medical Area as of March 2006.

	Hitachi Medical Center	Hitachi Health Care Center
CT scanner	Multi detector row CT (mobile, 4 rows)	Single slice spiral CT
Screening participants	Local residents, 50 years or older	Employees, retired persons, and their spouses, 50–69 years old
Start of screening program	April 2001	April 1998
Baseline screening		
Participants	11,204	14,181
Lung cancer cases	109	60
Detection rate (%)	0.97	0.42
Mean diameter, mm	18.5	17.9
Stage IA (%)	83	83
Repeat screening		
Examinations	4387	32,142
Lung cancer cases	20	21
Detection rate (%)	0.46	0.07
Mean diameter, mm	13.1	15.1
Stage IA (%)	90	86

Table 2
Characteristics and outcome of lung cancer cases detected on CT screening according to the type of screening.

	Initial screening (n = 169)	Repeat screening (n = 41)	Total (n = 210)
Age, years (mean ± SD)	62.2 ± 7.8	62.2 ± 7.5	62.4 ± 7.6
Sex (male/female)	76/93	25/16	101/109
Smoking history (%)	63 (37.3)	21 (51.2)	84 (40)
Nodule size, mm (mean ± SD)	18.3 ± 9.7	14.1 ± 7.0	17.5 ± 9.3
Nodule appearance in thin-section CT			
Nonsolid	61	14	75
Part-solid	69	11	80
Solid	39	16	55
Pathology			
Adenocarcinoma	159	36	195
Others	5	0	5
Stage[a]			
IA	142	36	178
IB	12	1	13
IIA to IV	6	2	8
Treatment			
Surgical resection	164	39	203
Other than surgery	4	2	6
No treatment	1	0	1
Time from screening to treatment, days (mean ± SD)	160 ± 147	164 ± 159	161 ± 149

[a] Disease stage was defined according to the UICC 5th edition of TNM staging system.

Table 3
Kaplan–Meier 5-year survival rate and hazard ratio of all cause death among patients with lung cancer detected on CT screening.

	n	Survival rate (95% CI)	*P*[a]	Age- and sex-adjusted HR (95% CI)	Multivariable adjusted HR (95% CI)[b]
All patients					
Total	210	90 (84, 93)			
Initial	169	91 (85, 94)	0.39	1 (reference)	
Repeat	41	84 (68, 93)		1.1 (0.4, 2.9)	
Women	109	97 (92, 99)	<0.001	1 (reference)	1 (reference)
Men	101	81 (72, 88)		5.9 (2.1, 17.3)	1.0 (0.3, 3.9)
Nonsmoker	126	98 (93, 99)	<0.001	1 (reference)	1 (reference)
Smoker[c]	84	77 (66, 85)		2.4 (0.7, 8.3)	4.7 (1.3, 16.5)
Nodule size, mm					
<11	52	98 (87, 100)	0.001	1 (reference)[d]	1 (reference)[d]
11 to <21	102	93 (85, 96)			
21+	56	76 (63, 86)		3.3 (1.5, 7.2)	1.9 (0.7, 5.1)
Nodule appearance					
Nonsolid	75	100	<0.001	1 (reference)[e]	1 (reference)[e]
Part-solid	80	96 (89, 99)			
Solid	55	66 (51, 77)		10.3 (3.7, 28.2)	4.6 (1.6, 13.9)
Stage IA					
Total	178	97 (92, 98)			
Initial	142	97 (92, 99)	0.69	1 (reference)	
Repeat	36	94 (79, 99)		1.2 (0.2, 6.1)	
Women	100	98 (92, 100)	0.35	1 (reference)	
Men	78	95 (86, 98)		2.0 (0.5, 8.5)	
Nonsmoker	112	99 (94, 100)	0.03	1 (reference)	1 (reference)
Smoker	66	92 (82, 97)		12.8 (1.4, 115.3)	3.4 (0.6, 18.8)
Nodule size, mm					
<11	52	98 (87, 100)	0.46	1 (reference)[d]	
11 to <21	93	96 (89, 98)			
21+	33	97 (79, 100)		0.6 (0.1, 4.7)	
Nodule appearance					
Nonsolid	74	100	0.03	1 (reference)[e]	1 (reference)[e]
Part-solid	72	97 (89, 99)			
Solid	32	87 (69, 95)		4.5 (1.0, 19.9)	3.2 (0.7, 14.1)

