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Multicentre prospective phase II trial of gefitinib for advanced non-small cell lung cancer with epidermal growth factor receptor mutations: results of the West Japan Thoracic Oncology Group trial (WJTOG0403)

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The purpose of this study was to evaluate the efficacy of gefitinib and the feasibility of screening for epidermal growth factor receptor (EGFR) mutations among select patients with advanced non-small cell lung cancer (NSCLC). Stage IIIB/IV NSCLC, chemotherapy-naive patients or patients with recurrences after up to two prior chemotherapy regimens were eligible. Direct sequencing using DNA from tumour specimens was performed by a central laboratory to detect EGFR mutations. Patients harbouring EGFR mutations received gefitinib. The primary study objective was response; the secondary objectives were toxicity, overall survival (OS), progression-free survival (PFS), 1-year survival (1Y-S) and the disease control rate (DCR). Between March 2005 and January 2006, 118 patients were recruited from 15 institutions and were screened for EGFR mutations, which were detected in 32 patients – 28 of whom were enrolled in the present study. The overall response rate was 75%, the DCR was 96% and the median PFS was 11.5 months. The median OS has not yet been reached, and the 1Y-S was 79%. Thus, gefitinib chemotherapy in patients with advanced NSCLC harbouring EGFR mutations was highly effective. This trial documents the feasibility of performing a multicentre phase II study using a central typing laboratory, demonstrating the benefit to patients of selecting gefitinib treatment based on their EGFR mutation status.
British Journal of Cancer (2008) 98, 907–914. doi:10.1038/sj.bjc.6604249 www.bjcancer.com
Published online 19 February 2008
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Gefitinib, a tyrosine kinase inhibitor (TKI), is an orally active small molecule that functions as a selective epidermal growth factor receptor (EGFR) inhibitor (Ranson et al, 2002). Two phase II trials (Fukuoka et al, 2003; Kris et al, 2003) for previously treated non-small cell lung cancer (NSCLC) (IDEAL-1 and -2, respectively) have documented favourable objective responses in 14–18% of patients. However, in a phase III

trial (Thatcher et al, 2005), no survival benefit of gefitinib was observed when compared with best-supportive care (BSC) for previously treated NSCLC. In contrast, we have seen a significant survival benefit of erlotinib compared with BSC as a salvage therapy (BR21); erlotinib is also an EGFR-TKI and its chemical structure, which is based on quinazoline, is quite similar to that of gefitinib (Shepherd et al, 2005). Although we do not know whether differences between gefitinib and erlotinib were responsible for these different outcomes, appropriate patient selection to identify good responders is likely crucial for revealing the clinical benefits of the EGFR-TKI family.

Patient subset analyses of these randomised phase III trials or retrospective trials (Kaneda *et al*, 2004; Miller *et al*, 2004) clearly show the existence of populations that are more likely to respond to gefitinib and erlotinib, including women, patients with adenocarcinoma (especially with bronchial alveolar carcinoma (BAC)), nonsmokers and Asian patients (compared with Caucasians). Somatic mutations in specific regions of exons 18, 19 and 21 of the ATP-binding domain of *EGFR* have recently been shown to have strong associations with sensitivity to gefitinib or erlotinib (Lynch *et al*, 2004; Paez *et al*, 2004; Pao *et al*, 2004). Consistent with these findings, the frequencies of these *EGFR* mutations were higher in women, patients with adenocarcinoma, nonsmokers and Asians, all of whom are among the more frequent responders, as mentioned above (Shigematsu *et al*, 2005). There are two characteristic types of *EGFR* mutations. One is the presence of in-frame deletions, including the amino acids at codons 746–750 in exon 19, and the other is an amino-acid substitution at codon 858 (L858R) in exon 21. Recent analyses (Bell *et al*, 2005) of phase II and III trials for *EGFR*-TKI, in which patients were not selected based on their mutation status, have suggested that *EGFR* mutations are correlated with response to therapy but are not correlated with overall survival (OS). Furthermore, *EGFR* gene amplification/copy number (Cappuzzo *et al*, 2005; Hirsch *et al*, 2005) or overexpression (Hirsch *et al*, 2003) has been shown to be a more useful prognostic marker of response to gefitinib treatment. Patient selection according to *EGFR* mutation status may yield a superior survival rate by excluding patients who are unlikely to respond to gefitinib treatment. However, other populations that might obtain a clinical benefit from gefitinib treatment, even in the absence of *EGFR* mutation, may exist.

Three Japanese groups (Asahina *et al*, 2006; Inoue *et al*, 2006; Yoshida *et al*, 2007) have reported prospective phase II studies of gefitinib for advanced-stage NSCLC that were designed to consider the *EGFR* mutation status of the patients. All of these studies have reported a high response rate and extended progression-free survival (PFS) period, compared with historical controls. However, all of these studies had a relatively short observation period, making the data preliminary. Moreover, the original sample size was calculated after patient selection, and a critical consideration of the suitability of the assay used to detect the mutations (which was performed using small paraffin-embedded specimens obtained from bronchoscopic biopsies), and the estimated *EGFR*-positive rate were lacking. Additionally, all the trials were conducted at single institutions located in one small area of Japan. Thus, the published data may not be representative of the situation found in general clinical practice throughout Japan and therefore may not directly translate to the general feasibility of gefitinib treatment in Japan.

In view of this situation, we performed a multicentre prospective phase II trial of gefitinib for advanced NSCLC harbouring *EGFR* mutations. We prospectively registered patients from 15 different institutes in Japan at the beginning of *EGFR* mutation screening using a central database. Whether or not tissue was available from a bronchoscopic biopsy or surgery was not an inclusion criterion. All the clinical samples from the registered patients were delivered to a central laboratory that then determined the *EGFR* mutation status or the histological BAC features. The analysis of the survival data was based on a minimum observation period of at least 15 months from the time of entry of the last patient.

MATERIALS AND METHODS

Eligibility criteria

Eligible patients had histologically confirmed stage III NSCLC for which thoracic irradiation was not indicated or were stage IV. Chemotherapy-naive patients or those who had previously received up to two prior chemotherapy regimens, including those performed in an adjuvant setting, were eligible. Other eligibility criteria included an age $\geqslant 20$ years, measurable disease, the availability of sufficient amounts of tumour specimen for *EGFR* mutation analysis, an Eastern Cooperative Oncology Group performance status of 0–2, adequate organ function (WBC $\leqslant 3000\,\mu l^{-1}$, platelets $\geqslant 75\,000\,\mu l^{-1}$, AST and ALT $\leqslant 100\,IU\,l^{-1}$, serum creatinine $\leqslant$ twice the upper limit of the reference range; $P_{aO2} \geqslant 60\,mm\,Hg$). The exclusion criteria included pulmonary fibrosis, the presence of symptomatic brain metastasis, active concomitant malignancy, severe heart disease, active gastrointestinal bleeding and continuous diarrhoea. All the patients signed a written informed consent form. Approval of this study and the gene analyses were obtained from the Institutional Review Board and the Ethics Committee of each hospital.

