

UCSF

UC San Francisco Previously Published Works

Title

Detection of E2A-PBX1 fusion transcripts in human non-small-cell lung cancer

Permalink

<https://escholarship.org/uc/item/25h0395g>

Journal

Journal of Experimental & Clinical Cancer Research, 32(1)

ISSN

0392-9078

Authors

Mo, Min-Li
Chen, Zhao
Zhou, Hai-Meng
[et al.](#)

Publication Date

2013-12-01

DOI

10.1186/1756-9966-32-29

Peer reviewed



RESEARCH

Open Access

Detection of E2A-PBX1 fusion transcripts in human non-small-cell lung cancer

Min-Li Mo^{1†}, Zhao Chen^{1,2†}, Hai-Meng Zhou^{1,3*}, Hui Li², Tomomi Hirata^{2,4}, David M Jablons² and Biao He^{2*}

Abstract

Background: E2A-PBX1 fusion gene caused by t(1;19)(q23;p13), has been well characterized in acute lymphoid leukemia (ALL). There is no report on E2A-PBX1 fusion transcripts in non-small-cell lung cancer (NSCLC).

Methods: We used polymerase chain reaction (PCR) to detect E2A-PBX1 fusion transcripts in human NSCLC tissue specimens and cell lines. We analyzed correlation of E2A-PBX1 fusion transcripts with clinical outcomes in 76 patients with adenocarcinoma in situ (AIS) and other subgroups. We compared mutation status of k-ras, p53 and EGFR in 22 patients with E2A-PBX1 fusion transcripts.

Results: We detected E2A-PBX1 transcripts in 23 of 184 (12.5%) NSCLC tissue specimens and 3 of 13 (23.1%) NSCLC cell lines. Presence of E2A-PBX1 fusion transcripts correlated with smoking status in female patients ($P = 0.048$), AIS histology ($P = 0.006$) and tumor size ($P = 0.026$). The overall survival was associated with gender among AIS patients ($P = 0.0378$) and AIS patients without E2A-PBX1 fusion transcripts ($P = 0.0345$), but not among AIS patients with E2A-PBX1 fusion transcripts ($P = 0.6401$). The overall survival was also associated with status of E2A-PBX1 fusion transcripts among AIS stage IA patients ($P = 0.0363$) and AIS stage IA female patients ($P = 0.0174$). In addition, among the 22 patients with E2A-PBX1 fusion transcripts, 12 (54.5%) patients including all four non-smokers, showed no common mutations in k-ras, p53 and EGFR.

Conclusions: E2A-PBX1 fusion gene caused by t(1;19)(q23;p13) may be a common genetic change in AIS and a survival determinant for female AIS patients at early stage.

Keywords: NSCLC, Fusion gene, E2A-PBX1

Background

Lung cancer has been the leading cause of cancer-related deaths in developed countries [1]. Non-small-cell lung cancer (NSCLC) accounts for around 80% of all lung cancer cases. Somatic events, such as point mutation, genomic rearrangements (e.g. translocation) and changes in copy number, usually cooperatively cause alterations in oncogenes, tumor-suppressor genes, and microRNA genes, and lead to the multi-step carcinogenesis [2,3]. During tumor initiation and/or progression, encoded oncogenic proteins activated by translocations or mutations can alter cell proliferation and/or apoptosis

[3], resulting in transformation events. Fusion transcripts can be caused by chromosomal translocations and may occur more frequently in solid tumors than previously understood [2-4].

E2A-PBX1 fusion protein contains the transactivation domain of E2A and the DNA-binding domain of PBX1 and is generated by t(1;19)(q23;p13) translocation [5]. t(1;19) occurs in 5% of pre-B-cell acute lymphoid leukemias (ALL) in children and adults [6] and E2A-PBX1 has been widely characterized in ALL [5-15]. E2A-PBX1 can cause transformation in several cell types in vitro and induce lymphoblastic lymphomas in transgenic mice [7-9]. Target genes of E2A-PBX1 includes fibroblast growth factor (FGF)-15 [13], WNT-16 [14], and some novel genes [10], etc. Bmi-1 regulation of INK4A-ARF was required for transformation of hematopoietic progenitors by E2A-PBX1 [15]. However, there has been no report on detection of E2A-PBX1 fusion transcripts in

* Correspondence: zhm-dbs@mail.tsinghua.edu.cn; biao.he@ucsfmedctr.org

†Equal contributors

¹School of Life Sciences, Tsinghua University, Beijing 10084, China

²Thoracic Oncology Program, Department of Surgery, Helen Diller Family Comprehensive Cancer Center, University of California, San Francisco, CA 94115, USA

Full list of author information is available at the end of the article

solid tumors. In this study, we investigated into the detection of E2A-PBX1 fusion transcripts in NSCLC and compared this genetic change with three other common mutations in NSCLC (i.e. k-ras, p53 and EGFR) [16-18]. These data suggest that E2A-PBX1 fusion transcripts caused by t(1;19)(q23;p13) may be a common somatic genetic change of importance in solid tumors and E2A-PBX1 may be a novel target for prognosis and therapy in adenocarcinoma in situ (AIS) [19].

Methods

Patients and tissue specimens

A total of 184 patients were chosen in this study. All eligible patients without preoperative chemotherapy or radiation treatment underwent surgical resection of a primary NSCLC and had adequate mediastinal lymph node staging at UCSF between July 1997 and January 2007. Their clinical information of patients was summarized in Table 1. Information on clinical variables and patient follow-up were obtained from a prospectively maintained database including all subjects with banked tissue in the study. Patients consented to tissue specimen collection prospectively, and the study was approved by UCSF Human Research Protection Program Committee on Human Research. Tissue specimens were snap-frozen in liquid nitrogen at the time of the operation and stored in -150°C freezer.

Cell lines

NSCLC cell lines A549, A427, H441, H838, H1975, H1650, H322, H358, H1666, H1703, H2170, H460 and H1299, acute lymphoblastic leukemia (ALL) cell line CCRF-CEM were obtained from ATCC and cultured as recommended. ALL cell line RCH-ACV was a kind gift from Dr. Mignon Loh (Department of Paediatrics, UCSF).

