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(Received February 7, 2012; Revised February 20, 2012; Accepted March 2, 2012)

J Anesth (2012) 26:711–720
DOI 10.1007/s00540-012-1397-3

ORIGINAL ARTICLE

Impact of remifentanil use on early postoperative outcomes following brain tumor resection or rectal cancer surgery

Kanji Uchida · Hideo Yasunaga · Hiroaki Miyata · Masahiko Sumitani · Hiromasa Horiguchi · Shinya Matsuda · Yoshitsugu Yamada

Received: 15 October 2011 / Accepted: 16 April 2012 / Published online: 4 May 2012

Abstract
Purpose Remifentanil, a mu-opioid receptor agonist, has important characteristics for neuroanesthesia, but data about its effects on postoperative recovery and mortality are currently lacking.
Methods Using the Japanese Diagnosis Procedure Combination database in 2007, we selected patients who underwent elective brain tumor resection with open craniotomy under general anesthesia using either remifentanil or fentanyl and divided them into two categories: remifentanil patients and non-remifentanil patients. After propensity score matching for potential confounders, we compared the in-hospital mortality and postoperative length of stay (LOS) between the two groups. For comparison, the same endpoints were evaluated for patients underwent rectal cancer surgery under general anesthesia with intraoperative epidural anesthesia.
Results In patients who underwent brain tumor resection (936 pairs), remifentanil patients had significantly lower in-hospital mortality (1.5 % vs. 3.0 %; $P = 0.029$). Logistic regression analysis revealed that the odds ratio for use of remifentanil for in-hospital mortality was 0.47 (95 % confidence interval, 0.25–0.91; $P = 0.025$). Remifentanil patients also showed earlier discharge from hospital (median LOS, 17 vs. 19 days; hazard ratio, 1.19, 95 % confidence interval, 1.08–1.30; $P < 0.001$). In contrast, in 2,756 pairs of patients undergoing rectal cancer surgery, no significant difference was seen in either in-hospital morality (1.2 % vs. 1.3 %; $P = 0.518$) or median LOS (19 vs. 19 days; $P = 0.148$) between the two groups.
Conclusions Our data suggest a possible association between use of remifentanil and better early postoperative recovery for patients undergoing neurosurgery with craniotomy. Further studies, including a randomized controlled trial, are required to confirm the present results.

Keywords Remifentanil · Brain neoplasm · Neurosurgery · Postoperative outcome

K. Uchida (✉) · M. Sumitani · Y. Yamada
Department of Anesthesiology, Tokyo University Graduate School of Medicine, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-8655, Japan
e-mail: uchidak-ane@h.u-tokyo.ac.jp

H. Yasunaga · H. Horiguchi
Department of Health Management and Policy, Tokyo University Graduate School of Medicine, Tokyo, Japan

H. Miyata
Department of Health Quality Assessment, Tokyo University Graduate School of Medicine, Tokyo, Japan

S. Matsuda
Department of Preventive Medicine and Community Health, University of Occupational and Environmental Health, Kitakyushu, Japan

Introduction

Remifentanil, a mu-opioid receptor agonist, has a unique pharmacokinetic profile characterized by rapid equilibration with the central compartment and a short half-life that is independent of infusion duration [1, 2]. Although the use of remifentanil is common in Western countries [3], it was only approved in Japan in December 2006, and clinical use commenced in January 2007. We previously evaluated the population who received remifentanil during general anesthesia in 2007 using a nationwide administrative database in Japan and found that remifentanil was used in more than 40 % of all general anesthesia [4]. Patients with preoperative comorbidities including diabetes mellitus, hypertension, liver cirrhosis, and chronic renal failure were positively associated, whereas those with cardiac disease and co-application of epidural anesthesia were negatively associated, with the use of remifentanil [4]. The pharmacokinetics of remifentanil allows easy titration against changing intraoperative conditions as well as quick and predictable emergence from anesthesia without prolonged respiratory depression [5, 6]. These characteristics are especially important in neuroanesthesia because rapid postoperative recovery is essential for assessment of neurological function, making remifentanil a potentially ideal neuroanesthetic agent [7, 8]. Indeed, our recent evaluation revealed that populations with remifentanil were exceptionally high in neurosurgery [4]. However, reports about the effects of remifentanil beyond the operating theatre, i.e., its effects on postoperative recovery and mortality, have been lacking.

In the present study, we hypothesized that general anesthesia with remifentanil is associated with better postoperative recovery, especially for neurosurgery. To confirm this hypothesis, we conducted propensity score matching analyses to compare the postoperative outcomes between remifentanil patients and non-remifentanil patients for brain tumor resection, with a retrospective survey of a large administrative claims database in Japan. To determine whether the results from patients with brain tumor are also applicable to patients with other non-cephalocervical malignancies, we selected patients undergoing rectal cancer surgery with both epidural and general anesthesia and evaluated the same endpoints.

Materials and methods

Data source

The Diagnosis Procedure Combination (DPC) database is a patient classification system that is similar to the diagnosis-related groups used by Medicare in the United States. In 2002, the Ministry of Health, Labour and Welfare of Japan launched this case-mix system, and linked it with a lump-sum payment system. All 82 university teaching hospitals are obliged to adopt the DPC system; community hospitals can adopt it voluntarily. A survey of the DPC hospitals is conducted between July 1 and December 31 of each year by the DPC Research Group, funded by the Ministry of Health, Labour and Welfare [9–11]. Not only administrative claims data, but also detailed patient data, are collected for all inpatients discharged from the participating hospitals. In 2007, the number of participating hospitals was 926, and the number of patients included was 3 million, representing approximately 45 % of all inpatient admissions to acute care hospitals in Japan.

The database includes the following data: hospital locations; patients' age and sex; diagnoses, comorbidities at admission, and complications after admission recorded using text data in the Japanese language and the International Classification of Diseases, 10th Revision codes; procedures coded using Japanese original codes; anesthesia duration (min); dates when each drug was used; and lengths of stay (LOS) and discharge statuses. Information on the level of consciousness at admission is recorded for all patients and evaluated using the Japan Coma Scale (JCS). The JCS, which is based on the degree of arousal, is widely used by Japanese clinical facilities, including emergency services, for assessment of the consciousness level. The JCS and Glasgow Coma Scale assessments are well correlated [12].

This study was based on a secondary analysis of the administrative claims data. Given the anonymous nature of the data, the need for informed consent was waived. Study approval was obtained from the Institutional Review Board of the University of Occupational and Environmental Health (Kitakyushu, Fukuoka, Japan).

Patient selection

From the 3 million inpatients recorded between July 1 and December 31 in 2007, we selected patients who underwent elective brain tumor resection with open craniotomy under general anesthesia or rectal cancer surgery under general anesthesia accompanied with epidural anesthesia. In this study, we only included patients whose consciousness level at admission was "alert" (JCS $= 0$) and excluded patients with consciousness disorders (JCS ≥ 1) [12]. We also excluded patients with cerebrovascular diseases, chronic renal failure, or liver cirrhosis. We then selected patients who received fentanyl or remifentanil during general anesthesia and divided them into two subgroups: (a) patients who received both remifentanil and fentanyl, and (b) patients receiving fentanyl alone.

Patient background data

Patient background data that could potentially affect the study endpoints, including age, sex, and comorbidities, were assessed. The comorbidities assessed included hypertension, diabetes, chronic heart disease (ischemic heart disease, valvular heart disease, cardiomyopathy, or congenital heart disease), and chronic lung disease (emphysema, chronic bronchitis, bronchiectasis, asthma, interstitial lung disease, or pulmonary hypertension). We also verified the use of volatile anesthetic agents (sevoflurane, isoflurane, enflurane, or halothane) for each patient. We assessed the hospital inpatient volumes for brain tumor resection and rectal cancer surgery because they could potentially affect the postoperative clinical outcomes, including mortality [9]. Hospital volumes were determined by the number of brain tumor resections or rectal cancer surgeries during the study period, using the unique identifier for each hospital.

Endpoints

The primary endpoint was in-hospital mortality. Postoperative LOS was assessed as a secondary endpoint.