EGFR gene analysis

Tumour specimens were obtained using bronchial fiberscope or surgical procedures. The specimens were fixed with formalin and embedded in paraffin. Four slices $(4-5\,\mu m)$ from the embedded block were sent to a central laboratory (Mitsubishi Chemical Safety Institute Ltd., Ibaraki, Japan) for genetic analysis. Most of the tumour specimens were available prior to the registration of this study. Genomic DNA was isolated from specimens using QIAamp Micro kits (QIAGEN KK, Tokyo, Japan). The *EGFR* mutations in exons 18, 19 and 21, as previously reported (Lynch *et al*, 2004; Paez *et al*, 2004), were determined using polymerase chain reaction (PCR) amplification and intron–exon boundary primers according to the published method. An *EGFR* registrant mutation in exon 20, which was reported by Pao *et al* (2005) was also examined using PCR and the previously reported primers. Polymerase chain reaction was performed using a Gene Amp PCR System 9700 (Applied Biosystems, Foster City, CA, USA), and the PCR products were confirmed using a Bioanalyzer 2100 (Agilent Technologies Inc., Santa Clara, CA, USA), then sequenced directly using the Big Dye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems) and ABI PRISM 3100 (Applied Biosystems). All sequencing reactions were performed in both forward and reverse directions and were analysed using the Basic Local Alignment Search Tool (BLAST); all the electropherograms were reanalysed by visual inspection to check for mutations. The presence of an *EGFR* mutation was confirmed using at least three independent PCR.

All sequence data were sent from the central laboratory to Kinki University. A principle investigator then confirmed whether or not the *EGFR* mutation status was positive, and the results were sent to the West Japan Thoracic Oncology Group (WJTOG) data centre. The data centre then informed each participating centre of the results of the genetic analysis and requested that the eligibility criteria of the patients be rechecked to insure that only *EGFR*-positive subjects were registered in the trial. Each tumour was categorised according to histology by a pulmonary pathologist (JF). The percentage of area exhibiting a BAC pattern was also examined to determine the WHO pathological category.

Treatment plan

Gefitinib $(250\,mg\,day^{-1})$ was administered once daily. Treatment was continued uninterrupted until disease progression or intolerable toxicity (grade 4 nonhaematological toxicities, any incidents of interstitial pneumonia or a treatment delay of more than 2 weeks because of adverse effects). Gefitinib administration was delayed if the patient's leukocyte and platelet counts were lower than 1500 and $5000\,\mu l^{-1}$, respectively, and was withheld until these counts had recovered. Gefitinib administration was also delayed if grade 3 or greater nonhaematological toxicities without nausea, vomiting or alopecia occurred and was withheld until recovery to grade 2.

Routine clinical and laboratory assessments and chest X-ray assessments were performed weekly or biweekly, where possible; CT examinations of the target lesion were performed every month, and magnetic resonance imaging of the whole brain and a bone scan were performed every 3 months. The objective responses of the patients were evaluated every month using the Response Evaluation Criteria in Solid Tumours (RECIST) guidelines (Therasse et al, 2000). Tumour response was centrally evaluated by independent reviewers at an extramural conference and was performed for the intent-to-treat population. All adverse effects that occurred during gefitinib treatment were reported, and the severity of the effects was graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events, version 3.0.

Statistical analyses

The primary end point of this study was the response rate. A one-stage design using the binominal probability was used to determine the sample size. Assuming that a response rate of 50% would indicate potential usefulness, whereas a rate of 25% would be the lower limit of interest, and with $\alpha = 0.10$ (two side) and $\beta = 0.20$, the estimated accrual number was 23 patients. Estimating that the EGFR-positive rate would be about 20%, the screening number required to accrue 23 EGFR-positive patients was 115. After assuming an inevaluability rate of <10%, the final required screening number was 125.

The secondary end points of this study were toxicity, OS, PFS, 1-year survival (1Y-S) and the disease control rate (DCR). Survival analyses were conducted on the intent-to-treat population using follow-up data available as of 30 April 2007. The survival curves were estimated using Kaplan–Meier plots.

RESULTS

Patient characteristics

Between March 2005 and January 2006, 118 patients were prospectively screened from 15 institutions; 117 of them underwent EGFR mutation analysis (tumour tissue was not available for one patient). The median time required for the EGFR mutation analysis was 12 days (range: 7–28 days). Among the 117 patients, EGFR mutations were detected in 32 patients (27%), 14 of whom had a deletion in or near E746-A750 (including one del E746-T751 ins A, two del L747-T751 and one del L747-T753 ins S) in exon 19. A further 17 had L858R, and one had a L861Q point mutation in exon 21 (Table 1).

Tissue samples from 17 patients (53%) were obtained by transbronchial biopsy. The EGFR detection rates for the surgical specimens and the bronchoscopic biopsy specimens were similar (30 vs 25%). The EGFR mutations were significantly more frequent in women ($P \leqslant 0.02$), in patients with adenocarcinoma ($P = 0.001$) and in people who had never smoked ($P < 0.001$) (Table 2). Finally, 28 patients (14 with deletions in exons 19 and 14 with point mutations in exon 21) were actually registered and received treatment with gefitinib, whereas four patients were dropped from the study as they became ineligible because of tumour progression during the time required for the mutation analysis.

Patient characteristics are listed in Table 3. In the initial screening, there were 56 female patients (48%), 97 patients (83%) with adenocarcinoma and 53 (45%) who had never smoked. The frequency of these characteristics was higher among the patients with EGFR mutations who were actually registered; namely, 18 patients (64%) were women, 27 (96%) had adenocarcinoma and 19 (68%) had never smoked. The median age of the 28 actually registered patients was 68 years; 24 patients (86%) had a good performance status (0–1), 22 (79%) had stage IV diseases and 17

(61%) were chemotherapy naive. Thoracic irradiation was contra-indicated in one patient with stage IIIA disease because of the large irradiation field that would have been required. All five patients with stage IIIB diseases had malignant effusions. Four patients had received adjuvant therapies; five had received platinum doublets or a combination of gemcitabine and vinorelbine as their first-line therapy. Two patients had received two regimens of platinum doublets followed by docetaxel or pemetrexed. One patient had received local radiation for pain control.

Response and survival

The objective tumour responses are listed in Table 4. The overall response rate and DCR were 75% (95% CI: 57.6–91.0%) and 96% (95% CI: 87.0–96.4%), respectively. Five out of ten male patients (50%), six out of nine smokers (67%) and five out of eight male smokers with adenocarcinoma (63%) achieved a PR. One female nonsmoker with squamous cell carcinoma also achieved a PR. Among the registered patients with EGFR mutations, the response rate was no different between current/former smokers and those who had never smoked (67 vs 79%) or between chemotherapy-naive and postchemotherapy patients (77 vs 73%). Female and patients with a mutational deletion in exon 19 tended to have a higher response rate than male (89 vs 50%) and patients with a missense mutation in exon 21 (86 vs 64%), respectively.