[a] Log-rank test.
[b] Variables adjusted for the multivariate model were sex, smoking, diameter of lesion, nodule appearance (which showed $P \leq 0.1$ in age- and sex-adjusted model), and cancer stage for all patients; smoking and nodule appearance (which showed $P \leq 0.1$ in age- and sex-adjusted model) for patients on stage IA.
[c] Including former and current smokers.
[d] Including nodules of <11 mm and 11 to <21 mm in size.
[e] Including nonsolid and part-solid nodules.

a 5-year survival rate for the 210 cases detected and according to the size of nodule (<11 mm, 11–<21 mm, or 21+ mm), features of nodule (solid, part-solid, or nonsolid), or the timing of detection (initial or repeat screening). Further, we examined clinical features of cases died from lung cancer detected on repeat screening.

2.3. Statistical analysis

Statistical analysis was done by using Stata version 10.0. Difference in continuous variable among groups was tested by *t* test or Mann–Whitney *U* test. Survival rate was estimated by using Kaplan–Meier method and its difference among groups was tested by using Log-rank test. Cox proportional hazard model was used to estimate hazard ratio and its 95 confidence interval. We calculated two types of hazard ratio: one using a model with adjustment of age and sex only and another using a model with adjustment of variables showing $P \leq 0.1$ in the age- and sex-adjusted model plus clinical stage of cancer. A two-sided *P* value of <0.05 was considered as statistically significant.

3. Results

The mean of follow-up period for all patients was 2076 days (5.7 years), with more than 70% of surviving patients being observed for at least 5 years. During the follow-up period, 25 (12%) died; 19 died from lung cancer and 6 died from other causes. Among 169 patients with lung cancer detected on initial screening, 19 (11%) died during follow-up period; of these, 14 died from the lung cancer detected. Causes of death other than lung cancer were colorectal cancer, esophageal cancer, ischemic heart disease (myocardial infarction), cerebrovascular infarction, and myeloid-fibrosis. Among 41 patients with lung cancer identified on repeat screening, 6 (15%) died; of these, 5 died from lung cancer and 1 from stomach cancer.

The estimated 5-year survival rate for death from all causes and hazard ratio and its 95% confidence interval were presented according to the characteristics of patients and nodule detected on screening (Table 3). Among all patients, the 5-year survival rate for death from all causes was 90%. The survival rate did not significantly differ between initial and repeat screenings (initial, 91%; repeat, 84%; $P = 0.39$). Male gender and smoking were each associated with a significantly poorer prognosis ($P < 0.001$ versus female gender and nonsmoking, respectively). Larger nodule and solid nodule on thin-section CT were significant predictors of lower survival. Patients with a lesion of 20 mm or smaller, compared with those with a lesion of 21 mm or larger in diameter, had a better prognosis (overall $P = 0.001$; Fig. 1). In age- and sex-adjusted model, a statistically significant increase in hazard of death was observed in association with male gender, smoking, larger nodule, and solid nodule. However, only smoking and solid nodule were associated with a statistically significantly increased hazard ratio after multivariate adjustment.

Patients with lung cancer on stage IA had a 5-year survival rate of 97% for death from all causes. Both solid nodule and smoking remained significant predictors of poor survival in this subgroup, whereas nodule size did not. Fig. 2 shows survival curves according to nodule density among patients with a lesion of 20 mm or smaller in diameter. Patients with a lesion of solid nodule had a 5-year

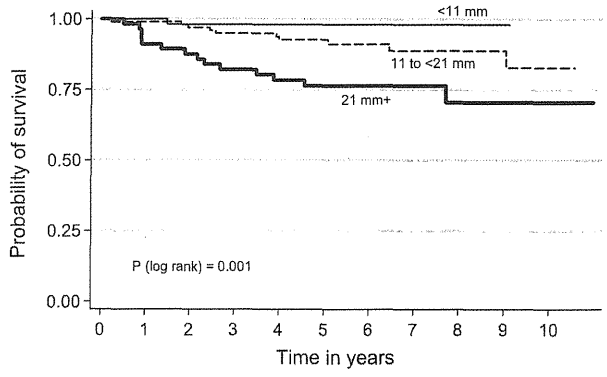

Fig. 1. Kaplan–Meier survival estimates for death from all causes by size of lung nodule.