Statistical analysis

We used propensity score matching [13] to adjust for differences in the baseline characteristics because the patients were not randomly assigned to receive remifentanil. We performed a one-to-one matched analysis on the basis of the estimated propensity scores for each patient. The log odds of the probability that a patient received remifentanil were modeled for potential confounders including age, sex, comorbidities (hypertension, diabetes mellitus, chronic lung diseases, or cardiovascular diseases), duration of anesthesia, and hospital volumes. C-statistics were calculated to evaluate the goodness of fit. The estimated logits were compared between the remifentanil patients and non-remifentanil patients, and a "match" occurred when one patient in the remifentanil group had an estimated logit within 0.6 SD of a patient in the non-remifentanil group. If two or more patients in the remifentanil group met this criterion, we randomly selected one patient for matching.

We compared the rates of in-hospital mortality between the remifentanil group and non-remifentanil group in brain tumor surgery and rectal cancer surgery using chi-square tests. For the logistic regression analyses, we performed univariate analyses between each covariate and in-hospital mortality in the first step. Then, age, sex, remifentanil use, and other covariates with a P value <0.10 were included in the final multivariate logistic regression models. The final models also adjusted for clustering of patients within hospitals using generalized estimating equations.

We compared the discharge rates of patients between the subgroups in each covariate using the Kaplan–Meier method and log-rank tests. Cox regression analyses were performed to model the concurrent effects of various factors on discharge, where we included age, sex, remifentanil use, and other covariates with a P value <0.10 in the log-rank tests.

We presented odds ratios (OR) and 95 % confidence intervals (95 % CI) for the logistic regressions and hazard ratios (HR) and 95 % CI for the Cox regressions. For the categorical variables, the OR (or HR) for the reference subgroup was 1.00, and the OR (or HR) for each of the other subgroups was presented in comparison with the reference subgroup. The threshold for significance was a P value <0.05. All statistical analyses were conducted using IBM SPSS version 19.0 (Statistical Package for Social Sciences, Chicago, IL, USA).

Results

Of the 3 million inpatients, we identified a total of 3,550 brain tumor resections and 11,142 rectal cancer surgeries between July and December of 2007. After inclusion of patients who were administered remifentanil or fentanyl and exclusion of those with consciousness disorders, cerebrovascular diseases, chronic renal failure, or liver cirrhosis, we selected 2,830 patients who underwent brain tumor resection under general anesthesia (1,891 with both remifentanil and fentanyl and 939 with fentanyl alone) and 8,205 patients who underwent rectal cancer surgery with general and epidural anesthesia (2,778 with both remifentanil and fentanyl and 5,427 with fentanyl alone). Using one-to-one propensity score matching, we selected 936 pairs of the remifentanil group and non-remifentanil group for brain tumor resection and 2,756 pairs for rectal cancer surgery. The C-statistics were calculated to be 0.592 and 0.541 for brain tumor resection and rectal cancer surgery, respectively.

Table 1 shows the patient background data of the 1,872 selected cases from the brain tumor resection and 5,512 from the rectal cancer surgery (including 4,610 low anterior resection and 902 abdominal perineal resection), divided into remifentanil group and non-remifentanil group. There were no significant differences in the patient background data between the two groups in each surgery.

Table 1 also shows the differences in the use of volatile agents between the two groups after propensity score matching. Overall, 1,351 patients received sevoflurane and 162 received isoflurane during brain tumor resection, whereas 4,344 received sevoflurane and 108 isoflurane during rectal cancer surgery. No patients received enflurane or halothane. The percentage of remifentanil patients receiving volatile agents was significantly lower than that of non-remifentanil patients in both the brain tumor resection group (68.9 % vs. 90.0 %; $P < 0.001$) and the rectal surgery group (73.9 % vs. 87.1 %; $P < 0.001$).

With regard to in-hospital mortality, a chi-square test revealed a significant difference between the remifentanil group and non-remifentanil group (1.5 % vs. 3.0 %; $P = 0.029$) in brain tumor resection but not in rectal cancer surgery (1.2 % vs. 1.3 %; $P = 0.630$). Table 2 shows results of logistic regression analyses for in-hospital mortality following brain tumor resection. In the multivariate model, the remifentanil group showed a significantly lower mortality than the fentanyl-alone group (odds ratio, 0.47,

Table 1 Patient background and use of volatile agents

	Brain tumor resection			Rectal cancer surgery		
	Fentanyl alone (n = 936)	Fentanyl and remifentanil (n = 936)	P	Fentanyl alone (n = 2,756)	Fentanyl and remifentanil (n = 2,756)	P
Patient background						
Age (mean ± SD)	55.2 ± 18.1	55.2 ± 17.0	0.876	65.1 ± 12.6	64.9 ± 13.5	0.645
Sex (male) (n, %)	427 (45.6 %)	427 (45.6 %)	1.000	1,741 (63.2 %)	1,755 (63.7 %)	0.695
Comorbidities (n, %)						
Hypertension	118 (12.6 %)	107 (11.4 %)	0.434	329 (11.9 %)	361 (13.1 %)	0.193
Diabetes	66 (7.1 %)	71 (7.6 %)	0.657	273 (9.9 %)	295 (10.7 %)	0.330
Cardiovascular diseases	39 (4.2 %)	33 (3.5 %)	0.471	254 (9.2 %)	258 (9.4 %)	0.853
Chronic lung diseases	7 (0.7 %)	8 (0.9 %)	0.795	71 (2.6 %)	80 (2.9 %)	0.458
Duration of anesthesia (min, mean ± SD)	434 ± 193	436 ± 181	0.853	323 ± 123	321 ± 122	0.624
Hospital volume for colorectal surgery (per 6 months; mean ± SD)	19.3 ± 15.7	18.3 ± 15.3	0.164	40.0 ± 39.8	39.8 ± 39.1	0.840
Use of volatile agents						
Nitrous oxide	230 (24.6 %)	57 (6.1 %)	<0.001	351 (12.7 %)	142 (5.2 %)	<0.001
Sevoflurane	751 (80.2 %)	600 (64.1 %)	<0.001	2,341 (84.9 %)	2,003 (72.7 %)	<0.001
Isoflurane	109 (11.6 %)	53 (5.7 %)	<0.001	64 (2.3 %)	44 (1.6 %)	0.052
Either or both: sevoflurane/isoflurane	842 (90.0 %)	645 (68.9 %)	<0.001	2,401 (87.1 %)	2,038 (73.9 %)	<0.001
Propofol	702 (75.0 %)	826 (88.2 %)	<0.001	2,158 (78.3 %)	2,462 (89.3 %)	<0.001

95 % CI, 0.25–0.91; $P = 0.025$). Older age was significantly associated with higher in-hospital mortality. Duration of anesthesia was not a significant predictor of in-hospital mortality. Other anesthetic agents including nitrous oxide, isoflurane, sevoflurane, or propofol were not significantly associated with in-hospital mortality.

The chi-square test showed no significant difference in in-hospital mortality following colorectal cancer surgery between the remifentanil group and non-remifentanil group (1.2 % vs. 1.3 %; $P = 0.518$). Table 3 shows results of logistic regression analyses for in-hospital mortality following rectal cancer surgery. Again, older age was a significant predictor of higher hospital mortality. Higher hospital volume was significantly associated with lower mortality. Remifentanil use was not associated with mortality.

Table 4 shows the results of log-rank tests for each covariate and the Cox proportional hazard regression analysis for discharge from hospital following brain tumor surgery. The median (95 % CI) values for LOS were 17 (16.2–17.8) days for the remifentanil group and 19 (17.8–20.2) days for the non-remifentanil group, and a log-rank test revealed a significant difference between the two groups ($P < 0.001$). In the log-rank tests, diabetes, cardiac diseases, hospital volume, nitrous oxide, isoflurane, and propofol showed $P > 0.10$, and therefore were not included in the Cox regression. In the Cox regression model, the remifentanil group showed significantly earlier discharge from hospital (hazard ratio, 1.19, 95 % CI, 1.08–1.30; $P < 0.001$) compared with the non-remifentanil group. Consequently, the postoperative LOS was significantly shorter for the remifentanil group than for the non-remifentanil group. Use of sevoflurane was not significantly associated with LOS. Male sex, older age, and longer duration of anesthesia were significantly associated with longer LOS.