RNA extraction and polymerase chain reaction (PCR)

Total RNA from cell lines and tissues were extracted using TRIzol reagent (Invitrogen) according to manufacturer's handbook. Adult normal lung total RNA was purchased at Biochain (CA). 1µg RNA was used for cDNA synthesis (BioRad). 1µL cDNA, 0.2mM for each dNTP, 0.4µM forward (5'-caccagcctcatgcacaa-3', according to NM_003200 1398-1416) and reverse (5'-tttctccagctccgtatggt-3', according to NM_002585 605-624) primers, magnesium with final concentration of 2mM, the PCR buffer, Q-solution and 2U Taq enzyme provided (Qiagen) were used in the first round PCR. The reaction cycles were 95°C for 5min, followed by 30 cycles of 95°C 30s, 55°C 30s, 72°C 30s, with final extension of 7min. 1µL PCR product was used in the second round PCR. The conditions were the same except forward primer (5'-gcacaaccacgcgccc-3', according to NM_003200

1407-1423) and reverse primer (5'-ccacgccttcgctaacagc-3', according to NM_002585 456-475). PCR products were run on 1.5% agarose gels and dyed with ethidium bromide. GAPDH was used as internal control. Sequencing was performed using PCR primers by Quintara (CA).

DNA extraction and mutation analysis in K-ras, p53 and EGFR

Genomic DNA was extracted from snap-frozen tissue specimens using Qiagen genomic DNA purification kit. Mutations in K-ras codon 12, p53 exons 4-8, EGFR exons 19-21 were analyzed by direct sequencing as previously reported [20-22].

Statistical analysis

The associations between the status of E2A-PBX1 fusion transcripts and clinical values were analyzed with Pearson Chi-square test and student *t* test for category variables and continuous variables, respectively. Median survival, 95% confidence intervals (CI) was calculated by Kaplan-Meier model and the log-rank test. A Cox regression model was used in AIS patients to assess the effects of E2A-PBX1 fusion transcripts, adjusted for gender, tumor stage, smoking status, race and Eastern Cooperative Oncology Group (ECOG) performance status. All *p* values reported were from two-sided tests. All analysis was performed by using SPSS 13.0. A *p*-value ≤ 0.05 was considered as significant.

Results

Detection of E2A-PBX1 fusion transcripts in NSCLC

We performed nested PCR and detected E2A-PBX1 in 23/184 (12.5%) NSCLC patients as well as in positive control (RCH-ACV cell line [23,24]), but not in negative control (CEM cell line [23,24]) or adult normal lung (Figure 1A). For the 23 patients with E2A-PBX1 fusion transcripts in their tumor tissues, we did not detect the E2A-PBX1 fusion transcripts in their paired adjacent normal tissues (figures not shown). We searched the sequencing results for all the PCR products in NCBI nucleotide/translated nucleotide/protein databases by BLAST (Basic Local Alignment Search Tool). The alignments showed that all the products we obtained were human E2A-PBX1 fusion gene with 100% identities. We also detected and confirmed E2A-PBX1 fusion transcripts in 3/13 (23.1%) NSCLC cell lines (Figure 1B). Furthermore, we found that all the junction sites in these specimens were the same as that reported by Nourse J, et al. [5] (sequencing examples of the sequence around the junction site in one positive NSCLC tissue sample and cell line were shown in Figure 1C).

Table 1 Characteristics of NSCLC patients in the study cohort

	Total (%)	E2A-PBX1 positive (%)	E2A-PBX1 negative (%)	P value	Median overall survival (95% CI)	P value
Total	184 (100)	23 (12.5)	161 (87.5)		105.60 (55.41 ~ 155.79)	
Age						
Mean (years)	66.9 ± 12.0	66.0 ± 11.7	67.0 ± 12.1	0.698*		
Range (years)	25-91	39-84	25-91			
<71	109 (100)	13 (11.9)	96 (88.1)	0.777	69.00 (43.73 ~ 94.27)	0.7069
≥71	75 (100)	10 (13.3)	65 (86.7)		105.60 (18.53 ~ 192.67)	
Gender				0.215		
Male	78 (100)	7 (9.0)	71 (91.0)		64.70 (NA)	0.0889
Female	106 (100)	16 (15.1)	90 (84.9)		105.60 (57.58 ~ 153.62)	
Race				0.606		0.1430
Caucasian	136 (100)	17 (12.5)	119 (87.5)		81.70 (52.59 ~ 110.81)	
Asian	27 (100)	3 (11.1)	24 (88.9)		64.70 (45.79 ~ 83.61)	
Hispanic	7 (100)	2 (28.6)	5 (71.4)		NR	
African-American	7 (100)	0	7 (100)		NR	
Others	7 (100)	1 (14.3)	6 (85.7)			
Smoking				0.174		0.0868
Smoker	127 (100)	19 (15.0)	108 (85.0)		69.00 (42.36 ~ 95.64)	
Non-smoker	53 (100)	4 (7.5)	49 (92.5)		105.60 (35.86 ~ 175.34)	
Unknown	4 (100)	0	4 (100)			
Pack/Year (smoker)						
Mean	41.6 ± 23.5	46.3 ± 26.7	30.9 ± 35.9	0.623*		
Range	1-160	5-90	1-160			
Gender × Smoking				0.097		0.0258
Male, Smoker	59 (100)	5 (8.5)	54 (91.5)	0.733 ¹	56.20 (27.25 ~ 85.15)	0.0749 ¹
Male, Non-smoker	18 (100)	2 (11.1)	16 (88.9)		NR	
Female, Smoker	68 (100)	14 (20.6)	54 (79.4)	0.048 ²	81.70 (41.68 ~ 121.72)	0.6714 ²
Female, Non-smoker	35 (100)	2 (5.7)	33 (94.3)		105.60 (35.04 ~ 176.16)	
Unkown	4 (100)	0	4 (100)			
Histology				0.276		0.6013
AIS	76 (100)	17 (22.4)	59 (77.6)	0.006 ³	105.60 (57.93 ~ 153.27)	0.1208 ³
Invasive adenocarcinoma	76 (100)	5 (6.6)	71 (93.4)		53.10 (NA)	
Squamous cell carcinoma	18 (100)	0	18 (100)		NR	
Carcinoid	6 (100)	0	6 (100)		NR	
Large	4 (100)	1 (25.0)	3 (75.0)		NR	
Others	4 (100)	0	4 (100)			
Tumor Size				0.026*		
Mean	3.3 ± 1.9	4.1 ± 2.8	3.2 ± 1.7			
Range	0.5-13.0	0.9-12.0	0.5-13.0			
Pathological TNM Classification						
pt						
pt1	74 (100)	9 (12.2)	65 (87.8)	0.408	105.60 (NA)	0.0915
pt2	81 (100)	9 (11.1)	72 (88.9)		69.00 (44.22 ~ 93.78)	
pt3	8 (100)	0	8 (100)		40.20 (26.06 ~ 54.34)	
pt4	18 (100)	4 (22.2)	14 (77.8)		30.50 (NA)	
Unknown	3 (100)	1 (33.3)	2 (66.6)			