The median follow-up time was 18.6 months (range: 13.8–23.4 months). The median PFS time was 11.5 months (95% CI: 7.3 months to -) (Figure 1A). The median OS has not yet been reached, and the 1Y-S was 79% (95% CI: 63.4–93.8%) (Figure 1B).

Table 1 Type of EGFR mutations (n = 32)

Characteristics	No. of patients	%
Exon 18	0	0
Exon 19	14	44
del E746-A750	10	32
del E746-T751 ins A	1	3
del L747-T751	2	6
del L747-T753 ins S	1	3
Exon 21	18	56
L858R	17	53
L861Q	1	3

EGFR = epidermal growth factor receptor.

Table 2 Relationship between patient characteristics and EGFR mutation status

Characteristics	EGFR mutation positive (n = 32)		EGFR mutation negative (n = 85)		
	No. of Patients	%	No. of Patients	%	P
Sex					
Male	11	34	50	59	
Female	21	66	35	41	<0.02
Histology					
Adenocarcinoma	31	97	66	78	
Nonadenocarcinoma	1	3	19	22	=0.001
Smoking status					
Never	21	66	31	36	
Current/former	11	34	54	64	<0.001

EGFR = epidermal growth factor receptor.

Clinical Studies

Table 3 Patient characteristics of all registered patients (n = 28)

Characteristics	No. of patients (%)
Age	
Median	68
Range	49–89
Performance status	
0	11 (39)
1	13 (47)
2	4 (14)
Sex	
Male	10 (36)
Female	18 (64)
Histology	
Adenocarcinoma	27 (96)
Squamous cell carcinoma	1 (4)
Large cell carcinoma	0 (0)
Adenosquamous carcinoma	0 (0)
Other	0 (0)
Smoking status	
Never	19 (68)
Current/former	9 (32)
Stage	
IIIA[a]	1 (3)
IIIB	5 (18)
IV	22 (79)
Prior cancer therapy	
Chemotherapy	
No	17 (61)
One regimen (adjuvant)	4 (14)
One regimen (not adjuvant)	5 (18)
Two regimens	2 (7)
Recurrence after surgery	11 (39)
Radiation	1 (4)

[a]Unresectable, no indication for thoracic radiation because of a large radiation field.

Table 4 Response rate (n = 28)

Response	No. of patients	Response rate (%)	95% CI
Complete response	1	3.6	
Partial response	20	71.4	
Stable disease	6	21.4	
Progressive disease	0	0.0	
Not evaluable[a]	1	3.6	
Overall response	21	75.0	57.6–91.0
Disease control rate	27	96.4	87.0–96.4

CI = confidence interval. [a]One patient was not evaluable because of a poor evaluation of efficacy.

Safety and toxicity

Toxicity was evaluated in all eligible patients (Table 5). The most frequent adverse events were rash, dry skin, diarrhoea, stomatitis and elevated AST/ALT levels. Two patients experienced grade 3 rash and one patient experienced grade 3 keratitis; however, these patients all achieved a PR, and the adverse effects subsided after pausing gefitinib treatment for around 2 weeks. Four patients experienced grade 3 hepatotoxicity; three of these patients had to discontinue treatment for this reason.

One patient developed interstitial lung disease (ILD) (Ando *et al*, 2006). Ground-glass opacity was detected in the right upper lobe 19 days after the start of gefitinib administration, resulting in the cessation of treatment. However, the lesion enlarged into bilateral

lung fields on day 25, and steroid therapy was initiated. Nonetheless, the patient died of respiratory failure on day 48. Two patients also experienced grade 1 ILD. They recovered without steroid administration.

Subsequent treatment after disease progression

Of the 14 patients who become refractory to gefitinib and exhibited disease progression, 10 received chemotherapy as their first treatment regimen after gefitinib (Table 6); 5 patients received platinum doublets and 1 patient received vinorelbine as a second-line treatment; and 3 received docetaxel and 1 received platinum doublet as a third-line treatment. In all, 4 out of the 10 patients (40%) had a PR. Of the nine patients who become refractory to the first treatment regimen after gefitinib, six received chemotherapy as their second regimen after gefitinib, including one who received gemcitabine, one who received docetaxel, and one who was re-treated with gefitinib as a third-line therapy; two other patients received docetaxel and one was re-treated with gefitinib as a fourth-line therapy. Two of the six patients (33%) had a PR. The two patients who received gefitinib re-treatment both had SD.

## BAC features, *EGFR* amplification and T790M mutation in exon 20

A total of 110 tissue samples were available for pathological review, of which 90 were from adenocarcinoma; 33 of these specimens (37%) revealed proportional BAC components in the specimen. Among them, 15 were considered extensive and the remaining 18 were found to have minor BAC components. The 39 surgical specimens included 36 from adenocarcinomas. The *EGFR* mutations were detected in 12 out of the 36 adenocarcinoma specimens. None of the samples with a BAC component, micropapillary pattern or mucin production was associated with an *EGFR* mutation (Table 7).

Data on *EGFR* gene copy numbers were available in only 12 samples. We used the criteria for defining a high *EGFR* gene copy number (gene amplification or high polysomy, as determined using FISH) that were described in a previous report (Cappuzzo *et al*, 2005). A total of 7 out of the 12 samples had a high gene copy number (FISH positive), and 6 (3 with *EGFR* mutations) out of the 7 samples had proportional BAC components. In all, 5 out of the 12 samples were FISH negative, only 1 (with no *EGFR* mutation) of which had a BAC component. Two patients that were FISH negative, BAC negative and *EGFR* mutation positive had SD when treated with gefitinib.

Another *EGFR* mutation, T790M in exon 20, has been reported to be associated with resistance to gefitinib (Kobayashi *et al*, 2005; Pao *et al*, 2005). We checked for this mutation in six patients who did not respond to gefitinib; however, the mutation could not be identified in any of the patients.

DISCUSSION

We performed a multicentre phase II study examining the use of gefitinib for advanced NSCLC in patients with *EGFR* mutations, prospectively recruiting patients at the time of genetic screening and avoiding a selection bias. All patients were registered in a central database. All tissues were delivered from the local participants to the central facility, where they were reviewed by a pathology specialist and the *EGFR* mutation status was evaluated. The median time for the *EGFR* mutation detection analysis was 12 days, which is probably an acceptable time lag before the start of treatment for advanced NSCLC. However, a shorter period would clearly be desirable for routine clinical practice. Indeed, 4 out of the 32 *EGFR*-positive patients were dropped from the study because of disease progression before their actual registration