Table 5 shows the results of log-rank tests for each covariate and the Cox regression analysis for rectal cancer surgery. No significant difference of median LOS was shown between the remifentanil group and non-remifentanil group (19 vs. 19 days; $P = 0.148$) No significant difference in discharge rates was seen between the remifentanil group and non-remifentanil group (hazard ratio, 1.04, 95 % CI, 0.99–1.10; $P = 0.141$).

Discussion

In this study, propensity score matching analyses revealed that patients who underwent brain tumor resection under general anesthesia with remifentanil showed reduced postoperative LOS and lower in-hospital mortality compared with non-remifentanil patients. In contrast, patients who underwent rectal surgery did not show any difference in postoperative LOS and in-hospital mortality.

Table 2 Logistic regression analyses for in-hospital mortality following brain tumor resection

	Univariate analysis			Multivariate analysis		
	OR	95 % CI	*P*	OR	95 % CI	*P*
Age (years)						
≤59	1.00			1.00		
60–74	1.40	0.53–3.65	0.497	1.21	0.45–3.25	0.698
≥75	5.70	2.29–14.2	<0.001	4.80	1.65–14.0	0.004
Sex						
Male	1.00			1.00		
Female	0.56	0.30–1.05	0.071	0.57	0.30–1.05	0.073
Diabetes	1.34	0.47–3.82	0.580			
Hypertension	1.48	0.65–3.37	0.352			
Cardiac diseases	2.73	0.95–7.86	0.063	1.77	0.66–4.71	0.253
Duration of anesthesia (h)	0.88	0.77–0.98	0.023	0.90	0.80–1.01	0.063
Hospital volume (per 6 months)						
Low (≤9)	1.00					
Medium (10–23)	0.75	0.37–1.53	0.433			
High (≥24)	0.51	0.23–1.14	0.102			
Remifentanil	0.49	0.26–0.94	0.032	0.47	0.25–0.91	0.025
Nitrous oxide	1.11	0.49–2.52	0.808			
Isoflurane	1.44	0.56–3.72	0.451			
Sevoflurane	1.95	0.86–4.43	0.109			
Propofol	1.34	0.57–3.25	0.490			

OR odds ratio, *CI* confidence interval

As expected, older age was a significant contributor to higher in-hospital mortality and longer postoperative LOS. Several preoperative and intraoperative factors were also associated with the outcomes. After adjustment for these variables, our data indicated that use of remifentanil was an independent factor for earlier discharge from hospital. Therefore, based on these data, use of remifentanil may lead to better early postoperative recovery in patients undergoing neurosurgery with craniotomy.

Limitations

Because the present data were based on the administrative claim database, several limitations of this study should be acknowledged and, therefore, we should interpret these results carefully. Most importantly, it was based on a nonrandomized retrospective study. Although we used propensity score matching to adjust for differences in the baseline characteristics, the results could have been biased by several unmeasured confounders. For instance, no data were available regarding tumor size or anatomical location. Although we included patients undergoing elective neurosurgery whose preoperative consciousness was alert (JCS = 0) and adjusted for duration of anesthesia because of its presumed association with the level of surgical procedure difficulty, the tumor size or anatomical location should be a direct indicator of the difficulty or invasiveness of the neurosurgical procedures, which may affect postoperative recovery.

We should also be aware of intangible factors such as the clinician's choice for rather newly introduced drugs. Anesthesiologists in Japan may be prudent in choosing remifentanil and apply it for those patients with fewer comorbidities, although that seems unlikely in neurosurgery, because they chose remifentanil for more than 60 % of the patients [4]. After adjusting patients' backgrounds by propensity score matching, use of remifentanil favorably affected postoperative outcome in neurosurgery but not in rectal cancer surgery. These results suggest that the experience or preference of the anesthesia care provider was not linked to remifentanil use and a better postoperative outcome. Nevertheless, we cannot completely neglect these possible effects.

Second, we could not evaluate the doses of anesthetics and concurrent effects of various other drugs that could potentially have affected postoperative outcomes. Although we performed regression analyses for other anesthetics and found no other agent significantly contributed to early postoperative outcomes, further studies, including a randomized controlled trial, are required to confirm the present results and to explore the underlying mechanism behind the better postoperative recoveries observed in the remifentanil group.

Third, postoperative LOS is much longer in Japan compared with other advanced nations. Nearly 80 % of

Table 3 Logistic regression analyses for in-hospital mortality following rectal cancer surgery

	Univariate analysis			Multivariate analysis		
	OR	95 % CI	*P*	OR	95 % CI	*P*
Age (years)						
≤59	1.00			1.00		
60–74	2.14	0.96–4.81	0.064	1.89	0.88–4.09	0.104
≥75	6.18	2.88–13.3	<0.001	5.43	2.54–11.6	<0.001
Sex						
Male	1.00			1.00		
Female	0.79	0.48–1.32	0.369	0.76	0.44–1.31	0.318
Diabetes	1.12	0.54–2.36	0.756			
Hypertension	0.77	0.35–1.70	0.523			
Cardiac diseases	1.26	0.60–2.65	0.536			
Chronic lung diseases	3.42	1.46–8.04	0.005	2.46	1.06–5.67	0.035
Procedure						
Low anterior resection	1.00			1.00		
Abdominoperineal resection	1.65	0.95–2.87	0.074	1.64	0.92–2.93	0.094
Hospital volume (per 6 months)						
Low volume (≤20)	1.00		0.007	1.00		
Medium volume (21–39)	0.61	0.35–1.04	0.071	0.67	0.37–1.20	0.176
High volume (≥40)	0.38	0.20–0.71	0.003	0.46	0.24–0.86	0.016
Remifentanil	1.06	0.84–1.34	0.631	1.09	0.68–1.75	0.727
Nitrous oxide	0.76	0.36–1.59	0.465			
Isoflurane	2.28	0.70–7.35	0.169			
Sevoflurane	1.30	0.70–2.44	0.406			
Propofol	0.77	0.43–1.39	0.384			

OR odds ratio, *CI* confidence interval

patients undergoing intracranial parenchymal tumor resection are discharged within 7 days postoperatively in the United States [14]. Generally, the average postoperative LOS is much longer in Japan than in most medical centers in the United States, reflecting differences in the expectations of patients and, more so, in the healthcare delivery systems (i.e., the predominantly managed care in the United States versus a highly centralized, government-funded healthcare program in Japan) [15]. Even with the different healthcare delivery systems, the present results showed that older age contributed negatively to earlier discharge, which coincides with other reports from Western countries [16, 17].

Fourth, we cannot predict the long-term outcomes of patients using this database. Opioids are generally recognized as suppressors of natural killer cell activities and potentially contribute to tumor metastasis [18]. Although remifentanil is quickly eliminated from the bloodstream, we should also be careful for the long-term outcomes of patients receiving high-dose opioids during surgery.

Speculations for the mechanisms

We can speculate on several possible mechanisms for the current results.

General anesthesia with remifentanil may provide more suitable conditions for neurosurgery compared with general anesthesia with other drugs. Remifentanil patients were anticipated to be exposed to a lesser amount of volatile anesthetics than non-remifentanil patients. Opioids, including remifentanil and fentanyl, do not have any effects on intracranial pressure and carbon dioxide reactivity [19–21], whereas volatile anesthetics contribute to brain swelling because of their vasodilatory effect [22–24]. Remifentanil-based anesthesia may suppress intraoperative increases in blood glucose [25, 26] that could damage intact and/or ischemic neurons. Remifentanil is known to strongly suppress surgical stress responses, sustaining the early postoperative period in comparison to fentanil-based or sevoflurane anesthesia [25, 27–29].