Table 1 Characteristics of NSCLC patients in the study cohort (Continued)

pn	pn0	144 (100)	18 (12.5)	126 (87.5)	0.924	105.60 (65.68 ~ 145.52)	0.0038
	pn1	19 (100)	3 (15.8)	16 (84.2)		47.80 (32.55 ~ 63.05)	
	pn2	17 (100)	2 (11.8)	15 (88.2)		45.50 (NA)	
	pn3	2 (100)	0	2 (100)		5.20 (NA)	
	Unknown	2 (100)	0	2 (100)			
pm	pm0	171 (100)	20 (11.7)	151(88.3)	0.179	105.60 (55.99 ~ 155.21)	0.2605
	pm1	12 (100)	3 (25.0)	9 (75.0)		56.20 (35.26 ~ 77.14)	
Pathological Stage					0.426		0.0167
	Stage I	119 (100)	13 (10.9)	106 (89.1)		105.60 (65.47 ~ 145.73)	
	Stage II	22 (100)	2 (9.1)	20 (90.9)		NR	
	Stage III	29 (100)	5 (17.2)	24 (82.8)		33.60 (0.00 ~ 73.11)	
	Stage IV	12 (100)	3 (25.0)	9 (75.0)		56.20 (35.26 ~ 77.14)	
	Unknown	2 (100)	0	2 (100)			
Recurrence					0.435		<0.001
	Yes	63 (100)	6 (9.5)	57 (90.5)		39.30 (30.45 ~ 48.15)	
	No	103 (100)	14 (13.6)	89 (86.4)		NR	
	Unknown	18 (100)	1 (5.6)	17 (94.4)			

* student t test.

1 between male smoker and male non-smoker.

2 between female smoker and female non-smoker.

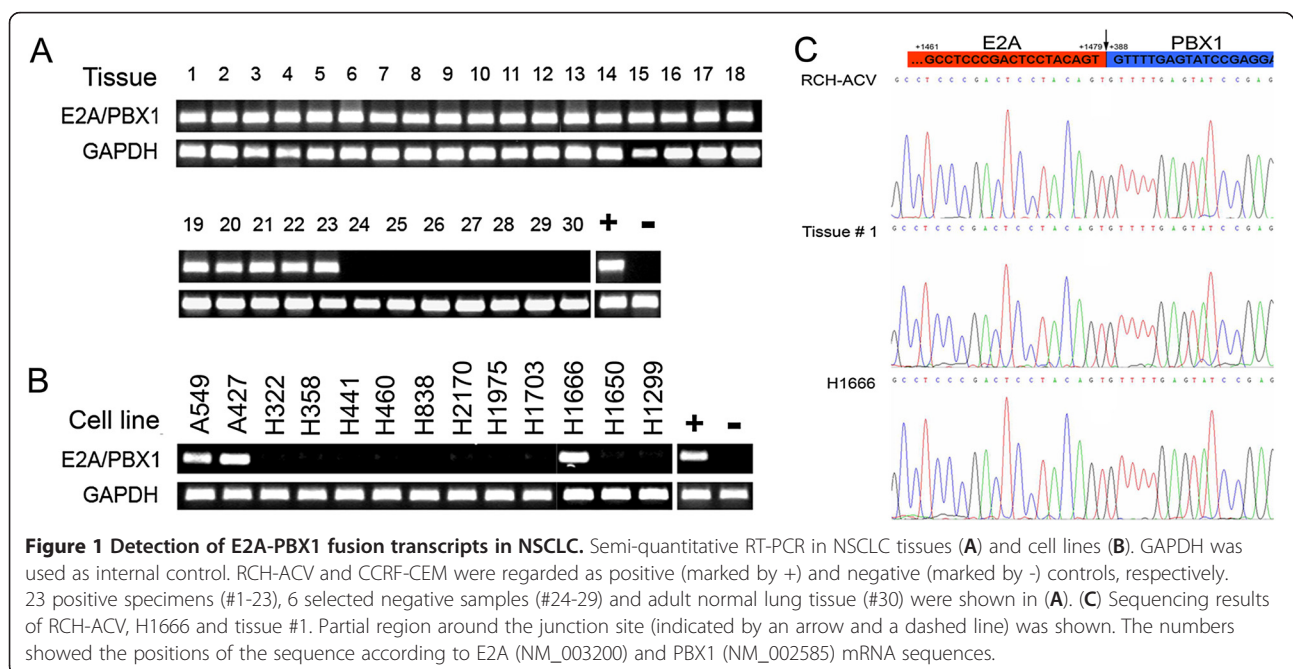
3 between AIS and invasive adenocarcinoma.

AIS: adenocarcinoma in situ; NR: not reached; NA: not available.