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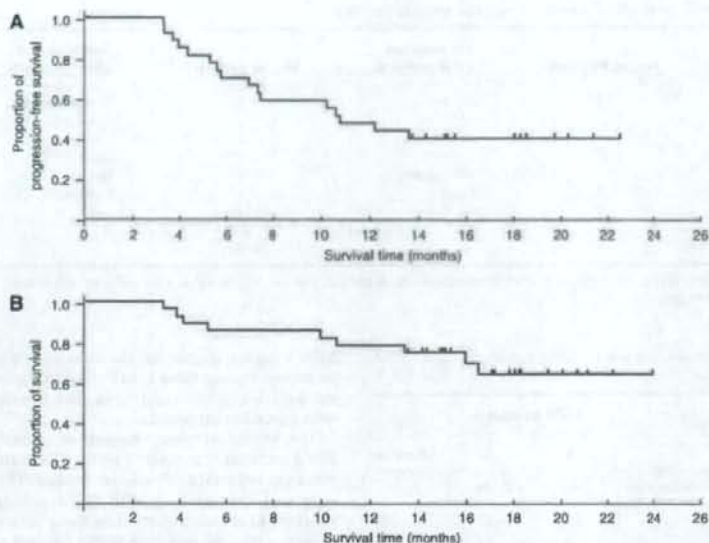
Figure I (A) Progression-free survival (PFS) and (B) overall survival (OS) of all eligible patients (n = 28). The median PFS was 11.5 months. The median OS has not yet been reached. The 1-year survival rate was 79%.

Table 5 Common adverse events (n = 28)

Adverse events	No. of patients (%)			
	Grade 1	Grade 2	Grade 3	Grade 4
Haematologic				
Anaemia	12 (43)	3 (11)	0 (0)	0 (0)
Leucopaenia	4 (14)	1 (4)	2 (7)	0 (0)
Neutropaenia	4 (14)	1 (4)	1 (4)	0 (0)
Thrombocytopaenia	3 (11)	0 (0)	0 (0)	0 (0)
Nonhaematologic				
Rash	10 (36)	11 (39)	2 (7)	0 (0)
Dry skin	9 (32)	10 (36)	0 (0)	0 (0)
Nail changes	5 (18)	2 (7)	0 (0)	0 (0)
Keratitis	0 (0)	0 (0)	1 (4)	0 (0)
Fever	0 (0)	1 (4)	0 (0)	0 (0)
Fatigue	3 (10)	3 (10)	3 (10)	0 (0)
Diarrhoea	7 (25)	1 (4)	0 (0)	0 (0)
Constipation	1 (4)	0 (0)	0 (0)	0 (0)
Stomatitis	8 (29)	1 (4)	0 (0)	0 (0)
Gastritis	1 (4)	0 (0)	0 (0)	0 (0)
Anorexia	2 (7)	1 (4)	0 (0)	0 (0)
Nausea	3 (11)	1 (4)	0 (0)	0 (0)
Vomiting	2 (7)	2 (7)	1 (4)	0 (0)
Dyspnoea	2 (7)	0 (0)	1 (4)	0 (0)
ILD	2 (7)	0 (0)	0 (0)	1 (4)ᵃ
Vertigo	1 (4)	1 (4)	0 (0)	0 (0)
Dysgeusia	0 (1)	1 (4)	0 (0)	0 (0)
Elevated AST/ALT	10 (36)	2 (7)	4 (14)	1 (4)ᵃ
Elevated creatinine	2 (7)	1 (4)	2 (7)	0 (0)

ALT = alanine transaminase; AST = aspartate transaminase; ILD = interstitial lung disease. ᵃSame patient.

could occur. Yatabe *et al* (2006) has developed a rapid assay to detect *EGFR* mutations, and we have decided to use this assay in a phase III trial. The *EGFR* mutation rates in transbronchial biopsy samples were found to be the same as those in surgical specimens, suggesting that this assay can also accommodate stage IV NSCLC. We detected the two characteristic types of *EGFR* mutations (in exons 19 and 21) in 44 and 56% of the patients, respectively (Table 1); these percentages are identical to those in previous reports from Japan (Shigematsu *et al*, 2005; Asahina *et al*, 2006; Inoue *et al*, 2006; Yatabe *et al*, 2006; Yoshida *et al*, 2007). In summary, we confirmed the feasibility of using the *EGFR* detection assay in daily practice.

The overall response rate was 75%, which was comparable to those of other phase II studies of gefitinib in patients with *EGFR* mutations (Asahina *et al*, 2006; Inoue *et al*, 2006), despite our study permitting the entry of patients who had previously received up to two chemotherapy regimens. The DCR of 96% was relatively high, and the median PFS of 11.5 months and 1Y-S of 79% were also very promising. In a Korean study, Lee *et al* (2006) also reported a very promising response rate (56%) and 1Y-S (76%) for gefitinib in a prospective study of selected NSCLC patients with adenocarcinoma and never/light smokers, defined as having smoked no more than 100 cigarettes during one's lifetime. In the screening process for the present study, *EGFR* mutations were significantly more frequent in women, patients with adenocarcinoma and those who had never smoked. However, among the patients who were selected according to their *EGFR* mutation status, no differences in response were observed between never smokers and current/former smokers or between chemotherapy-naive and postchemotherapy patients. In a retrospective study, Han *et al* (2006) directly compared clinical predictors (smoking history, gender and histology) and the *EGFR* mutation status for their ability to predict response and survival. They showed that female never smokers with adenocarcinoma (three clinical predictors) had a 33% response rate, whereas patients with a positive *EGFR* mutation status had a 62% response rate. Furthermore, in a multivariate analysis, only a positive *EGFR* mutation status was associated with an improved OS, suggesting that the *EGFR* mutation status should be analysed whenever possible to optimise response predictions based on clinical

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Table 6 Subsequent treatments after failure to respond to gefitinib (n = 28)

Gefitinib treatment	No. of Patients	1st regimen after gefitinib	No. of patients	2nd regimen after gefitinib	No. of patients
1st line	17	Plt doublet	5	Gem or Doce	2
				Gefitinib[a]	1
		VNR	1	—	-
2nd line[b]	4	Doce	2	Doce	1
		Plt doublet	1	Doce	1
2nd line	5	Doce	1	Gefitinib[a]	1
3rd line	2	—		—	—
Total	28		10		
Response			4/10		2/6

Doce = docetaxel; Gem = gemcitabine; Plt = platinum; VNR = vinorelbine. [a]Both patients had an SD response after gefitinib re-treatment. [b]First regimen as systemic chemotherapy after adjuvant treatment.

Table 7 Bronchial alveolar carcinoma (BAC) features and *EGFR* mutation status

	EGFR mutation		
	+	−	P-value
Surgically resected adenocarcinoma case	12	24	
BAC component			
Yes	8	17	1.0
No	4	7	
Micropapillary pattern			
Yes	4	12	0.48
No	8	12	
Mucin production			
Yes	1	5	1.0
No	11	19	

EGFR = epidermal growth factor receptor.

background factors. In the present study, *EGFR* mutations were detected in 16 out of 40 (40%) female never smokers with adenocarcinoma who underwent the screening process, and 14 out of these 16 patients (88%) achieved a response after undergoing gefitinib therapy. We could not compare the predictive powers of clinical predictors and the *EGFR* mutation status with regard to the clinical benefits of gefitinib in this study. Thus, the need for *EGFR* mutation testing among clinically favourable patients remains uncertain. Decisions regarding the first-line therapy of choice for patients with *EGFR* mutations or a clinically favourable profile (nonsmoker with adenocarcinoma) must also await the results of an ongoing randomised phase III study in an Asian population (IPASS: Iressa Pan-Asian Study) comparing platinum doublets with gefitinib.