In contrast, the use of remifentanil did not cause any significant difference in postoperative outcomes for rectal cancer surgeries that were conducted under general anesthesia with intraoperative epidural anesthesia. This neuraxial blockade is used for blocking afferent noxious stimuli from surgical sites to the central nervous system and reduces the total amount of volatile anesthetics used. Epidural anesthesia also attenuates the surgical stress response and reduces postoperative morbidity [30] after major abdominal surgery [31], coronary artery bypass grafting

Table 4 Log-rank tests and Cox regression analysis for discharge from hospital following brain tumor resection

	Log-rank tests			Cox regression[a]		
	Median LOS	95 % CI	*P*	Hazard ratio	95 % CI	*P*
Age (years)						
≤49	17	15.9–18.1	<0.001	1.00		
50–69	18	17.2–18.8		0.90	0.81–1.00	0.049
≥70	21	18.8–23.2		0.70	0.61–0.80	<0.001
Sex						
Male	19	17.7–20.3	<0.001	1.00		
Female	17	16.3–17.7		1.25	1.14–1.37	<0.001
Diabetes						
No	18	17.3–18.7	0.450			
Yes	18	15.2–20.8				
Hypertension						
No	17	16.3–17.7	0.013	1.00		
Yes	22	19.1–24.9		0.89	0.77–1.03	0.131
Cardiac diseases						
No	18	17.3–18.7	0.784			
Yes	19	15.4–22.6				
Chronic lung diseases						
No	18	17.4–18.6	0.099	1.00		
Yes	29	15.1–42.9		0.67	0.40–1.12	0.130
Hospital volume (per 6 months)						
Low (≤9)	18	16.8–19.2	0.607			
Medium (10–23)	18	17.0–19.0				
High (≥24)	17	15.8–18.2				
Duration of anesthesia (min)						
≤240	15	14.1–15.9	0.002	1.00		
241–360	16	15.1–16.9		0.93	0.79–1.09	0.389
≥361	19	18.0–20.0		0.76	0.66–0.89	<0.001
Remifentanil						
Non-users	19	17.8–20.2	<0.001	1.00		
Users	17	16.2–17.8		1.19	1.08–1.30	<0.001
Nitrous oxide						
Non-users	18	17.3–18.7	0.666			
Users	18	16.5–19.5				
Isoflurane						
Non-users	18	17.3–18.7	0.595			
Users	18	16.0–20.0				
Sevoflurane						
Non-users	17	16.1–17.9	0.012	1.00		
Users	18	17.1–18.9		0.91	0.82–1.02	0.095
Propofol						
Non-users	17	15.3–18.7	0.169			
Users	18	17.3–18.7				

LOS length of stay, *CI* confidence interval

[a] Before evaluating hazard ratio for a specific confounding factor, effects of all other factors are excluded

[32], and labor/delivery [33]. Subclinical increases in blood glucose are also attenuated with epidural anesthesia [34]. For patients who underwent rectal surgery, we believe that adequate suppression of the stress response may have been achieved with epidural anesthesia, and as a consequence, the use of supplemental remifentanil would not have added any further benefit.

Both volatile anesthetics and opioids have neuroprotective properties for ischemia [35–37]. Remifentanil is known to have *N*-methyl-D-aspartate receptor (NMDAR)

Table 5 Log-rank tests and Cox regression analysis for discharge from hospital following rectal cancer surgery

	Log-rank tests			Cox regression[a]		
	Median LOS	95 % CI	*P*	Hazard ratio	95 % CI	*P*
Age (years)						
≤49	18	17.4–18.6	<0.001	1.00		
50–69	19	18.4–19.6		0.97	0.92–1.04	0.408
≥70	21	20.2–21.8		0.87	0.81–0.93	<0.001
Sex						
Male	20	19.5–20.5	<0.001	1.00		
Female	18	17.5–18.5		1.11	1.05–1.17	<0.001
Diabetes						
No	19	18.6–19.4	0.022	1.00		
Yes	19	17.7–20.3		0.94	0.86–1.03	0.186
Hypertension						
No	19	18.6–19.4	0.127			
Yes	18	17.2–18.8				
Cardiac diseases						
No	19	18.6–19.4	0.550			
Yes	19	17.6–20.4				
Chronic lung diseases						
No	19	18.6–19.4	0.151			
Yes	20	18.3–21.7				
Procedure						
Low anterior resection	17	16.6–17.4	<0.001	1.00		
Abdominoperineal resection	28	26.7–29.3		0.60	0.56–0.64	<0.001
Hospital volume (per 6 months)						
Low (≤20)	22	21.3–22.7	<0.001	1.00		
Medium (21–39)	18	17.4–18.6		1.18	1.10–1.26	<0.001
High (≥40)	17	16.4–17.6		1.40	1.31–1.49	<0.001
Remifentanil						
Non-users	19	18.4–19.6	0.148	1.00		
Users	19	18.5–19.5		1.04	0.99–1.10	0.141
Nitrous oxide						
Non-users	18	17.2–18.8	0.225			
Users	19	18.5–19.5				
Isoflurane						
Non-users	19	18.6–19.4	0.557			
Users	19	16.8–21.2				
Sevoflurane						
Non-users	18	17.3–18.7	0.167			
Users	19	18.5–19.5				
Propofol						
Non-users	19	18.1–19.9	0.125			
Users	19	18.6–19.4				

LOS length of stay, *CI* confidence interval

[a] Before evaluating hazard ratio for specific confounding factor, effects of all other factors are excluded

agonist activity [38] and is associated with opioid-induced hyperalgesia [39]. NMDAR agonists are known to enhance neuronal activity and have been considered to contribute to ischemic neuronal damage [40]. On the other hand, NMDAR antagonists also exerted detrimental effects in patients who had a stroke [41]. Recently, a small dose of NMDA was reported to have preconditioning effect [42]. Based on these publications, optimal NMDA receptor activity is crucial for neuroprotection. General anesthesia with remifentanil, usually combined with other NMDA antagonists such as sevoflurane and propofol, may possibly (coincidentally) provide an optimal NMDA signaling balance for neuroprotection.

Based on these lines of evidence, general anesthesia with remifentanil may provide optimal surgical conditions, reduce ischemic tissue damage, and attenuate postoperative as well as intraoperative stress responses, resulting in better early postoperative conditions for neurosurgical patients, although we should be aware of methodological limitations related to a retrospective survey.

In conclusion, the present data indicate a possible association between remifentanil use and earlier postoperative recovery in patients undergoing neurosurgery, and this finding warrants further prospective investigations.

Acknowledgments This study was funded by Grants-in-Aid for Research on Policy Planning and Evaluation (Grant Number: H19-Policy-001) from the Ministry of Health, Labour and Welfare, Japan.

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Commentary

DOI: 10.5582/ddt.2012.v6.2.108

Standardization of perioperative management on hepato-biliary-pancreatic surgery

Jianjun Gao[1,2], Peipei Song[1], Sumihito Tamura[1], Kiyoshi Hasegawa[1], Yasuhiko Sugawara[1], Norihiro Kokudo[1], Kanji Uchida[3], Ryo Orii[3], Fanghua Qi[1,4], Jiahong Dong[5,*], Wei Tang[1,*]

[1] *Department of Surgery, Graduate School of Medicine, The University of Tokyo, Tokyo, Japan;*
[2] *China-Japan Cooperation Center for Drug Discovery & Screening, Shandong University, Ji'nan, China;*
[3] *Department of Anesthesiology, Faculty of Medicine, The University of Tokyo, Tokyo, Japan;*
[4] *Provincial Hospital Affiliated to Shandong University, Ji'nan, China;*
[5] *Hepato-Biliary-Pancreatic Surgery Division, The General Hospital of PLA, Beijing, China.*

ABSTRACT: Japan-China Joint Medical Workshop (2012) on standardization of perioperative management on hepato-biliary-pancreatic surgery was held by the Center for Medical Standards Research, IRCA-BSSA Group in Japan on April 15-16, 2012. Experts in the fields of surgery, anesthesia, pharmacy, and public health from 21 health institutions from Japan and China presented their research achievements and shared their medical experience of perioperative management on hepato-biliary-pancreatic surgery, which should facilitate building of guidelines for hepatocellular carcinoma and be expected to promote standardized management of liver cancer in Asia.

Keywords: Guideline, standardized management, liver surgery, liver transplantation

From advocacy of evidence-based medicine to establishment of clinical pathways, the concept of "standardized management of care" has garnered substantial attention globally and has been fully implemented in several countries. Clinical practices prove that standardized management of care contributes to normalized medical practices, optimized health care programs, decreased medical risks, increased efficiency of medical services, and reduced health care costs (*1,2*). In this context, the Center for Medical Standards Research (CMSR) was established by the IRCA-BSSA Group in Japan in 2011, aiming to promote the advancement of evidence-based medicine, standardization of medical care, and systematic cataloging of research findings in related disciplines. Many research programs on standardized management of diseases have been promoted by CMSR since the establishment, especially for the research between Japan and China. Concerning liver cancer, the Japan-China Joint Team for Medical Research and Cooperation on Hepatocellular Carcinoma was established in 2011 with the support of CMSR. The team carried out a retrospective study and analyzed the published guidelines for hepatocellular carcinoma (HCC) worldwide, and clarified principles of evidence-, resource-, and population-based guidelines that should be given a great deal of attention in constructing prospective guidelines for HCC, especially for China and other Asian countries (*3,4*).