Association of E2A-PBX1 fusion transcripts with clinicopathological characteristics of NSCLC patients

We next analyzed association of the expression of E2A-PBX1 fusion transcripts and patients' characteristics (Table 1). Smoking status was not significantly associated

with the frequency of E2A-PBX1 fusion transcripts in all patients (19/127 (15.0%) in smokers and 4/56 (7.5%) in non-smokers ($p = 0.174$)), or in male patients (5/59 (8.5%) in smokers and 2/18 (11.1%) in non-smokers ($p = 0.733$)). On the other hand, the frequency of E2A-PBX1 fusion



transcripts in female smokers (14/68 (20.6%)) was significantly higher than that in female non-smokers (2/35 (5.7%)) ($p = 0.048$). The odds ratio for female smoker/non-smoker was 4.278, and 95% CI was from 0.914 to 20.026, also suggesting that the expression of E2A-PBX1 fusion transcripts correlated with smoking status among female patients with NSCLC.

The frequencies of E2A-PBX1 fusion transcripts in adenocarcinomas, squamous cell carcinomas, carcinoids and large cell carcinomas were 22/152 (14.5%), 0/18 (0%), 0/6 (0%), 1/4 (25%), respectively ($p = 0.276$) (Table 1). Interestingly, the frequency of E2A-PBX1 fusion transcripts in patients with AIS (17/76 (22.4%)) was significantly higher ($p = 0.006$) than that in patients with invasive adenocarcinoma (5/76 (6.6%)) (Table 1). The odds ratio for AIS/invasive adenocarcinoma was 4.092, and 95% CI was from 1.424 to 11.753, suggesting significant correlation between the expression of E2A-PBX1 fusion transcripts and patients with AIS. Moreover, the mean tumor size in patients with E2A-PBX1 fusion transcripts (4.1 ± 2.8 cm) was significantly larger than that in patients without E2A-PBX1 fusion transcripts (3.2 ± 1.7 cm) ($p = 0.026$) (Table 1). There was no significant association between expression of E2A-PBX1 fusion transcripts and age, gender, race, stage, or recurrence status (Table 1).

Association of E2A-PBX1 fusion transcripts with overall survival in AIS patients

In our study cohort of patients with AIS, females had significantly better overall survival (OS) than males

($p = 0.0378$; hazard ratio 0.3647; 95% CI, 0.1395 ~ 0.9532) (Table 2, Figure 2A), consistent with known data [25]. When these AIS patients were grouped by gender and expression of E2A-PBX1 fusion transcripts, no significant difference in OS was found between females and males in AIS patients with E2A-PBX1 fusion transcripts ($p = 0.6401$) (Figure 2B). In patients without E2A-PBX1 fusion transcripts, however, females had significantly better OS than males ($p = 0.0345$; hazard ratio 0.2687; 95% CI, 0.07945 ~ 0.9089) (Figure 2C). In addition, Kaplan-Meier analysis demonstrated an association between expression of E2A-PBX1 fusion transcripts and OS by stage. A statistically significant difference in OS was not observed in stage I patients (Figure 2D). OS was significantly better in E2A-PBX1 fusion transcripts (-) group than that in E2A-PBX1 fusion transcripts (+) group in stage IA patients with AIS ($p = 0.0363$; hazard ratio 0.04104; 95% CI, 0.002065 ~ 0.8157) (Figure 2E) and female stage IA patients with AIS ($p = 0.0174$; hazard ratio 0.02174; 95% CI, 0.0009266 ~ 0.5100) (Figure 2F). A multivariate analysis also showed that the status of E2A-PBX1 fusion transcripts ($P = 0.050$; hazard ratio 3.447; 95% CI, 1.002 ~ 11.857), gender ($p = 0.005$; hazard ratio 0.212; 95% CI, 0.071 ~ 0.628) and stage IA ($p = 0.004$; hazard ratio 0.011; 95% CI, 0.001 ~ 0.237) were significantly associated with overall survival.

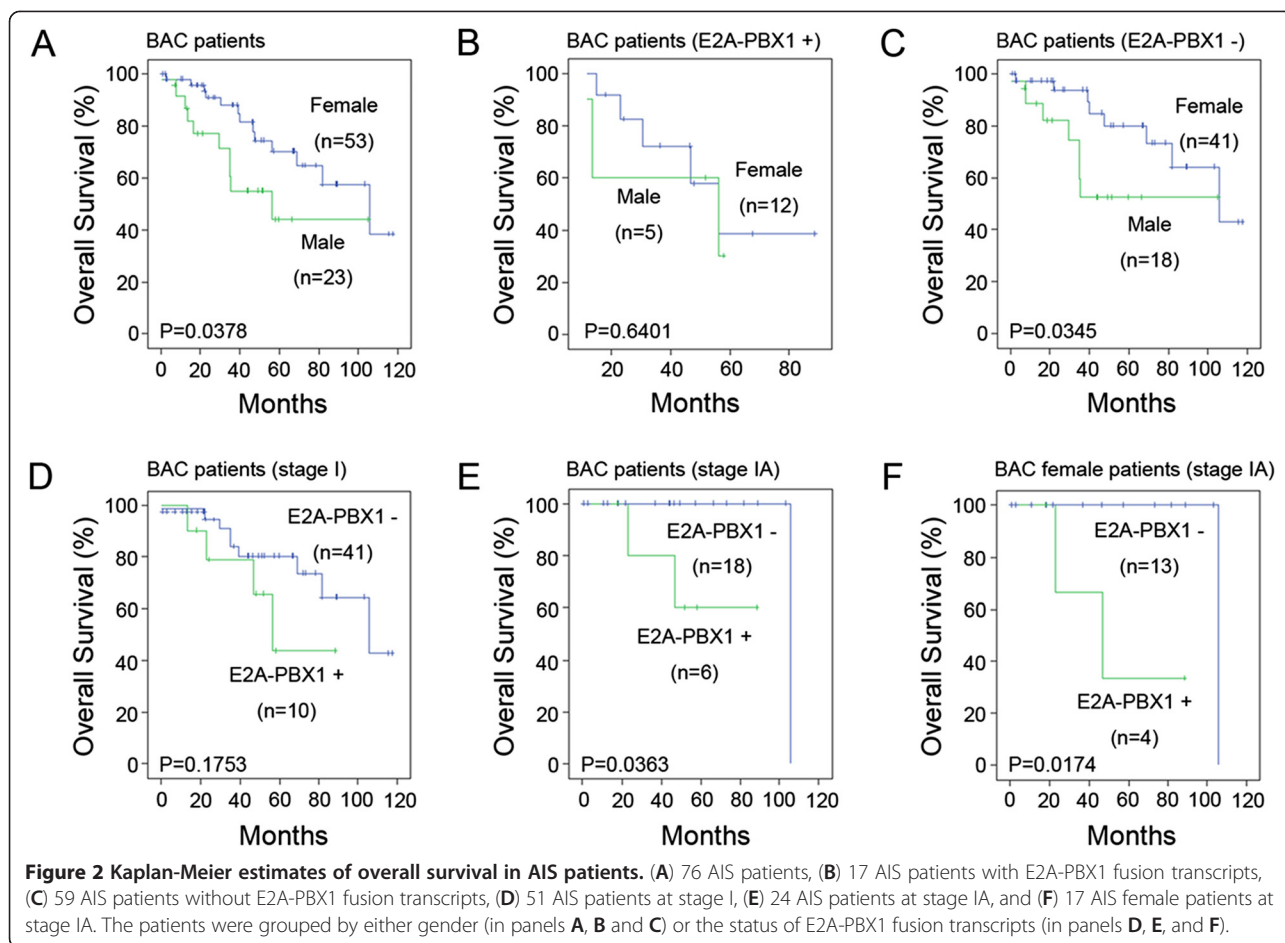
E2A-PBX1 fusion transcripts as a potential genetic biomarker in AIS