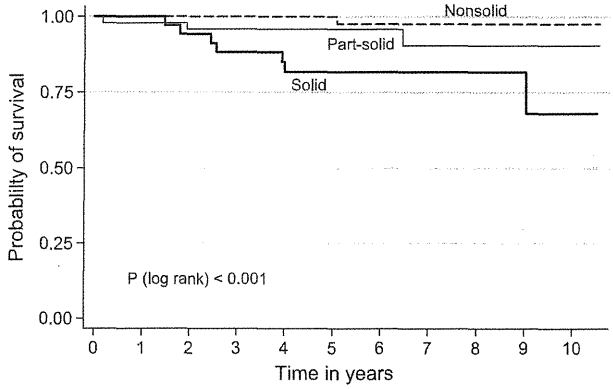

Fig. 2. Kaplan–Meier survival estimates for death from all causes in patients with small lung nodule (<21 mm in diameter).

survival of 82%, a value significantly lower than that among those with a lesion of combined nonsolid and part-solid shadow (98%; $P = 0.001$). In age- and sex-adjusted model, hazard ratio of death was statistically significantly increased in association with smoking and solid nodule. After multivariate adjustment, none of these variables remained statistically significant.

As smoking was a strong predictor of increased mortality among patients whose lung cancer was detected on CT screening, we presented background factors according to smoking status (Table 4). Smokers including past smokers were more likely to be male, had a larger nodule and a higher proportion of solid nodule, and tended to be on advanced clinical stage than nonsmokers.

Table 4
Clinical features of lung cancer detected on CT screening by smoking status.

	Smoker[b] ($n = 84$)	Nonsmoker ($n = 126$)	P[a]
Women	7 (8)	102 (81)	<0.001
Cancer stage			
IA	66 (79)	112 (89)	0.06
IB	9 (11)	4 (3)	
II to VI	9 (11)	10 (8)	
Mean (SD) nodule size, mm	19.4 (10.5)	16.2 (8.3)	0.01
Nodule appearance			
Nonsolid	19 (23)	56 (44)	<0.001
Part-solid	29 (35)	51 (40)	
Solid	36 (43)	19 (15)	

Figures in the table are number and percentage (in parenthesis) unless stated otherwise.

[a] Chi-square test for categorical variable and t-test for continuous variable.

[b] Including former and current smokers.

Among 14 deceased patients with lung cancer detected on baseline screening, the mean diameter of nodule was 32.1 mm and the proportion of advanced cancer showing solid shadow was high. Of these, 12 (86%) were male and 9 (64%) had a smoking history; 12 underwent surgical operation, 1 received chemotherapy, and 1 refused receiving medical therapy. Mean period from initiation of medical therapy to death was 3.0 years. Included in the 14 patients who subsequently died from lung cancer were one patient who chose alternative medicine and another patient who was under the care of physician due to interstitial pneumonia at the time of diagnosis. All five patients who died from the lung cancer detected on repeat screening were male, had a history of smoking, and showed a solid nodule. Of these, 4 had a lesion of 20 mm or smaller in diameter and only one had a nodule (4 mm in diameter and solitary) detectable at the time of initial screening.

4. Discussion

We investigated the prognosis of 210 patients with lung cancer detected on low-dose chest CT screening in two medical facilities in Hitachi City, Japan, with a 5.7-year mean follow-up period. Our study showed that lung cancer cases detected on CT screening had a fairly good prognosis, with a 5-year survival rate of 90%. A total of 19 patients, including 3 who refused or delayed medical therapy, died from lung cancer detected on the screening. No premature death was documented associated with therapeutic intervention. Patients with a lesion of solid shadow, indicative of invasive cancer, had a 5-year survival of 82% if the lesion detected was 20 mm or less in diameter.