As part of the standardized management of liver cancer, the Japan-China Joint Team for Medical Research and Cooperation on Hepato-Biliary-Pancreatic Surgery was also established with the support of CMSR. In order to promote information exchange between experts from Japan and China, the Japan-China Joint Medical Workshop (2012) on Standardization of Perioperative Management on Hepato-Biliary-Pancreatic Surgery was held by CMSR at the University of Tokyo, Tokyo, Japan on April 15-16, 2012. Experts in the fields of surgery, anesthesia, pharmacy, and public health from 21 health institutions from Japan and China presented their research achievements and shared their medical experience of perioperative management on hepato-biliary-pancreatic surgery.

In this workshop, experts from the Chinese PLA General Hospital, Peking Union Medical College Hospital, Peking University People's Hospital, and other institutes introduced the current Chinese research status and their experience on the topics of protection of remnant liver function, perioperative management for abdominal surgery, and so on. Specifically, the experts' research and experience on perioperative management of safe liver resection from the University

**Address correspondence to:*
The "Japan-China Joint Team for Medical Research and Cooperation"
Dr. Jiahong Dong (E-mail: dongjh301@163.com) and Dr. Wei Tang (E-mail: tang-sur@h.u-tokyo.ac.jp)

of Tokyo Hospital (UTH) was impressive since the short-term mortality after hepatectomy in this hospital is nearly 0 which is far less than that in representative hospitals of other countries (ranges: approximate 4-6%) (*5-11*). Their research and experience are shared as the following. First, in preparation for safe hepatectomy, they thought that evaluation of liver function, volumetric analysis, and keeping sufficient functional liver volume were thought to be important, and asserted that *i*) the indocyanine green (ICG) test is a key method to estimate liver function (*12-14*); *ii*) volumetric analysis using preoperative simulation is useful (*15-17*), and *iii*) portal vein embolization (PVE) (*16,18,19*) and venous reconstruction can contribute to securing sufficient functional liver volume (*20-22*). Second, in intra-operative management, control of central vein pressure (CVP), transection technique, and inflow occlusion should be paid close attention to. They proposed that *i*) low CVP should be achieved by some techniques such as infusion restriction, reducing TV (*23*), and blood salvage (*24*); *ii*) clamp crushing remains the first choice (*25-28*); and *iii*) intermittent inflow occlusion is safe and useful (*29,30*). Third, in post-operative management, prevention of infection including proper placement and management of drainage tubes (*31*) administration of antibiotics, control of ascites including exact control of water balance and proper administration of diuretics should be considered (*32*). The experience of UTH on management of safe hepatectomy may provide a valuable referencefor standardization of perioperative management of liver surgery (*11*).

Experts from UTH also introduced their research and experience in living donor liver transplantation (LDLT). They emphasized that most important in LDLT is safety of the living donor with good quality of life after donation (*33-35*). To assure this, a careful protocol based evaluation process under a multidisciplinary approach (*36,37*) and a tailored graft selection algorithm (*38*) have been established. As for the recipients, three important points in pre-operative examination have been stressed. First is exclusion of pulmonary hypertension. Specifically, when right ventricular systolic pressure (RVSP) is evaluated to be more than 50 mmHg by ultrasound imaging (US), the pressure must be measured directly. If the pressure is actually more than 50 mmHg, the patient should be contraindicated for LDLT. Second is exclusion of infectious disease that may seriously compromise survival. Bacterial, viral, and fungal checks are necessary for successful LDLT. Third is the check for esophageal varices and treatment, and endoscopic ligation if necessary. In postoperative care, four major complications including infection, acute cellular rejection (ACR), biliary stenosis, and thrombosis occur with rates of approximately 40%, 30%, 10%, and 8%, respectively. Close monitoring and early intervention is mandatory in every aspect for better survival of the recipient. In case of suspected infectious complications, treatment with prophylactic administration of antibiotics, and antiviral or antifungal drugs may be justified (*39-41*). Of note is the novel approach with plasma (1 → 3) β-D-glucan measurement for preemptive treatment of fungal diseases that may often become fatal once becoming symptomatic (*41*). Meticulous adjustment of immunosupppressive drug levels (*42*), regularly performed US to confirm intact blood flow, in combination with adequate anticoagulation may further contribute to early sound recovery of the recipient. The experience of UTH on LDLT may also contribute to the standardization of liver surgery.

In conclusion, the Japan-China Joint Medical Workshop (2012) discussed the hot topic of standardization of perioperative management on hepato-biliary-pancreatic surgery, with information exchange from Japan and China. The experts' research and experience presented in this workshop should facilitate building of guidelines. Furthermore, these achievements are expected to promote standardized management of liver cancer in Asia.

Acknowledgements

This work was supported by the Center for Medical Standards Research, IRCA-BSSA Group. We are grateful to Dr. Takeaki Ishizawa, Dr. Yosuke Inoue, and Dr. Atsushi Shimizu (Department of Surgery, Graduate School of Medicine, The University of Tokyo) for their support of this conference.

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(Received April 23, 2012)

Tetraspanin CD151 Protects against Pulmonary Fibrosis by Maintaining Epithelial Integrity

Kazuyuki Tsujino[1], Yoshito Takeda[1], Toru Arai[2], Yasushi Shintani[3], Ryosaku Inagaki[4], Hiroyuki Saiga[5], Takeo Iwasaki[1], Satoshi Tetsumoto[1], Yingji Jin[1], Shoichi Ihara[1], Toshiyuki Minami[1], Mayumi Suzuki[1], Izumi Nagatomo[1], Koji Inoue[1], Hiroshi Kida[1], Takashi Kijima[1], Mari Ito[4], Masanori Kitaichi[2], Yoshikazu Inoue[2], Isao Tachibana[1], Kiyoshi Takeda[5], Meinoshin Okumura[3], Martin E. Hemler[6], and Atsushi Kumanogoh[1,7]

[1]Department of Respiratory Medicine, Allergy, and Rheumatic Diseases, [3]Department of Thoracic Surgery, and [5]Laboratory of Immune Regulation, Department of Microbiology and Immunology, Osaka University Graduate School of Medicine, Suita, Osaka, Japan; [2]Department of Diffuse Lung Diseases and Respiratory Failure, National Hospital Organization Kinki-Chuo Chest Medical Center, Sakai, Osaka, Japan; [4]Drug Research Division, Dainippon Sumitomo Pharma Co., Ltd, Osaka, Japan; [6]Dana-Farber Cancer Institute, Boston, Massachusetts; and [7]Department of Immunology, Immunology Frontier Research Center (iFrec), Osaka University, Suita, Osaka, Japan

Rationale: Idiopathic pulmonary fibrosis (IPF) is a chronic pulmonary disorder of unknown etiology with few treatment options. Although tetraspanins are involved in various diseases, their roles in fibrosis have not been determined.
Objectives: To investigate the role of tetraspanin CD151 in pulmonary fibrosis.
Methods: CD151 knockout (KO) mice were studied by histological, biochemical, and physiological analyses and compared with wild-type mice and CD9 KO mice. Further mechanistic analyses were performed _in vitro_, _in vivo_, and on samples from patients with IPF.
Measurements and Main Results: A microarray study identified an enrichment of genes involved in connective tissue disorders in the lungs of CD151 KO mice, but not in CD9 KO mice. Consistent with this, CD151 KO mice spontaneously exhibited age-related pulmonary fibrosis. Deletion of CD151 did not affect pulmonary fibroblast functions but instead degraded epithelial integrity via attenuated adhesion strength on the basement membrane; CD151-deleted alveolar epithelial cells exhibited increased α-SMA expression with activation of p-Smad2, leading to fibrotic changes in the lungs. This loss of epithelial integrity in CD151 KO lungs was further exacerbated by intratracheal bleomycin exposure, resulting in severe fibrosis with increased mortality. We also observed decreased numbers of CD151-positive alveolar epithelial cells in patients with IPF.
Conclusions: CD151 is essential for normal function of alveolar epithelial cells; loss of CD151 causes pulmonary fibrosis as a result of epithelial disintegrity. Given that CD151 may protect against fibrosis, this protein represents a novel target for the treatment of fibrotic diseases.