In contrast, 50% of the men, 67% of the smokers and 63% of the men who were smokers achieved a PR in this study. Furthermore, one female nonsmoker with squamous cell carcinoma also responded to gefitinib. The histological type of this tumour was reassigned by a pulmonary pathologist, and the tumour was finally confirmed to be a squamous cell carcinoma. Squamous cell carcinoma harbouring an *EGFR* mutation is rarely seen but has been previously reported (Asahina et al, 2006). In a Japanese phase II trial of gefitinib for unselected chemotherapy-naive patients (Niho et al, 2006), the response rates among smokers, men, and patients with nonadenocarcinoma were 19, 13 and 10%, respectively. Thus, NSCLC patients who are either smokers, men or have a nonadenocarcinoma histology are unlikely to receive gefitinib treatment as a first-line treatment instead of standard chemotherapies (platinum doublets), which yield a response rate of about 30% (Schiller et al, 2002). Therefore, *EGFR* mutation screening may

have a higher impact on the selection of responders to gefitinib treatment among these kinds of Asian patient subset (for example, smokers with adenocarcinoma, and nonsmoking men or women with nonadenocarcinoma).

The benefit of chemotherapy in general among patients with *EGFR* mutations, compared with *EGFR* mutation-negative patients, remains uncertain. Previous studies (Bell et al, 2005) have suggested that patients with *EGFR* mutations tend to be more sensitive to chemotherapy than those with wild-type *EGFR*. In the present study, 40 and 33% of the patients responded to first- and second-line chemotherapy regimens after gefitinib, respectively. These relatively high response rates for refractory NSCLC suggest that patients with an *EGFR* mutation-positive status are generally sensitive to chemotherapy. Large-scale multivariate analyses, using pooled data from prospective phase II or III trials in which the *EGFR* mutation status was clearly confirmed, are needed to clarify this point.

The toxicities observed in the present study were mostly tolerable. Most of the common adverse events, like rash, diarrhoea or hepatotoxicity, were mild and subsided after gefitinib administration was paused for a short period. One male smoker with adenocarcinoma died of ILD. Thus, even among patients who are selected based on their *EGFR* mutation status, men or smokers may still be at risk for developing ILD; therefore, biomarkers to predict ILD are needed.

Patients with exon 19 mutations tended to have a higher response rate than those with a missense mutation in exon 21, consistent with the findings of previous reports (Jackman et al, 2006; Riely et al, 2006). The Spanish Lung Cancer Group also reported on a prospective phase II study of erlotinib in advanced NSCLC patients with *EGFR* mutations (Paz-Ares et al, 2006). The overall response rate was 82%. They also showed a difference in response rates between patients with mutations in exons 19 and 21 (95 and 67%, respectively). Exon 11 c-kit mutations are more closely correlated with a good prognosis in patients with gastrointestinal stromal tumour, who may benefit from lower doses of imatinib, whereas patients with exon 9 mutations may require higher doses (Debiec-Rychter et al, 2006). In the case of *EGFR*, functional differences between mutation types may also exist.

We found no discernible associations between the *EGFR* mutation frequency and the presence of a BAC component. Several reports, including that of Hirsch et al (2005) suggest that a higher *EGFR* copy number is correlated with BAC histological features. We also found an association between a high *EGFR* copy number and the presence of a BAC component, even though the number of specimens examined was relatively small. In a study on erlotinib, the presence of a BAC component was clearly associated with *EGFR* amplification. As the *EGFR* mutation rate is lower in western populations than in Asian populations, the *EGFR* gene copy number might be a more useful biomarker in western populations, especially with regard to the use of erlotinib.

In conclusion, gefitinib treatment for patients with advanced NSCLC harbouring an *EGFR* mutation demonstrated a promising activity in patients with a good performance status. Patient screening according to *EGFR* mutation status may be a useful tool in daily practice and will likely have a great impact on the selection of patients who are likely to benefit from gefitinib treatment.

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Clinical Studies

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MURINE PULMONARY RESPONSE TO CHRONIC HYPOXIA IS STRAIN SPECIFIC

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☐ *Information concerning the effects of genetic variation between different background strains on hemodynamic, morphometric, and gene expression response to hypoxia would be useful. Three strains of mice were kept in hypoxia and phenotyped followed by gene profiling analysis. Among the variables examined, hematocrit, right heart muscularization, and right ventricular systolic pressure showed a strain-specific effect. Increased gene expression of inflammatory, muscle, and angiogenesis genes were seen in all strains, though the specific genes changed varied among groups. These results suggest that different strains use different gene expression mechanisms to adapt to the challenge of chronic hypoxia, resulting in modified phenotypic changes.*

Keywords   gene expression, hypoxia pulmonary circulation, strain difference,

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Chronic hypoxia as a contributing factor to pulmonary arterial hypertension (PAH) is associated with a variety of human diseases. Chronic obstructive pulmonary disease [1], cystic fibrosis [2], interstitial fibrosis [3], upper airway obstruction, and bronchopulmonary dysplasia [4], among others, are all associated with exposure to chronic hypoxia. Understanding the effects of and potential treatments for chronic hypoxia is thus relevant to many human disorders [5].

Hypoxic pulmonary hypertension is often studied in mouse models [6, 7]. These studies, examining molecular mechanisms or interventions, use many of the same phenotypic markers of PAH. Standard measurements include right ventricular systolic pressure (RVSP), cardiac output, right ventricular hypertrophy, muscularization of small pulmonary vessels, counts of alveoli, hematocrit, and blood gas measurements. Together, these are used to both gauge extent of PAH, as well as physiologic response to it.

Three of the most widely used experimental mouse strains are FVB/N, C57BL/6, and SV129. The goal of our current study was to determine whether these 3 strains had a differential response to chronic hypoxia according to the metrics listed above, and if so, whether this could be correlated with a differential response by gene expression. This would be useful to the mouse modeling community in understanding the differences among strains in previous studies of hypoxic pulmonary hypertension and in planning future studies. Moreover, strain-specific differences in gene expression may suggest modifier genes if correlated to a less severe phenotype.

We measured 16 different phenotypic variables in each of the 3 strains, under either normoxia or 4 weeks' hypobaric hypoxia. Although most variables had significant changes with hypoxia, and some had significant differences at baseline, only 3 had significant differences in hypoxic response among strains. SV129 had greater increase in RVSP with hypoxia, FVB/N had less right ventricular hypertrophy, and C57BL/6 had less of an increase in hematocrit, although from a higher baseline.

By gene array, we found that all of the strains had changes in similar pathways, including muscle, angiogenesis, growth, adhesion, G-protein, and differentiation pathways, and that all strains had changes in some common genes within these pathways, but that each strain also had a large number of changed genes within these pathways that were strain specific.