The high survival rate among patients with lung cancer detected on CT screening observed in the present study is consistent with those in Japanese studies [4,6] as well as multi-country study [5]. However, lower survival rate have been reported in some Western studies [11,12], in which the proportion of stage I cancer of all cases detected was less than those in Japanese studies [2–4]. This may be attributed in part to the different characteristics of target population; Western studies have recruited persons with a history of smoking only, whereas Japanese studies also included persons without smoking experience. Moreover, there is ethnic difference in histological types of lung cancer; the proportion of adenocarcinoma among lung cancer patients in Japanese CT screening studies [2–4] is much higher than that observed in Western CT screening study [19]. Therefore, an extrapolation of findings obtained in Western populations to Japanese or vice versa requires caution. The analysis of data from an on-going Japanese cohort [20] using a simulation approach [21,22] may reveal the effectiveness of CT screening for lung cancer for Japanese populations.

We observed no measurable difference in survival rate between lung cancers detected on initial screening and those detected on repeat screening, similar to findings in previous studies [4–6]. Clinical characteristics of lung cancer cases differ according to whether the cancer was detected on initial or repeat examination [19]. At initial screening, not only cases showing small, vaguely delineated nodule but also those on advanced stage will be identified, leading to a wide variation in the nature of cancers ranging from non-invasive, slow-growing type to advanced one. High survival rate of patients with screening-detected cancer has been ascribed to well known bias; namely, lead time bias, length bias, and over-diagnose bias [23]. The effects of these types of bias are serious if cancers detected are mainly non-invasive and slow-glowing. However, given that 64% of patients with lung cancer detected at initial screening in the present study showed solid or part-solid nodule, which are likely invasive cancer [24], we believe that the observed high survival rate cannot fully be explained by these types of bias only. We should note that there was no death observed among patients with lung cancer with nonsolid nodule of 20 mm or less

in diameter at initial screening. More research is required to examine whether in-depth work-up for such a small, nonsolid nodule can be suspended until the next CT screening.

As regards repeat screening, lung cancers are detected due mainly to the emergence of new nodule or enlargement and change in concentration of CT image of the nodule detected on the previous screening. Although cancers detected on repeat screening are on average smaller than those detected on initial screening, they probably progress rapidly and thus are life-threatening if left untreated. In other words, the aforementioned bias inherent to the evaluation of screening may exert to a lesser extent in the survival of cases detected on repeat CT screening. Therefore, the present finding showing a good prognosis of these cases adds to evidence that repeat chest CT screening can prevent early death from lung cancer.

In the present study, smokers had a significantly poorer survival than nonsmokers even among stage IA patients, and all the three patients with small lung cancer (20 mm or less in diameter) detected on repeat screening who subsequently died from the lung cancer were current smokers. Poorer survival of smokers compared with nonsmokers is compatible with results of previous studies, including one among Japanese patients with CT-screen detected lung cancer [6]. These findings suggest that smoking-related lung cancers are likely aggressive and incurable even if detected on early stage, and thus underscore the importance of providing smoking cessation program at all settings including CT screening to decrease overall mortality [25].

Our study has several strengths including larger number of lung cancer patients who were detected on low-dose chest CT screening and longer follow-up period (mean 5.7 years) relative to most previous studies. In addition, the present study provided data not only for high-risk group (ever-smokers) but also for low risk group (lifetime nonsmokers), which makes it possible to compare survival of patients with lung cancer detected on CT screening between smokers and nonsmokers. The present study is limited due to bias inherent to screening studies of one arm design, as discussed above. Besides, we acknowledge two other limitations. First, our study was done only among patients whose cancer was detected at the time of CT screening and did not obtain any information about lung cancer diagnosed between the screenings. However, such interim cancers are probably few, and the inclusion of such cases may not greatly distort the result. Another limitation is that, as Hitachi CT screening program covered both ever-smokers and lifetime nonsmokers, overall result may not be applied to high-risk populations with a history of smoking.

CT screening for lung cancer has been performed at community and occupational settings in Hitachi City. If we assume 76% of screening participants (the proportion of residents in Hitachi City among lung cancer patients detected on CT screening) reside in Hitachi City, it is estimated that 18,115 residents (nearly 30% of residents aged 50–69 years [26]) of Hitachi City had participated into the CT screening program as of May 2006. The number of screening participants is increasing constantly, with some 700 individuals and another 2000 individuals receiving the screening at Hitachi Health Care Center and Hitachi Medical Center, respectively, each year. Given this wide-spread practice of CT screening in this community, it would be of interest whether lung cancer mortality among residents of Hitachi City will decrease more rapidly than that in other areas. Such time-trend analysis may provide valuable data for assessing the effectiveness of CT screening at population level.