(_Received in original form January 22, 2012; accepted in final form May 4, 2012_)

Supported by a Grant-in-Aid for Scientific Research (C) No. 21590992, the Takeda Science Foundation, the Okamoto Satoshi Memorial Fund for Pulmonary Fibrosis Research, the Kato Memorial Trust For Nambyo Research (Y.T.), National Institutes of Health grant CA42368 (M.E.H.), the Funding Program for Next Generation World-Leading Researchers (NEXT Program), and Special Coordination Funds for Promoting Science and Technology (A.K.).

Author Contributions: Conception and design, K. Tsujino, Y.T., I.T., A.K.; analysis and interpretation, K. Tsujino, Y.T., R.I., H.S., T.I., S.T., Y.J., S.I., T.M., M.S., I.N., K.I., H.K., T.K., M.I., K. Takeda; collection of human samples, K. Tsujino, T.A., Y.S., M.K., Y.I., M.O.; preparing the manuscript, K. Tsujino, Y.T., M.E.H., A.K.

Correspondence and requests for reprints should be addressed to Yoshito Takeda, M.D., Ph.D., Department of Respiratory Medicine, Allergy, and Rheumatic Diseases, Osaka University Graduate School of Medicine, 2-2 Yamadaoka, Suita, Osaka, 565-0871, Japan. E-mail: yoshito@imed3.med.osaka-u.ac.jp

This article has an online supplement, which is accessible from this issue's table of contents at www.atsjournals.org

Am J Respir Crit Care Med Vol 186, Iss. 2, pp 170–180, Jul 15, 2012

Originally Published in Press as DOI: 10.1164/rccm.201201-0117OC on May 16, 2012
Internet address: www.atsjournals.org

AT A GLANCE COMMENTARY

Scientific Knowledge on the Subject

Although tetraspanin CD151 is abundantly expressed in the lung and plays a critical role in epithelial cells, its role in pulmonary fibrosis has not been determined.

What This Study Adds to the Field

Deletion of CD151 in mice results in progressive pulmonary fibrosis. The underlying mechanism could involve loss of epithelial integrity: deletion of CD151 in alveolar epithelial cells attenuates the strength of adhesion to the basement membrane, resulting in mesenchymal-like changes and increased p-Smad2 signaling.

Keywords: alveolar epithelial cell; adhesion strength; epithelial-to-mesenchymal transition; Smad2; CD9

Idiopathic pulmonary fibrosis (IPF) is a chronic pulmonary disorder of unknown etiology; the condition has a poor prognosis, and there are no effective treatments. IPF is characterized by progressive deposition of collagen and other extracellular matrix (ECM) molecules (1). Previously, IPF was viewed as a "smoldering" inflammatory response that ultimately led to chronic lung injury with subsequent fibrosis. However, inflammation is now not regarded as crucial to the etiology, largely because current antiinflammatory therapies for IPF have provided little benefit for patients (2). Instead, it has become clear that abnormal behavior of alveolar epithelial cells (AECs) is a primary source of the development of pulmonary fibrosis (3, 4). The disease process is initiated through repetitive injury of AECs, causing AEC activation, which in turn leads to recruitment of immune cells and fibroblasts within the lung microenvironment. Aberrant activated AECs, in cooperation with migrated immune cells and fibroblasts, secrete and activate latent transforming growth factor (TGF)-β_1, as well as other profibrotic factors, which promote the differentiation of fibroblasts and AECs to myofibroblasts, resulting in overproduction of ECM in the lung.

The tetraspanins are a protein family comprising at least 33 members in mammals. Tetraspanins contain four transmembrane regions and associate with a variety of membrane proteins, including integrins, growth factors, and intracellular signaling molecules (5). Tetraspanins facilitate the formation of these multimolecular complexes, thereby regulating cell activation, fusion, motility, and signaling (6). Thus, tetraspanins are involved in a number of normal and pathological processes, such as tumor progression, angiogenesis, and infectious diseases (7). For example, tetraspanins CD9 and CD151 regulate tumor progression, either negatively (CD9) or positively (CD151), in multiple tumor types (7, 8). Specifically regarding lung diseases, CD9 plays protective roles in lung inflammation and emphysema, which we have studied using genetic models in mice (9, 10).

In contrast to CD9, which is ubiquitously expressed at the apical and lateral sides of the cell, CD151 are predominantly expressed at the basolateral surface of epithelial and endothelial cells. Moreover, CD151 and a homologous tetraspanin, TSP-15, are essential for maintenance of epithelial characteristics (11, 12), which is crucial for the development and maintenance of lung structure. However, the involvement of CD151 in lung diseases has remained unclear. Recently, close attention has been paid to β_1 integrins in AECs and fibroblasts, focusing on their potential roles in pulmonary fibrosis (13, 14). Therefore, we investigated whether CD151, the most robust partner of laminin-binding integrins among tetraspanins, is involved in lung diseases.

In this study, we found that CD151 is essential in order for AECs to maintain epithelial integrity via firm adhesion to the basement membrane. The deletion of CD151 promotes mesenchymal-like changes and activation of TGF-β signaling in AECs and ultimately leads to pulmonary fibrosis.

METHODS

See the online supplemental for additional information.

Animal Model

CD151 knockout (KO) mice were generated as previously described (15). CD9 KO mice were kindly provided by Eisuke Mekada (16). The mice were backcrossed to C57BL/6 mice for more than eight generations. All experiments comparing CD151 KO and WT mice were performed with age- and sex-matched littermates.

Microarrays

mRNA was extracted from WT (n = 4), CD151 KO (n = 5), and CD9 KO (n = 5) lungs at 20 weeks of age. After reverse transcription, cDNA was hybridized to GeneChip Mouse Genome 430 2.0 Arrays (Affymetrix, Santa Clara, CA). Array data were analyzed using Ingenuity Pathway Analysis (Ingenuity Systems, www.ingenuity.com), comparing the gene expression lists of CD151 KO/CD9 KO lungs and WT lungs; significantly differentially regulated genes were classified into subcategories by gene functions.

Bleomycin-induced Pulmonary Fibrosis Model

Eight- to 10-week-old mice were anesthetized with 13 μl/g of 4% Avertin. Intratracheal instillation of saline or 1.2 U/kg of bleomycin (BLM) (Nippon Kayaku Co., Tokyo, Japan) was performed after cervical incision. Mice were killed 7 to003F21 days after instillation, and lungs were removed for further analysis.

Assessment of Pulmonary Fibrosis

Hydroxyproline was measured as previously described (17). Lung compliance of mice was measured using a whole-body plethysmograph as part of the Pulmonary Maneuvers System (Buxco Electronics, Wilmington, NC), as previously described (10).

Isolation and Culture of Primary Alveolar Epithelial Type II Cells

Mouse AECs were isolated as previously described (18) with minor modifications. Because it was difficult to maintain the phenotype of primary epithelial cells *in vitro*, we used AECs isolated from H-$2K^b$-tsA58 transgenic mice (Immortomouse, Charles River, Wilmington, MA) (19).

Human Lung Tissues

Lung tissue samples from patients with IPF were obtained from diagnostic surgical lung biopsies, surgical lung resection from patients with lung cancer, or explanted lung obtained during lung transplantation procedures performed at the Kinki-Chuo Chest Medical Center or Osaka University Hospital. All cases were diagnosed as IPF/usual interstitial pneumonia according to the American Thoracic Society/European Respiratory Society Consensus Conference (20). Normal controls were obtained from healthy tissue derived from surgical lung resection from patients with lung cancer. We performed quantitative polymerase chain reaction using homogenates from 5 IPF and 6 healthy lung tissue samples and immunohistochemical analyses using sections from 10 IPF and 3 healthy lungs. The protocol was approved by the Institutional Review Board of both institutes; written informed consent was obtained from all subjects.