Mutations in K-ras, p53 and EGFR are commonly found in NSCLC [16-18]. We next screened for mutations in

Table 2 Overall survival analysis in AIS patients and subgroups

Group	Gender	E2A-PBX1 status	Patient number	Median survival (months)	95% CI	P value
AIS patients	Female		53	105.60	63.95 ~ 147.25	0.0378
	Male		23	56.20	22.34 ~ 90.06	
AIS patients with E2A-PBX1	Female		12	56.20	37.46 ~ 74.94	0.6401
	Male		5	56.20	0.00 ~ 122.80	
AIS patients without E2A-PBX1	Female		41	105.60	63.45 ~ 147.75	0.0345
	Male		18	NR	NA	
AIS patients		+	17	56.20	44.37 ~ 68.03	0.1235
		-	59	105.60	63.95 ~ 147.25	
AIS stage I patients		+	10	56.20	38.38 ~ 74.02	0.1753
		-	41	105.60	63.65 ~ 147.55	
AIS female patients		+	12	56.20	37.46 ~ 74.94	0.0747
		-	41	105.60	63.45 ~ 147.75	
AIS stage IA patients		+	6	NR	NA	0.0363
		-	18	NR	NA	
AIS stage IA female patients		+	4	46.70	8.77 ~ 84.63	0.0174
		-	13	105.60	NA	

NR: not reached; NA: not available.



codon 12 of K-ras, exons 4-8 of p53, and exons 19-21 of EGFR in the specimens from patients with expression of E2A-PBX1 fusion transcripts. We also compared the status of E2A-PBX1 fusion transcripts and mutation status of K-ras, p53 and EGFR in the NSCLC cell lines used in our study (mutation information was obtained from The COSMIC (Catalogue of Somatic Mutations in Cancer) database and website [25]) (Table 3 and Table 4). 8 of these 22 (36.4%) patients with E2A-PBX1 fusion transcripts had K-ras mutations; 3 of the 22 (13.6%) patients had p53 mutations; only 1 of the 22 (4.5%) patient had EGFR mutation. K-ras and EGFR mutations in these patients were mutually exclusive to each other, same as previously reported [26]. Notably, 12 of the 22 (54.5%) patients had none of these three common mutations in lung cancer (Table 3 and Table 4). This subgroup of patients included all four non-smokers (patient # 4, 8, 13 and 22) and 8 stage I patients with AIS (Table 3). Among all thirteen NSCLC cell lines that we examined, only H1666 that was derived from a female patient with BAC (non-smoker) had no mutations in K-ras, p53 and EGFR (Note: the histology information of H1666 from ATCC still uses BAC). Taken together, our results suggest

that the E2A-PBX1 fusion gene may be a genetic biomarker in NSCLC, especially in its subtype AIS.

Discussion

Somatic genetic changes have been believed to play important roles in human tumorigenesis, but the cancer type in which somatic rearrangement occurs is limited to leukemias, lymphomas and soft tissue tumors [2]. Overexpression of Notch3 was found to be associated with chromosome 19 translocation in lung cancer [27]. EML4-ALK fusion gene [28] and ETS fusion genes [29,30] exist in NSCLC and prostate cancer, respectively. It is still unclear whether chromosome aberrations are important in the initiation of epithelial tumorigenesis.

AIS (formerly named BAC) is a subset of adenocarcinoma characterized by non-invasive growth along alveolar septae [19,25]. It is more prevalent in women, non-smokers, and Asians [25]. Despite the lack of stromal, vascular, or pleural invasion, AIS is malignant and surgical resection is currently the mainstay of curative treatment. We previously discussed about a multi-step model of lung cancer development, especially AIS as carcinoma in situ [31]. Genetic changes can sequentially accumulate

Table 3 Mutational analysis of K-ras, p53 and EGFR in NSCLC patients with E2A-PBX1 fusion transcripts and NSCLC cell lines