5. Conclusion

Patients with lung cancer detected on low-dose CT screening had a fairly good prognosis, with the estimated 5-year survival rate for all patients and for those on stage IA being 90% and 97%, respectively. Besides cancer stage, smoking and nodule appearance were independent predictors of a poor survival. It is anticipated that chest CT screening program combined with anti-smoking campaign could effectively decrease risk of deaths from lung cancer. The impact of CT screening on mortality at community level needs to be clarified by monitoring lung cancer deaths.

6. Conflicts of interest

There are no conflicts of interest to disclose.

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The 37th Diagnostic Imaging Seminar

胸部CT（肺結節の存在診断）

中川　徹[1]

Thoracic CT (Diagnosis of Existence for Pulmonary Nodule)

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ABSTRACT —— I commented on lung cancer cases for low dose CT screening in the seminar.

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KEY WORDS —— Low dose CT screening for lung cancer, Diagnosis of existence

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要旨 —— 目的．低線量肺癌CT検診における肺癌発見事例を中心に結節の存在診断について解説した．**方法．**日立健康管理センタ低線量肺癌CT検診の実施状況とともに発見肺癌症例を供覧した．

索引用語 —— 低線量肺癌CT検診，存在診断

目　的

職域での働き盛りの癌死亡原因のトップである肺癌死亡率を減少させるために，総合健康診断の胸部画像検査に低線量CT検査を組み入れた．今回は職域肺癌CT検診の10年間の実施状況および，CT検診がひらく健康診断の可能性について言及する．

対　象

50～69歳までの総合健康診断受診者を対象にした．1998年4月～2007年3月の10年間にCT検診を受診した実人数は15,525名（男性13,032名・女性2,493名；平均年齢57歳），10年間の総検査件数は55,570件であった．

方　法

1998年から5年間は，50歳以上の総合健康診断受診者に，胸部単純X線検査と低線量CT検査（検査にかかわる追加費用なし）を選択させた．2003年からの5年間は，CT検診はオプション検査として追加費用9,000円で受診いただいた．

撮影条件は，シングルスライスCT：120 kV・50 mA・10 mm collimation・pitch 2（経年検診より管電流25 mA），2006年1月からは4列MDCT：120 kV・20 mA・0.8秒/回転・pitch 5に変更した．

放射線専門医および呼吸器内科医が二重読影し，経年検診は比較読影を行った．

成　績

初回検診から60例，経年検診では31例の肺癌が検出された．発見率は初回0.386%，経年0.077%，腫瘍直径20 mm未満肺癌の割合は84%，臨床病期IA期癌の割合は初回83.3%，経年93.5%であった．初回検診発見肺癌の典型症例（図1）と進行癌症例（図2）を供覧する．