METHODS

Animals

FVB/NJ, C57BL/6J, or 129X1/SvJ mice, 6 to 8 weeks old, were ordered from The Jackson Laboratory (Bar Harbor, MA) and allowed to acclimatize for 1 week to Denver altitude. Fifteen mice from each strain were kept at either Denver altitude (~85 kPa) or at 50 kPa in hypobaric hypoxia

chambers. After 4 weeks, animals were phenotyped by echocardiography, followed by RVSP measurement and blood and tissue collection. All animal studies were preapproved by the University of Colorado Health Sciences Center (UCHSC) Institutional Animal Care and Use Committee (IACUC).

Echocardiographic Measurements

Transthoracic echocardiography was performed using 10- and 13-MHz ultrasound probes with a Vivid Five System (General Electrics Vingmed Ultrasound, Horton, Norway). Echocardiographic data were analyzed with EchoPac 6.3.6 software (General Electrics Vingmed Ultrasound). Left heart dimensions were obtained in short-axis view. The diameters of the aorta and pulmonary artery as well as flow in the pulmonary artery were obtained in parasternal longitudinal axis. The flow in the aorta was measured using a suprasternal longitudinal view. Cardiac output and pulmonary vascular resistance was calculated in standardized fashion [8–10]. All echocardiographic measurements were performed in triplicate by one investigator and averaged.

RVSP Measurements

Animals were anesthetized with intraperitoneal injections of ketamine 200 mg/kg and xylazine 10 mg/kg. If further anesthesia was necessary, repeat doses of ketamine 100 mg/kg and xylazine 5 mg/kg were administered. Studies were conducted with mice positioned supine on a heated operating table while spontaneously breathing room air. Right ventricular (RV) pressure was directly measured with a 1.4-French Pressure Volume Conductance System SPR-839 (Millar Instruments, Houston, TX) inserted into the right ventricle via the surgically exposed right jugular vein. Hemodynamics were continuously recorded with a Millar MPVS-300 unit coupled to a Powerlab 8-SP A/D converter, acquired at 1000 Hz, and captured to a Macintosh G4 computer utilizing Chart5.3 software. Blood was drawn for gas measurements by cardiac puncture, and immediately transferred to a Radiometer Medical (Copenhagan, Denmark) ABL5 blood gas analyzer. After lethal injection of pentobarbital, the heart and lungs were removed. Lungs were divided and processed for immunohistochemistry or molecular studies.

Morphometry

Tissue blocks were made from the left lungs. The embedded tissue was cut at 4 μm on an AO 820 microtome (American Optical, Southbridge, MA) and placed onto Superfrost Plus slides (Fisher Scientific, Pittsburgh, PA). Slides were baked for 1 hour at 60°C and then deparaffinized through

Citrisolv (Fisher Scientific, Pittsburgh, PA) and a graded alcohol series. Antigen retrieval was then performed by heating the slides 4×3 minutes at 30% power in a 1650-watt microwave in a solution containing 16 mM sodium citrate–trisodium and 4 mM citric acid, pH 5.6. Slides were then rehydrated in phosphate-buffered saline (PBS) and incubated in a blocking solution of PBS/4% serum/0.1% Triton X-100 for 30 minutes and then in primary antibody overnight at 4°C in PBS/4% serum/0.1% Triton X-100 (PBS). Polyclonal anti-smooth muscle actin was purchased from Abcam (Cambridge, MA) and used at a dilution of 1:1000.

Images from 12 random 20× fields per slide were taken using a Zeiss Axioscop II microscope, and medial thickness, fully muscularized (FM) vessels of $<100~\mu$m diameter, partially muscularized (PM vessels) of $<100~\mu$m diameter, and alveoli per field were counted in a blinded fashion using tools included in the Axioscop software.

Affymetrix Arrays

Samples were prepared for Affymetrix arrays using 2.5 μg of total RNA. First- and second-strand complimentary DNA was synthesized using standard techniques. Biotin-labeled antisense complimentary RNA was produced by an in vitro transcription reaction. Mouse Genome 430 2.0 microarrays (Affymetrix, Foster City, CA) were hybridized with 20 μg cRNA. Target hybridization, washing, staining, and scanning probe arrays were done following an Affymetrix Gene Chip Expression Analysis Manual. All array results have been submitted to the National Center for Bioinformatics (NCBI) gene expression and hybridization array data repository (GEO; http://www.ncbi.nlm.nih.gov/geo/), as series GSE7823.

Array Analysis

Affymetrix Cel files were loaded into dChip 2005 array analysis software. The dChip algorithm is capable of detecting significant differences at signal strengths lower than those usable in Microarray Suite(11) (Affymetrix, Santa Clara, CA). Overall signal strength from arrays was normalized to the median array, and expression levels determined using the perfect match/mismatch (PM/MM) algorithm. Gene ontology was determined using the Classify Genes tool within dChip(12), with gene ontology files down loaded from the Gene Ontology Consortium (www.geneontology.org)(13). In order to avoid problems with either false negatives, or with determining an arbitrary fold-change cutoff, we set very loose definitions for changed genes (1.4×, with a minimum change of 200) and then determined statistically overrepresented gene ontology groups at a high stringency ($p < .001$) within the genes called as differentially regulated. By this method, the number of gene ontology groups produced by chance should be close to zero.

Other specific details of analytic methods are included in Results. Statistics for array analysis were handled by algorithms internal to dChip.

RESULTS

Murine Phenotypic Response to Chronic Hypoxia Is Strain Specific

Fifteen animals per group of each of FVB/N, SV129, or C57BL/6 were subjected to either hypobaric hypoxia (50 kPa) or normoxia (85 kPa). After four weeks, mice were weighed, and examined by echocardiography. Echocardiography was used to determine the diameters of the aorta and pulmonary artery (PA), measure cardiac output (CO), and calculate pulmonary vascular resistance (PVR). Next, closed-chested catheterization by pressure transducer was used to determine right ventricular systolic pressure (RVSP) and heart rate under anesthesia. Blood was drawn and immediately analyzed for hematocrit, pH, pCO_2, and PO_2. Hearts were dissected and weighed to determine right ventricular muscularization (RV/LV+S). Finally, lung sections were fixed and analyzed by immunohistochemistry for numbers of alveoli and vessel muscularization. Total numbers of mice used for each measurement varied between 6 and 15, with average and median of ~11. Results are summarized in Table 1 and Figure 1.

We found that 5 of these 16 metrics were different between strains in baseline animals at $P < .05$ by 2-way analysis of variance (ANOVA), 11 out of 16 were changed by hypoxia at $P < .05$, but only 3, hematocrit, RVSP, and RV/LV+S, showed a differential response to hypoxia by strain. Hematocrit was less increased in C57BL/6 mice, although starting from a higher baseline (Figure 1A); RVSP was more increased in SV129 mice (Figure 1B); right heart muscularization was less pronounced in FVB/N mice (Figure 1C).