#	E2A-PBX1	k-Kras codon 12	p53	EGFR	Age	Gender	Race	Smoking status	Stage	Histology
2	+				67	M	Middle Eastern	S	IIIB	Adc
12	+				72	F	Hispanic	S	IB	Adc
14	+				66	F	Caucasian	S	IIB	Adc
18	+	G12V			78	F	Caucasian	S	IB	Adc
20	+	G12C			63	F	Caucasian	S	IV	Adc
1	+				71	F	Caucasian	S	IA	AIS
3	+				41	M	Caucasian	S	IV	AIS
8	+				59	M	Caucasian	NS	IB	AIS
9	+				73	F	Caucasian	S	IIIB	AIS
11	+				84	M	Caucasian	S	IA	AIS
13	+				61	F	South Asian	NS	IB	AIS
21	+				82	F	Caucasian	S	IA	AIS
22	+				48	F	East Asian	NS	IB	AIS
16	+		G245S		63	F	Caucasian	S	IA	AIS
7	+		V272M	L858R	73	F	Caucasian	S	IIIB	AIS
6	+	G12C			68	M	Southeast Asian	S	IIIA	AIS
10	+	G12C			71	M	Hispanic	S	IA	AIS
17	+	G12C			67	F	Caucasian	S	IB	AIS
19	+	G12C			79	F	Caucasian	S	IIA	AIS
5	+	G12A			54	F	Caucasian	S	IIIB	AIS
15	+	G12V	exon 7(FS)		67	F	Caucasian	S	IV	AIS
4	+				39	M	Caucasian	NS	IB	LCC
23	+	ND	72	F	Caucasian	S	IA	AIS		
Cell line										
A549	+	G12S			58	M	Caucasian			Adc
A427	+	G12D			52	M	Caucasian			Adc
H441	-	G12V	R158L			M				Adc
H838	-				59	M	Caucasian	S	IIIB	Adc
H1975	-		R273H	L858R, T790M		F		NS		Adc
H1650	-			Exon 19 deletion	27	M	Caucasian	S	IIIB	Adc
H322	-		R248L		52	M	Caucasian			BAC
H358	-	G12C	Null			M	Caucasian			BAC
H1666	+				50	F	Caucasian	NS	III?	BAC
H2170	-		R158G			M		NS		Sqc
H1703	-		E285K		54	M	Caucasian	S	I	Sqc
H460	-	Q61H				M				LCC
H1299	-		Null		43	M	Caucasian			LCC

FS: frame shift; M: male; F: female; S: smoker; NS: non-smoker; Adc: adenocarcinoma; AIS: adenocarcinoma in situ; BAC: bronchioloalveolar carcinoma (histology information from ATCC still uses BAC); LCC: large cell carcinoma; Sqc: squamous carcinoma; ND: not determined.

Table 4 Summary of mutational analysis in NSCLC patients with E2A-PBX1 fusion transcripts

		Total (%)	K-P-E-	K+P-E-	K-P+E-	K+P+E-	K-P+E+	K+P+E+	K+P+E+
Total		22 (100)	12 (54.5)	7 (31.8)	1 (4.5)	1 (4.5)	1 (4.5)		
Gender	F	15 (100)	7 (46.7)	5 (33.3)	1 (6.7)	1 (6.7)	1 (6.7)		
	M	7 (100)	5 (71.4)	2 (28.6)					
Race	Caucasian	16 (100)	8 (50.0)	5 (31.3)	1 (6.3)	1 (6.3)	1 (6.3)		
	Asian	3 (100)	2 (66.7)	1 (33.3)					
	Middle eastern	1 (100)	1 (100)						
	Hispanic	2 (100)	1 (50.0)	1 (50.0)					
Smoking status	NS	4 (100)	4 (100)						
	S	18 (100)	8 (44.4)	7 (38.9)	1 (5.6)	1 (5.6)	1 (5.6)		
Stage	I	12 (100)	8 (66.7)	3 (25.0)	1 (8.3)				
	II	2 (100)	1 (50.0)	1 (50.0)					
	III	5 (100)	2 (40.0)	2 (40.0)			1 (20.0)		
	IV	3 (100)	1 (33.3)	1 (33.3)		1 (33.3)			
Histology	AIS	16 (100)	8 (50.0)	5 (31.3)	1 (6.3)	1 (6.3)	1 (6.3)		
	Invasive Adc	5 (100)	3 (60.0)	2 (40.0)					
	LCC	1 (100)	1 (100)						

K: k-ras codon 12; P: p53 exons 4-8; E: EGFR exons 19-21.

and cause bronchioalveolar stem cells to transform, leading to development of invasive phenotype in human cancers. However, it is unclear what is the cause for transformation of atypical bronchioalveolar cells into invasive adenocarcinoma or maintenance for the growth characterization in AIS. Several important players such as K-ras, p53, and survivin, etc. have been considered as tumor markers in AIS progression into invasive cancer [16-18,25], few are specific to AIS and “driver mutations” for AIS progression are still unidentified. Here in this study, we reported in NSCLC the expression of E2A-PBX1 fusion transcripts that have been well documented in leukemias [5-15]. This is the first report of detection of the E2A-PBX1 fusion transcripts in solid tumors. More interestingly, we observed that the E2A-PBX1 fusion transcripts were more frequently found in AIS than other subtypes of NSCLC, and the presence of E2A-PBX1 fusion transcripts were significantly associated with decreased overall survival in female and stage IA patients with AIS. These results suggest that the E2A-PBX1 fusion transcripts may play a critical role in AIS progression, especially for females and non-smokers. Supportive evidence also comes from our analysis of mutations in K-ras, p53 and EGFR that are common in NSCLC and considered as “driver mutations” [16-18]. Comparison of the mutational status of these genes in patients expressing the E2A-PBX1 fusion transcripts showed that approximately 55% patients examined in our study cohort were wild type in K-ras, p53 and EGFR. Majority of this subgroup were patients with AIS including all four non-smokers. Because E2A-PBX1 onco-protein has been proved to exhibit transformation

potentials by transcribing target genes [5-15], we argue that E2A-PBX1 may serve as one “driver mutation” in AIS and play critical roles during initiation and progression of at least a subset of AIS. E2A-PBX1 may represent a new therapeutic target for NSCLC, especially AIS. Further investigation is needed to evaluate the function of E2A-PBX1 fusion protein, as well as its therapeutic and prognostic values and its correlation with treatment resistance in AIS.