結　語

①胸部CT検診では腺癌などの肺野型肺癌の早期検出（存在診断）は容易だが，肺門型肺癌を検出しておらず検討すべき大きな課題である．

②経年受診における経過観察で肺野孤立性結節の質的

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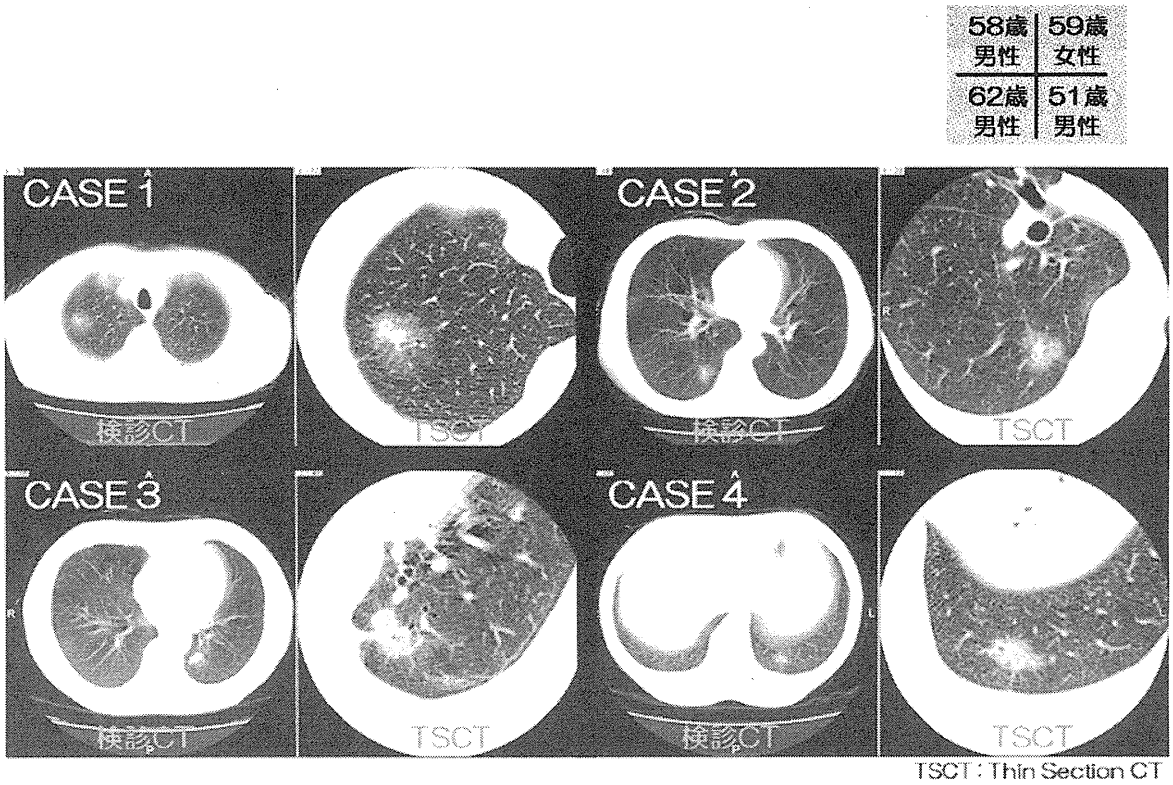

図 1. 初回検診発見肺癌：典型症例．高分化腺癌（全例）．pT1N0M0，Stage IA.

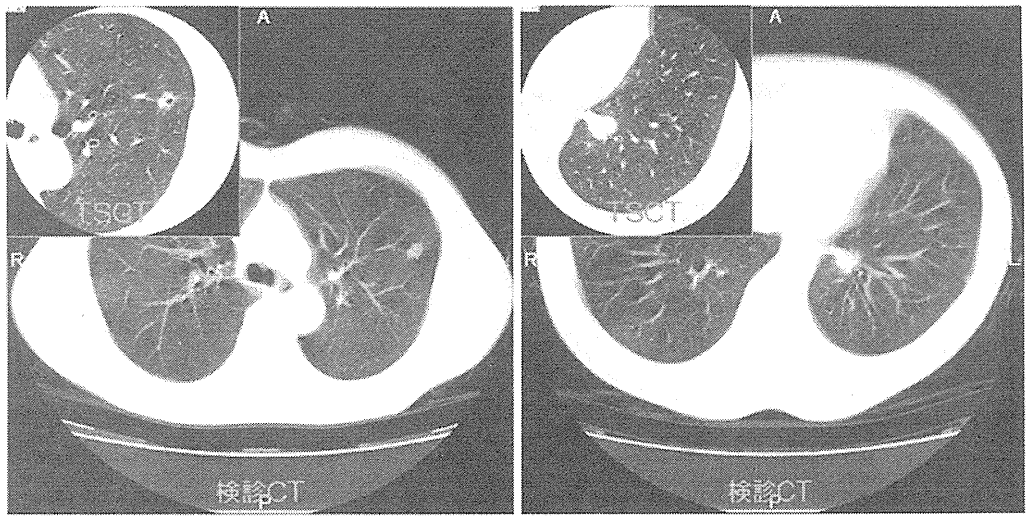

図 2. 初回検診発見肺癌：進行癌症例．pT1N1M0，Stage IIA.

診断が可能であるが，腫瘍直径増大を確認するための比較読影システム構築が必要である．

③孤立性肺結節の質的診断のための適切な観察間隔についてはさらに検討を加えていく必要がある．

④自験例では，検出された肺癌症例の 3/4 はすりガラス状濃度を伴っていた．

⑤低線量 CT 検診では，肺野型早期肺癌検出が可能で，死亡率減少に寄与しうる．

本論文内容に関連する著者の利益相反：なし