Statistical Analysis

All results are expressed as mean ± SEM. The significance of differences between two sample means was determined by two-tailed Student *t*-test. Analyses of survival were conducted using the Kaplan-Meier method; statistical differences between two groups were calculated using a log-rank test. $P < 0.05$ was considered statistically significant. All statistical analyses were performed using SPSS 11.0 for Windows (SPSS, Inc., Chicago, IL).

RESULTS

DNA Microarray Analyses Identify Genes Associated with Connective Tissue Disorders in CD151 KO Lungs

To determine the expression level of CD151 in human lung tissues, we first performed immunohistochemical analyses. In normal human lungs, CD151 was expressed abundantly in AECs, alveolar macrophages, and peripheral bronchial epithelial cells (*see* Figure E1 in the online supplement). mRNA expression of CD151 was confirmed in the lungs of WT mice; in contrast, in the lungs of CD151 KO mice, expression of CD151 was clearly absent, but there was no effect on expression of other tetraspanins or associated integrins (Figure E2).

To investigate the changes in mRNA expression levels in CD151 KO lungs in an unbiased and comprehensive manner, we performed DNA microarray analyses on mouse lungs. We analyzed the list of genes differentially expressed between WT lungs and CD151/CD9 KO lungs using Ingenuity Pathway Analysis and identified enriched functional categories. "Cancer" was ranked at the top of the functional categories in both CD151 KO and CD9 KO lungs (Figure 1A), consistent with previous reports (7, 8). Unexpectedly, two categories were ranked highly in CD151 KO lungs but not in CD9 KO lungs: "connective tissue disorders" and "connective tissue development and function" (Figure 1A). In the category "connective tissue disorders," there were 13 genes (detected by 16 probe sets) differentially expressed between WT lungs and CD151 KO lungs; most of these were collagen genes, such as collagen-1 and -3. As shown in Figure 1B, all of these genes were up-regulated in CD151 KO lungs. Moreover, expression levels of other ECM component genes, such as elastin and laminin, were also increased in CD151 KO lungs (data not shown). These data suggest that deletion of CD151 could result in fibrotic changes in the lung.

CD151 KO Mice Spontaneously Develop Age-related Pulmonary Fibrosis

No lung phenotype has ever been reported in CD151 KO mice, but these novel findings from microarrays led us to investigate the phenotype of CD151 KO mice in more detail. Consistent with our array results, Azan staining revealed slightly increased collagen depositions in the alveolar wall of CD151 KO lungs, although hematoxylin and eosin staining revealed minimal changes to alveolar structures (Figure 2A). Furthermore, collagen-1 expression

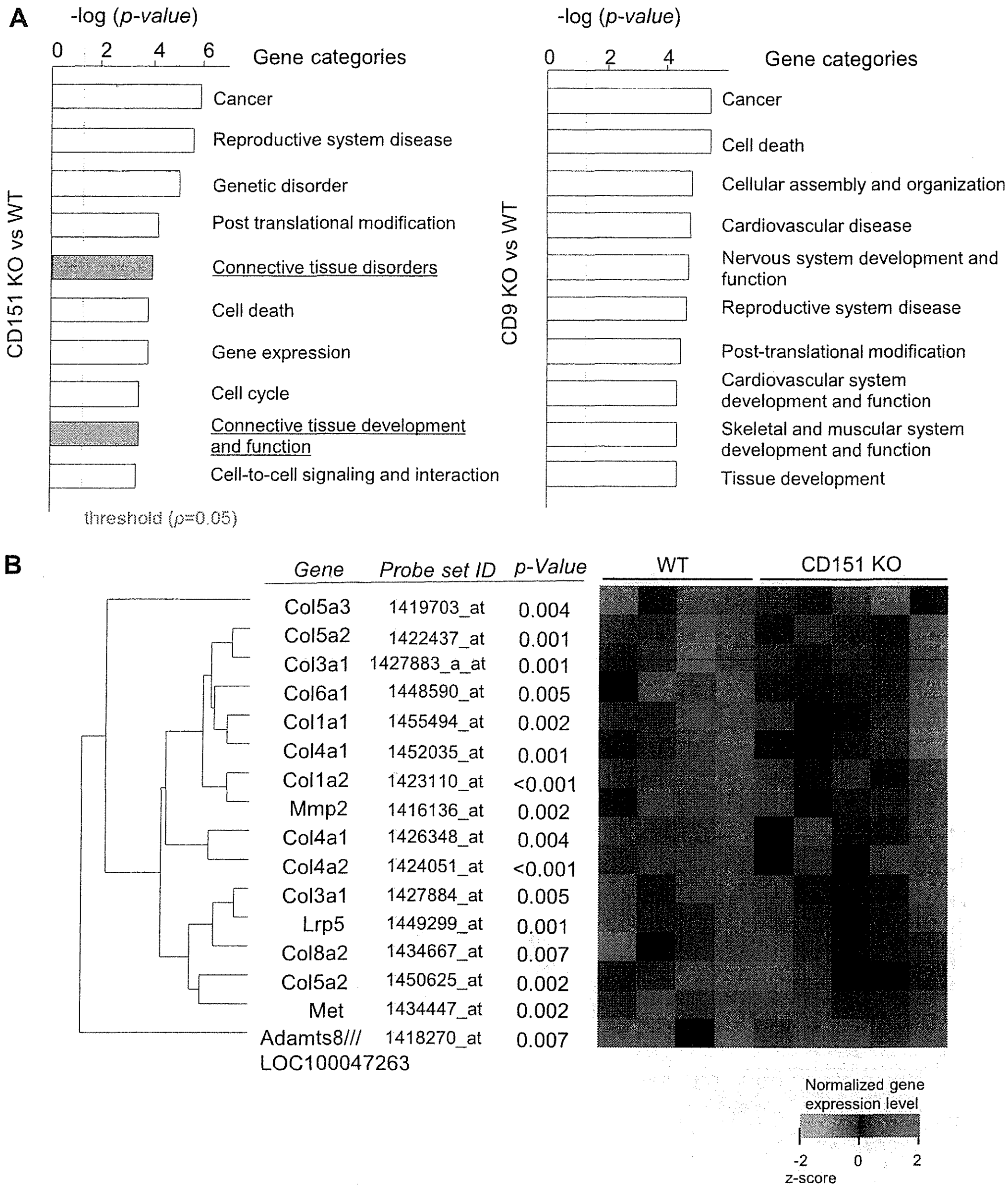

Figure 1. Microarray analysis indicated that genes associated with connective tissue disorders are differentially expressed in CD151 knockout (KO) lungs. (*A*) The list of genes differentially expressed between wild-type (WT) and CD151/CD9 KO lungs was analyzed using Ingenuity Pathway Analysis (IPA), and enriched functional gene categories were identified; results shown are the top 10 gene categories differentially expressed between CD151 KO and WT lungs (*left*) and between CD9 KO and WT lungs (*right*). Common logarithm of *P* value calculated by IPA (the length of the bar) reflects the significance of differential expression in each category. (*B*) Differentially expressed genes in the category "connective tissue disorders" are shown. A heat map shows the expression level of each gene (*red* = higher than mean value; *green* = lower than mean value). Adamts8 /// LOC100047263 = a disintegrin-like and metallopeptidase (reprolysin type) with thrombospondin type 1 motif, 8; Lrp5 = low-density lipoprotein receptor-related protein 5; Met = met protooncogene; MMP2 = matrix metallopeptidase 2.

in CD151 KO lungs was significantly increased relative to WT and CD9 KO lungs (Figure 2B). Likewise, hydroxyproline content of CD151 KO lungs was significantly increased compared with WT lungs (Figure 2C). Additionally, CD151 heterozygous mice also exhibited increased collagen-1 expression in their lungs. In lung function analyses, the lung compliance (C chord value) of CD151 KO mice was significantly lower than that of WT, CD9 KO, and CD151 heterozygous mice (Figure 2D), indicating that restrictive respiratory dysfunction occurred in CD151 KO lungs. These changes were obvious at 30 weeks of age but not at 10 weeks of age, suggesting that developmental abnormalities were only minimally involved. On the other hand, total cell numbers in bronchoalveolar lavage fluid were comparable between CD151 KO and WT lungs (Figure 2E). These data indicate that CD151 KO mice spontaneously develop age-related fibrotic phenotypes in the lungs.