In this study, we only examined in NSCLC specimens the conserved E2A-PBX1 fusion transcripts that are well documented in leukemias [5-15]. It is possible that other forms of E2A-PBX1 fusion transcripts also exist in NSCLC. TCGA (The Cancer Genome Atlas) data may be useful to analyze the frequency of E2A-PBX1 fusion transcriptions in NSCLC. Another limitation of this study is relatively small number of AIS specimens analyzed. Analysis of an independent large cohort of AIS is needed to validate our observation.

Conclusions

Our data demonstrated the presence of E2A-PBX1 fusion transcripts caused by t(1;19)(q23;p13) in lung adenocarcinomas, especially AIS. It may be a common genetic change in AIS and a survival determinant for female AIS patients at early stage. These data may be of significant clinical importance, because finding reliable genetic biomarkers for early-stage lung adenocarcinomas including AIS is becoming increasingly apparent for early identification and management of this deadly disease.

Consent

Written informed consent was obtained from the patient for publication of this report and any accompanying images.

Competing interest

The authors declare that they have no competing interests.

Authors' contributions

MLM carried out the RNA extraction, primer design and PCR. TH carried out the DNA extraction and sequencing analysis. ZC and HL performed the statistical analysis. DJ participated in the design of the study. HMZ and BH conceived of the study, and participated in its design and coordination and helped to draft the manuscript. All authors read and approved the final manuscript.

Acknowledgement

This work was supported by a Research Grant from The Joan's Legacy Lung Cancer Foundation and NIH Grant R01 CA125030 (to B. He); by the Larry Hall and Zygielbaum Memorial Trust, and the Kazan, McClain, Edises, Abrams, Fernandez, Lyons& Farrise Foundations (to D.M. Jablons); by the National Key Basic Research and Development (973) Program of China No. 2011CB910800 and No. 2012CB917304 (to H.M. Zhou); and by the China Natural Science Foundation No. 31170732 and No. 31270854 (to H.M. Zhou).

Author details

¹School of Life Sciences, Tsinghua University, Beijing 10084, China. ²Thoracic Oncology Program, Department of Surgery, Helen Diller Family Comprehensive Cancer Center, University of California, San Francisco, CA 94115, USA. ³Zhejiang Provincial Key Laboratory of Applied Enzymology, Yangtze Delta Region Institute of Tsinghua University, Jiaxing 314006, Zhejiang, China. ⁴Department of Surgery, Division of Thoracic Surgery, Nippon Medical School, Tokyo 113-8602, Japan.