Murine Transcriptional Response to Chronic Hypoxia Is Strain Specific

In order to examine transcriptional response to chronic hypoxia, Affymetrix arrays were probed with RNA from whole lung from hypoxic or normoxic FVB/N, SV129, or C57BL/6 mice. Array data were analyzed by dChip software and sorted into gene ontology groups. We found that gene expression changes in all strains fell into the same categories, and many of the same genes were differentially regulated in the same direction in all strains. However, some transcriptional changes were specific to a particular strain, or 2 of the 3 strains.

All strains had changes in muscle, angiogenesis, growth, adhesion, G-protein, differentiation, apoptosis, and stress-response genes. For most of these, there were many genes in common across strains (Table 2). However,

TABLE 1 Measurements ± SEM

		Echocardiogram				Blood gas		
	Pressure	Aorta (mm)	PA diameter	CO (mL/mm)	PVR (calculated)	pH	pCO_2	pO_2
C57BL/6	85 kPa	1.03 ± 0.03⁵	1.08 ± 0.05	6.8 ± 1.0‡	0.89 ± 0.23	7.38 ± 0.05	36 ± 3	70 ± 2⁵
	50 kPa	0.96 ± 0.03⁵*	1.01 ± 0.02*	6.8 ± 0.5	1.47 ± 0.16	7.37 ± 0.08	46 ± 3*	52 ± 3*
FVB/N	85 kPa	0.94 ± 0.02	1.09 ± 0.04	8.8 ± 0.8	0.91 ± 0.08	7.27 ± 0.05	39+2	76 ± 2
	50 kPa	0.90 ± 0.02*	1.01 ± 0.03*	5.7 ± 0.6‡	2.02 ± 0.19*	7.25 ± 0.09	41 ± 2	59 ± 2*
SV129	85 kPa	0.96 ± 0.02	1.04 ± 0.04	8.9 ± 0.9	0.79 ± 0.08	7.26 ± 0.05	38 ± 4	77 ± 2
	50 kPa	0.92 ± 0.02*	1.00 ± 0.03*	8.1 ± 0.7	1.73 ± 0.31	7.22 ± 0.08	43 ± 3	63 ± 3*

				Morphometry			
	Presssure	Body mass	Heart rate	FM/ field	PM/ field	Alveoli/ field	Medial thickness
C57BL/6	85 kPa	23.9 ± 0.3	184 ± 22	1.0 ± 0.2	5.5 ± 0.5	159 ± 9	9.3 ± 1.5
	50 kPa	22.0 ± 0.3*	196 ± 9	3.1 ± 0.3*	6.5 ± 0.6*	176 ± 8	9.6 ± 1.0
FVB/N	85 kPa	24.6 ± 0.3	217 ± 11	1.4 ± 0.4	3.9 ± 0.5	156 ± 5	7.6 ± 1.3
	50 kPa	23.4 ± 0.5*	174 ± 12	2.8 ± 0.6*	6.1 ± 0.6*	176 ± 6	9.2 ± 0.8
SV129	85 kPa	24.0 ± 0.4	195 ± 14	1.2 ± 0.2	5.4 ± 0.6	185 ± 10	8.7 ± 1.3
	50 kPa	21.3 ± 0.4*	189 ± 9	2.2 ± 0.3*	5.1 ± 0.5	169 ± 8	7.9 ± 0.5

All p values are for 2-way ANOVA with Fisher's poet hoc for differences caused by strain or hypoxia.

⁵$p < .01$ for difference compared across strains.

*$p < .01$ for differences caused by hypoxia, except pCO_2 ($p = .029$), PM ($p = .047$), PA diameter ($p = .029$).

‡0.94 for strain-specific and $p = .057$ for pressure specific differences in CO.

in the case of stress response and apoptosis, each strain activates different sets of genes (Table 3).

DISCUSSION

The goal of this study was to determine mouse strain-specific differences in phenotype and gene expression in response to chronic hypoxia. As a secondary benefit, we believe we have produced a more complete characterization of murine response to hypoxia than was previously available. Most of the changes to hypoxia we saw have been found in many previous studies: decreased body mass [14], increased hematocrit [14], increased RVSP [15], right heart and pulmonary vessel muscularization [15], and pulmonary vascular resistance [15]. Decreased aortic and pulmonary artery diameters are similar to results found in humans in the Operation Everest III study [16]. Because the mice were at room air for approximately half an hour prior to phenotyping, the increased pCO_2 and decreased pO_2 in chronically hypoxic animals likely resulted from ventilation-perfusion mismatch.

There were interesting baseline differences between strains, as well. We found that C57BL/6 mice had higher baseline hematocrit but lower baseline RVSP and right heart weight than the other strains, with a slightly
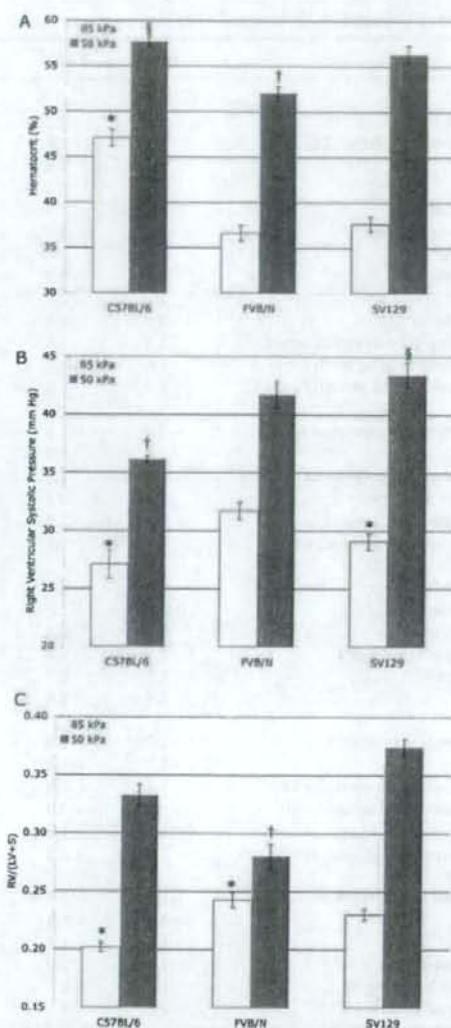
FIGURE 1 Hematocrit (*A*), RVSP (*B*), and RV/LV+S (*C*) all have significant differences dependent on pressure, strain, and pressure-dependent on strain by 2-way ANOVA. Differences in individual values were determined using Tukey's post hoc test. All values at 50 kPa are significantly different (*p* < .05) than the within-strain 85 kPa value. (*A*) Hematocrit in C57BL/6 is higher at 85 kPa than other strains (*) but increases less with 50 kPa hypoxia (§). Hematocrit in FVB/N at 50 kPa is less than in other strains (†). (*B*) RVSP at 85 kPA in C57BL/6 is significantly lower than in SV129 (*), but neither are different than FVB/N. RVSP at 85 kPa in C57BL/6 is lower than other strains (†), and increase in RVSP between 85 and 50 kPa is greater in SV129 than in other strains (§). (*C*) Right heart muscularization is lower at 85 kPa in C57BL/6 than FVB/N mice but neither is different than SV129. RV/LV+S is different at 50 kPa in all strains, and increase in RV/LV+S between pressures is lower in FVB/N than in other strains (†).