As expected, transmission electron microscopy revealed increased collagen deposition in the interstitium of CD151 KO lungs (Figure 2F). Of note, type II AECs of CD151 KO lungs exhibited alterations: these cells were hypertrophied and contained many dense cytoplasmic, lamellar body–like structures (Figure 2F). Furthermore, the basement membranes (BM) adjacent to these AECs were irregularly thickened (Figure 2F). No obvious changes were detected in macrophages, fibroblasts, or endothelial cells of CD151 KO lungs.

We next examined whether similar fibrotic changes could be observed in other organs. In CD151 KO mice, Azan staining revealed mild fibrotic changes not only in the glomeruli of kidneys but also around the intrahepatic bile ducts (Figure E3). However, fibrotic changes could not be detected in the heart (data not shown). These findings implicate CD151 in fibrosis not only in the lung but also in the liver and kidney.

Phosphorylation of Smad2 Is Enhanced in AECs in CD151 KO Mice

Because TGF-β signaling is crucial for inducing tissue fibrosis (21), we investigated TGF-β and related signaling pathways in

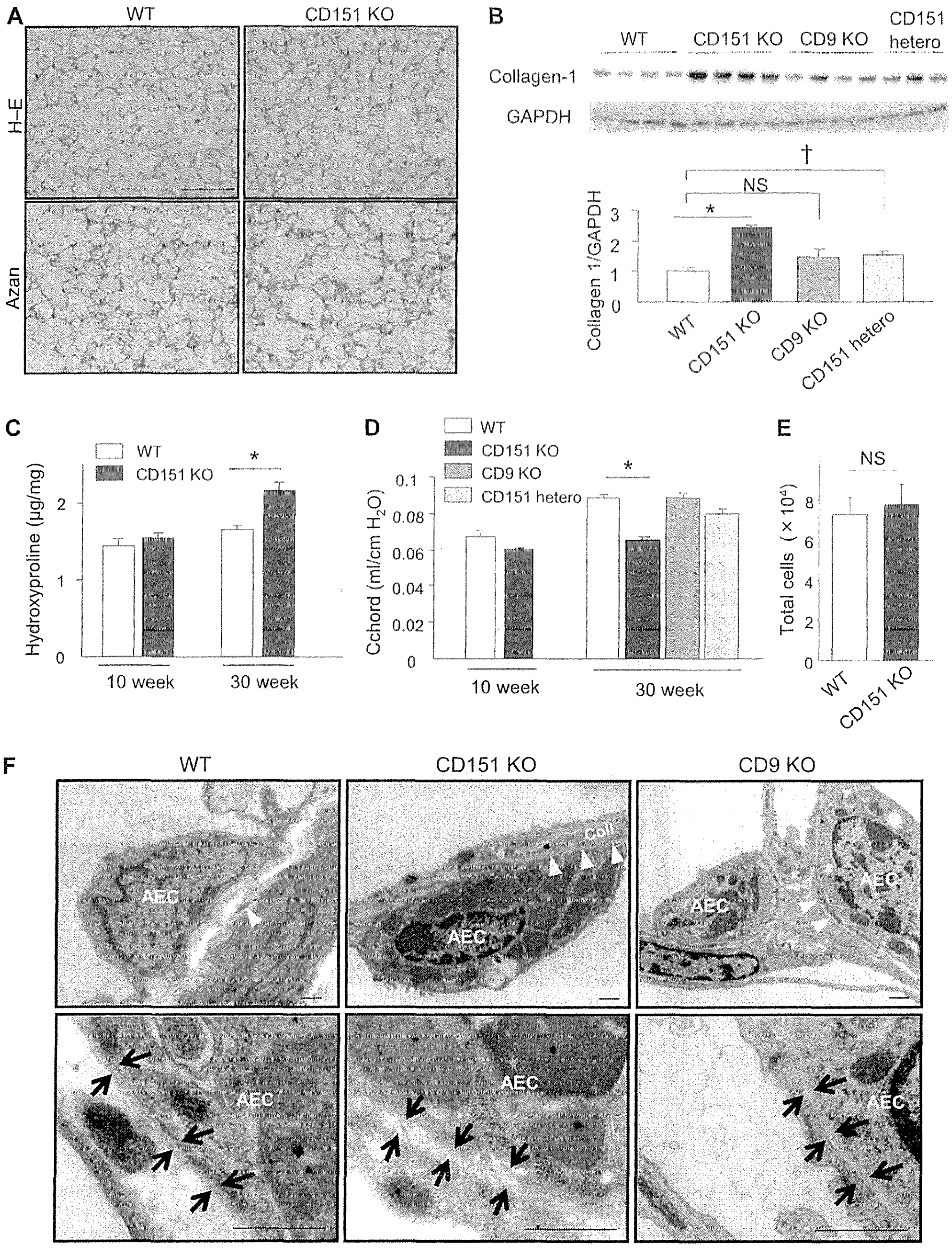

Figure 2. CD151 knockout (KO) mice spontaneously develop age-related pulmonary fibrosis. (*A*) Representative lung sections from wild-type (WT) and CD151 KO mice at 30 weeks of age stained with hematoxylin-eosin (H-E) and Azan. Bar = 100 μm. (*B*) Immunoblotting of total lung homogenate revealed increased expression of collagen-1 in CD151 KO and heterozygous (hetero) mice but not in CD9 KO mice compared with WT mice at 30 weeks of age. $^{*}P < 0.001$ (WT vs. CD151 KO), $^{\dagger}P < 0.05$ (WT vs. CD151 hetero). (*C*) Hydroxyproline contents of total lungs were measured and normalized against lung weight (μg/mg, n = 5 per group). $^{*}P < 0.05$. (*D*) Lung compliance was measured by a quasistatic pressure/volume maneuver, and is shown as C chord values (n = 4–5 per group). $^{*}P < 0.001$. (*E*) Total cell number in bronchoalveolar lavage fluids was comparable between CD151 KO and WT mice at 30 weeks of age (n = 4 per group). (*F*) Electron microscopy of CD151 KO lung at 16 weeks of age revealed increased collagen (coll) deposition in alveolar walls and hypertrophied type II alveolar epithelial cells (AECs) with many lamellar body–like structures. Basement membranes (BM) under these AECs were irregularly thickened. The number of lamellar body–like structures was also increased in CD9 KO lungs but not as prominently as in CD151 KO lungs. *Arrowheads* indicate coll in alveolar walls; *arrows* indicate BM. Bar = 1 μm.

CD151 KO lungs. Among TGF-β targets, phosphorylated Smad 2 (p-Smad2) was augmented in CD151 KO lungs. This finding became evident in CD151 KO mice at 30 weeks of age, but the tendency was already detectable at 10 weeks of age (Figure 3A), suggesting that this change is crucial in the development of fibrosis. However, no significant changes were observed in other signals, including Smad-7, p-Akt, p-Erk1/2, p-JNK, and p-p38 (Figure 3B and data not shown). To determine the main source of the elevated p-Smad2, we performed immunofluorescence analyses. As shown in Figure 3C, increased p-Smad2 was predominantly observed in nuclei of AECs but could scarcely be detected in alveolar macrophages (Figure 3D). In contrast, levels of activated TGF-β_1 in whole lungs were comparable between WT and CD151 KO (Figure 3E). Combined with the abnormal morphology of AECs observed by electron microscopy, these findings suggest that increased p-Smad2 signals arising from aberrant AECs underlie the fibrotic phenotype of CD151 KO lungs.

CD151 Is Required for Stable Adhesion of AECs on BM

To evaluate the role of CD151 in AECs, we knocked down the CD151 gene in AECs isolated from H-2K^{b}-tsA58 transgenic mice. The CD151 gene was knocked down specifically, without affecting other tetraspanins and associated integrins (Figure 4A). Because tight attachment to the BM is essential for epithelia to maintain their characteristics, we initially analyzed the role of CD151 on cell binding to Matrigel, which contains ECM components similar to those in BM (e.g., laminin, collagen). To investigate the ECM-dependent function of CD151, we also conducted the same analyses on fibronectin, which is not present in