Received: 20 February 2013 Accepted: 15 April 2013

Published: 20 May 2013

References

- Jemal A, Siegel R, Ward E, Hao Y, Xu J, Murray T, Thun MJ: **Cancer statistics, 2008.** *CA Cancer J Clin* 2008, **58**:71–96.
- Campbell PJ, Stephens PJ, Pleasance ED, O'Meara S, Li H, Santarius T, Stebbings LA, Leroy C, Edkins S, Hardy C, et al: **Identification of somatically acquired rearrangements in cancer using genome-wide massively parallel paired-end sequencing.** *Nat Genet* 2008, **40**:722–729.
- Croce CM: **Oncogenes and cancer.** *N Engl J Med* 2008, **358**:502–511.
- Mitelman F, Johansson B, Mertens F: **Fusion genes and rearranged genes as a linear function of chromosome aberrations in cancer.** *Nat Genet* 2004, **36**:331–334.
- Nourse J, Mellentin JD, Galili N, Wilkinson J, Stanbridge E, Smith SD, Cleary ML: **Chromosomal translocation t(1;19) results in synthesis of a homeobox fusion mRNA that codes for a potential chimeric transcription factor.** *Cell* 1990, **60**:535–545.
- Wiemels JL, Leonard BC, Wang Y, Segal MR, Hunger SP, Smith MT, Crouse V, Ma X, Buffler PA, Pine SR: **Site-specific translocation and evidence of postnatal origin of the t(1;19) E2A-PBX1 fusion in childhood acute lymphoblastic leukemia.** *Proc Natl Acad Sci USA* 2002, **99**:15101–15106.
- Dedera DA, Waller EK, LeBrun DP, Sen-Majumdar A, Stevens ME, Barsh GS, Cleary ML: **Chimeric homeobox gene E2A-PBX1 induces proliferation, apoptosis, and malignant lymphomas in transgenic mice.** *Cell* 1993, **74**:833–843.
- Kamps MP, Wright DD: **Oncoprotein E2A-Pbx1 immortalizes a myeloid progenitor in primary marrow cultures without abrogating its factor-dependence.** *Oncogene* 1994, **9**:3159–3166.
- Monica K, LeBrun DP, Dedera DA, Brown R, Cleary ML: **Transformation properties of the E2a-Pbx1 chimeric oncoprotein: fusion with E2a is essential, but the Pbx1 homeodomain is dispensable.** *Mol Cell Biol* 1994, **14**:8304–8314.
- Fu X, Kamps MP: **E2a-Pbx1 induces aberrant expression of tissue-specific and developmentally regulated genes when expressed in NIH 3T3 fibroblasts.** *Mol Cell Biol* 1997, **17**:1503–1512.
- Hunger SP, Galili N, Carroll AJ, Crist WM, Link MP, Cleary ML: **The t(1;19) (q23;p13) results in consistent fusion of E2A and PBX1 coding sequences in acute lymphoblastic leukemias.** *Blood* 1991, **77**:687–693.
- Kamps MP, Look AT, Baltimore D: **The human t(1;19) translocation in pre-B ALL produces multiple nuclear E2A-Pbx1 fusion proteins with differing transforming potentials.** *Genes Dev* 1991, **5**:358–368.
- McWhirter JR, Goulding M, Weiner JA, Chun J, Murre C: **A novel fibroblast growth factor gene expressed in the developing nervous system is a downstream target of the chimeric homeodomain oncoprotein E2A-Pbx1.** *Development* 1997, **124**:3221–3232.
- McWhirter JR, Neuteboom ST, Wancewicz EV, Monia BP, Downing JR, Murre C: **Oncogenic homeodomain transcription factor E2A-Pbx1 activates a novel WNT gene in pre-B acute lymphoblastoid leukemia.** *Proc Natl Acad Sci USA* 1999, **96**:11464–11469.
- Smith KS, Chanda SK, Lingbeek M, Ross DT, Botstein D, van Lohuizen M, Cleary ML: **Bmi-1 regulation of INK4A-ARF is a downstream requirement for transformation of hematopoietic progenitors by E2a-Pbx1.** *Mol Cell* 2003, **12**:393–400.
- Mounawar M, Mukeria A, Le Calvez F, Hung RJ, Renard H, Cortot A, Bollart C, Zaridze D, Brennan P, Boffetta P, et al: **Patterns of EGFR, HER2, TP53, and KRAS mutations of p14arf expression in non-small cell lung cancers in relation to smoking history.** *Cancer Res* 2007, **67**:5667–5672.
- Sonobe M, Manabe T, Wada H, Tanaka F: **Mutations in the epidermal growth factor receptor gene are linked to smoking-independent, lung adenocarcinoma.** *Br J Cancer* 2005, **93**:355–363.
- Sonobe M, Manabe T, Wada H, Tanaka F: **Lung adenocarcinoma harboring mutations in the ERBB2 kinase domain.** *J Mol Diagn* 2006, **8**:351–356.
- Travis WD, Brambilla E, Noguchi M, Nicholson AG, Geisinger KR, Yatabe Y, Beer DG, Powell CA, Riely GJ, Van Schil PE, et al: **International association for the study of lung cancer/american thoracic society/european respiratory society international multidisciplinary classification of lung adenocarcinoma.** *Journal of thoracic oncology : official publication of the International Association for the Study of Lung Cancer* 2011, **6**:244–285.
- Kim IJ, Kang HC, Shin Y, Park HW, Jang SG, Han SY, Lim SK, Lee MR, Chang HJ, Ku JL, et al: **A TP53-truncating germline mutation (E287X) in a family with characteristics of both hereditary diffuse gastric cancer and Li-Fraumeni syndrome.** *J Hum Genet* 2004, **49**:591–595.
- Lynch TJ, Bell DW, Sordella R, Gurubhagavatula S, Okimoto RA, Brannigan BW, Harris PL, Haserlat SM, Supko JG, Haluska FG, et al: **Activating mutations in the epidermal growth factor receptor underlying responsiveness of non-small-cell lung cancer to gefitinib.** *N Engl J Med* 2004, **350**:2129–2139.
- Sagawa M, Saito Y, Fujimura S, Linnoila RI: **K-ras point mutation occurs in the early stage of carcinogenesis in lung cancer.** *Br J Cancer* 1998, **77**:720–723.
- Curry JD, Glaser MC, Smith MT: **Real-time reverse transcription polymerase chain reaction detection and quantification of t(1;19) (E2A-PBX1) fusion genes associated with leukaemia.** *Br J Haematol* 2001, **115**:826–830.
- Mazieres J, You L, He B, Xu Z, Lee AY, Mikami I, McCormick F, Jablons DM: **Inhibition of Wnt16 in human acute lymphoblastoid leukemia cells containing the t(1;19) translocation induces apoptosis.** *Oncogene* 2005, **24**:5396–5400.
- Bamford S, Dawson E, Forbes S, Clements J, Pettett R, Dogan A, Flanagan A, Teague J, Futreal PA, Stratton MR, Wooster R: **The COSMIC (Catalogue of Somatic Mutations in Cancer) database and website.** *Br J Cancer* 2004, **91**:355–358.
- Shigematsu H, Takahashi T, Nomura M, Majumdar K, Suzuki M, Lee H, Wistuba II, Fong KM, Toyooka S, Shimizu N, et al: **Somatic mutations of the HER2 kinase domain in lung adenocarcinomas.** *Cancer Res* 2005, **65**:1642–1646.
- Dang TP, Gazdar AF, Virmani AK, Sepetavec T, Hande KR, Minna JD, Roberts JR, Carbone DP: **Chromosome 19 translocation, overexpression of Notch3, and human lung cancer.** *J Natl Cancer Inst* 2000, **92**:1355–1357.
- Soda M, Choi YL, Enomoto M, Takada S, Yamashita Y, Ishikawa S, Fujiwara S, Watanabe H, Kurashina K, Hatanaka H, et al: **Identification of the transforming EML4-ALK fusion gene in non-small-cell lung cancer.** *Nature* 2007, **448**:561–566.
- Tomlins SA, Laxman B, Dhanasekaran SM, Helgeson BE, Cao X, Morris DS, Menon A, Jing X, Cao Q, Han B, et al: **Distinct classes of chromosomal rearrangements create oncogenic ETS gene fusions in prostate cancer.** *Nature* 2007, **448**:595–599.

30. Tomlins SA, Rhodes DR, Perner S, Dhanasekaran SM, Mehra R, Sun XW, Varambally S, Cao X, Tchinda J, Kuefer R, et al: **Recurrent fusion of TMPRSS2 and ETS transcription factor genes in prostate cancer.** *Science* 2005, **310**:644–648.
31. Raz DJ, He B, Rosell R, Jablons DM: **Current concepts in bronchioloalveolar carcinoma biology.** *Clin Cancer Res* 2006, **12**:3698–3704.

doi:10.1186/1756-9966-32-29

Cite this article as: Mo et al.: Detection of E2A-PBX1 fusion transcripts in human non-small-cell lung cancer. *Journal of Experimental & Clinical Cancer Research* 2013 **32**:29.

**Submit your next manuscript to BioMed Central
and take full advantage of:**

- Convenient online submission
- Thorough peer review
- No space constraints or color figure charges
- Immediate publication on acceptance
- Inclusion in PubMed, CAS, Scopus and Google Scholar
- Research which is freely available for redistribution

Submit your manuscript at
www.biomedcentral.com/submit

 **BioMed** Central