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TABLE 2 Genes Changed In All Strains (Examples)

	BL6	FVBN	SV129
Muscle			
caldesmon 1	1.7	2.3	2.1
myocyte enhancer factor 2C	1.7	2.6	2.6
myosin X	1.7	2.4	1.7
restin	1.6	3.9	2.4
tropomodulin 2	1.6	2.4	2.4
tropomyosin 1, alpha	2.1	3.4	2
Angiogenesis			
angiopoietin 1	1.6	2.5	1.6
quaking	2	2.6	1.4
semaphorin 5A	2	2.1	1.7
transforming growth factor, beta 2	1.4	1.5	1.5
vascular endothelial growth factor A	1.5	1.5	1.4
vascular endothelial growth factor C	1.4	2.3	1.4
Growth			
cell division cycle associated 5	−1.8	−3.2	−1.7
ephrin B1	1.8	2.7	1.6
growth associated protein 43	2.4	2.2	1.8
large tumor suppressor 2	2.2	2.2	2.4
RAD21 homolog	−3.3	−2.2	−2.3
tumor necrosis factor receptor 5	1.7	3.1	1.6
Adhesion			
integrin alpha 8	1.4	2.5	1.4
integrin beta 6	3.2	1.8	2.4
integrin, beta-like 1	1.3	2.3	1.8
LIM and senescent cell 1	1.9	2.7	2.2
nephronectin	1.7	2.3	2.4
nidogen 1	1.4	2.8	1.5
G-Protein			
A kinase anchor protein 9	1.7	2.8	2.9
calmodulin 1	−1.7	−1.4	−1.4
guanine nucleotide bp alpha 13	1.8	1.8	1.4
guanine nucleotide bp alpha inh. 3	−3.9	−2.8	−2.9
phosphodiesterase 7A	1.6	1.8	1.4
protein kinase, cAMP dep, catalytic, b	1.8	2.2	1.4
Development			
GLI-Kruppel family member GLI2	−1.5	−1.5	−1.2
jagged 1	−3.1	−2.9	−1.9
MAP3K10	−1.5	−1.9	−1.4
muscleblind-like 3	1.5	1.9	1.6
Notch gene homolog 3	1.4	2	1.9
transducer of ErbB-2.1	−1.8	−3	−1.4

Note. Number is fold change from control.

larger aortic diameter. Because the study was performed at Denver ambient pressure (85 kPa), it is unclear whether any of these are an early response to mild hypoxia.

Only 3 of our metrics had a differential response to hypoxia by strain. Hematocrit had a smaller increase in C57BL/6 than the other strains, but to approximately the same final value. RVSP was increased to a greater extent

TABLE 3 Genes Changed Differentially Between Strains

	BL6	FVBN	SV129
Development			
BMP2	1.4	2	—
BMPR2	−1.4	2	—
forkhead box O3a	−1.6	—	−1.9
Kruppel-like factor 9	−2.3	—	—
SMAD1	−1.5	—	−1.4
spondin 2	5.3	1.8	—
Muscle			
adrenergic receptor, alpha 1a	2.4	—	1.6
chloride channel 3	—	−2.8	—
chloride channel 5	—	−4.2	—
myosin IB	—	2.1	—
myosin IC	—	2	—
phospholipase C, beta 4	−2.2	—	−1.5
Stress			
chemokine CXCL12	2.1	—	1.6
coagulation factor III	−2.4	—	−1.4
Fibronectin 1	—	1.4	1.5
heat shock protein 1B	3.3	1.4	−1.7
S100 calcium binding A8	−1.6	—	−2.9
sialophorin	—	—	−1.7
Growth			
c-fos induced growth factor	2.3	—	2.7
cyclin D2	—	2.8	1.4
cyclin T2	—	1.8	—
cysteine rich protein 61	—	−3.2	—
retinoblastoma-like 2	−1.9	—	−1.4
Zinc finger and BTB domain 16	−1.8	−1.7	−2.4
Apoptosis			
caspase recruitment domain 4	—	1.6	—
cytochrome c, somatic	—	−4.7	—
deoxyribonuclease II alpha	−1.9	−6.5	−1.7
phosphatidylinositol 3-kinase, a	−2	—	−2.3
programmed cell death 8	—	−3.6	—
TSC22 domain family 3	−2.2	—	−1.9

in SV129 than the other strains, by ∼14 mm Hg instead of 9 to 10 mm Hg in C57BL/6 and FVB/N mice. FVB/N mice showed less right heart muscularization than the other strains, with only a 20% increase in RV/LV+S, instead of the ∼60% increase seen in C57BL/6 and SV129.

Gene expression changes also generally followed expectation, with increased expression of muscle, angiogenesis, and adhesion-related genes, and differential regulation of growth, differentiation, G-protein, stress, and apoptosis genes. All of these are pathways long associated with hypoxic response[17–19].

The most interesting finding of the array data was that, whereas the overall pathways were the same, the specific genes dysregulated were strain specific. This suggests that it is possible to accomplish the same phenotypic

ends with different molecular means, and indeed that different strains use different genes to do so.

Although ours is the first study to reach this conclusion in comparisons among strains in the pulmonary vasculature of mice, similar results have been obtained in studies of the systemic vasculature and in comparisons between mice and rats.

In the systemic vasculature, Korshunov and Berk showed the magnitude of remodeling of the carotid artery after partial ligation is significantly different among the mouse strains using C3H/HeJ, SJL/J, DBA/2J, C57Bl/6J, and FVB/NJ strains. Their study suggested fundamental alterations in sensing or transducing hemodynamic signals among strains[20]. Similarly, Ward and colleagues showed that cerebral expression of the angiogenic factors, such as vascular endothelial growth factor (VEGF), angiopoietin (Ang)-1 and -2, are different in response to long-term hypoxia, and are variable among frequently used mouse strains, including CD1, C57Bl/6,129/SV, and Balb/c [21]. Both of these studies show that, in other organs, different mouse strains have similar but unique responses to hemodynamic stress.

Hoshikawa and colleagues compared some hemodynamic markers and gene expression between C57Bl6 mice and Sprague-Dawley rats [17]. They reported that mice showed less vascular remodeling than rat, though both demonstrated pulmonary hypertension. Although both had alterations in cell cycle and vasodilation-related genes, the precise changes were unique to each species.

Thus, the literature supports the hypothesis that although in broad terms the morphologic changes and molecular pathways involved in response to vascular stress are conserved, genotypic background determines the precise mechanisms used in this response. Because humans all have different genotypic background, human response to vascular stress is also likely to conserve pathways, but with different specific genes used depending on the individual genetic background, with implications for both modifier gene searches and the practice of molecular medicine.

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