短　報

気管切開が施行不能であった頸部海綿状血管腫の1症例

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キーワード：CICV，頸部海綿状血管腫，レミフェンタニル

血管腫は全体の約65%が頭頸部に発生し，大部分は鼻腔副鼻腔領域にできる[1]。気道および気道周辺に発生した血管腫に対し外科的処置が必要な場合，気道確保に際して綿密な計画が必要となる。今回，下咽頭・喉頭・気管周囲から縦隔まで連続する広範な病変を有し，気管切開が施行不能であった頸部海綿状血管腫の1症例を経験したので報告する。

1. 症　　例

48歳，女性，身長152 cm，体重45 kg

幼少時より嗄声を認め，38歳時に近医で下咽頭血管腫を指摘された。48歳となり呼吸困難が出現し，下咽頭血管腫に対する手術目的で当院耳鼻科を受診した。初診時喉頭ファイバーを施行したところ，左被裂部に拇指頭大の有茎性腫瘤を認めた。腫瘤は発声時に喉頭腔への嵌頓を認め，呼吸困難の原因と考えられた。頸部コンピュータ断層撮影（computed tomography：CT）では喉頭上部より左声門に膨隆する長径2.5 cmの腫瘤性病変を認めた（図1）。病変は前頸部より後頸部・縦隔まで連続し，甲状腺両葉にも浸潤を認めた。多数の静脈結石を認め，高血流の静脈性血管奇形を伴う頸部海綿状血管腫と診断された。前頸部は舌骨の下方1 cmより尾側に病変が連続しており（図2），病変を避けての気管切開は困難が予想されたが，全身麻酔下気管切開（第1気管輪レベル）および咽頭側切開下咽頭腫瘍摘出術が予定された。

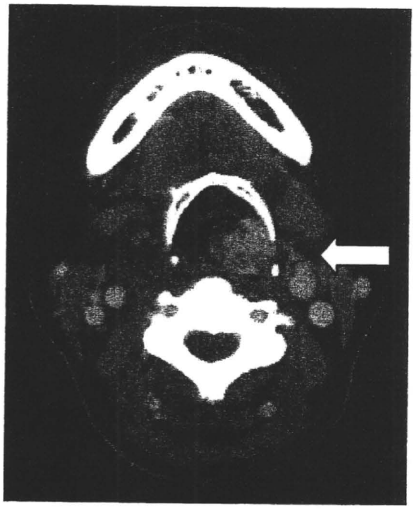

図1　喉頭上部より左声門に膨隆する長径2.5 cmの腫瘤性病変（矢印）を認める。

麻酔導入に際しては，下咽頭の有茎性血管腫による気道閉塞，換気困難・気管挿管困難（cannot intubate, cannot ventilate：CICV）の可能性が考えられた。また，術前検査より輪状甲状間膜切開および気管切開も困難が予想されたため，意識下での経口気管支ファイバー挿管を予定した。手術室入室後，硫酸アトロピン0.5 mgを静注，8%リドカイン0.9 mlを口腔内に噴霧し，酸素マスクで5分間の脱窒素化を行った。レミフェンタニルを0.1 $\mu g \cdot kg^{-1} \cdot min^{-1}$で持続投与開始し，患者に深呼吸を促しながら0.2 $\mu g \cdot kg^{-1} \cdot min^{-1}$まで漸増した。経鼻カニューレで酸素3 $l \cdot min^{-1}$を投与しながら口腔内に経口エアウェイ挿入し，口腔内分泌物吸引後に仰臥位で外径5.0 mmの気管支ファ

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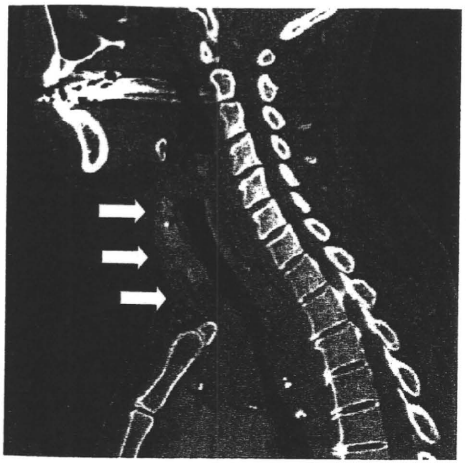

図 2　前頸部は舌骨の下方 1 cm より尾側に血管腫病変（矢印）が連続している。

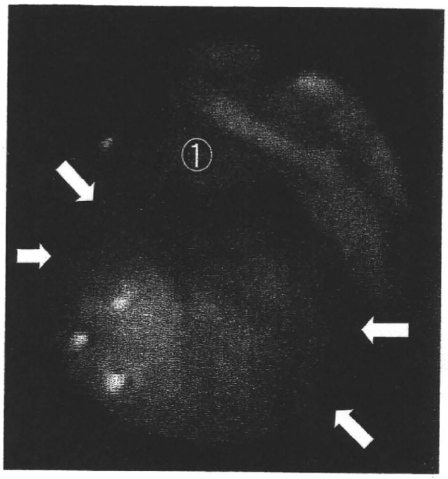

図 3　仰臥位でのファイバー挿管試行時血管腫（矢印）で閉塞され声門部は視認不能であった。①：喉頭蓋

イバーを挿入したが，血管腫で閉塞され声門部は視認不能であった（図 3）。患者を半坐位として再施行し，助手が下顎を挙上したところわずかに声門部が視認できた。4%リドカイン 5 ml を噴霧しつつ気管支ファイバーを気管分岐部まで進め，気管支ファイバーをガイドとして内径 6.5 mm のスタンダードチューブを気管挿管した。気管挿管後の麻酔は酸素 $2\,l \cdot \mathrm{min}^{-1}$，空気 $4\,l \cdot \mathrm{min}^{-1}$，セボフルラン 1.5-2%およびレミフェンタニル 0.2-0.25 $\mu\mathrm{g} \cdot \mathrm{kg}^{-1} \cdot \mathrm{min}^{-1}$の持続投与で維持した。

手術はまず全身麻酔下で気管切開を試みたが，気管前壁の血管腫より多量の出血があり視野確保に難渋した。エフェドリン総量 40 mg およびフェニレフリン総量 50 μg の間歇的静注およびヒドロキシエチルデンプン注射液 1,500 ml の補液により血圧を維持していたが，手術開始 3 時間後には出血が 1,000 g を超え，収縮期血圧が 70 mmHg，ヘモグロビン（hemoglobin：Hb）が $7.4\,\mathrm{g} \cdot \mathrm{dl}^{-1}$まで低下したため赤血球濃厚液の輸血を開始した。その後も出血は続き，手術進行不可能と考えられたため気管切開せずに止血して手術終了となった。手術時間は 4 時間 10 分，術中出血は 1,880 g，術中輸血は赤血球濃厚液 4 単位であった。

患者は気管挿管したまま集中治療室（intensive care unit：ICU）に帰室し，鎮静下に人工呼吸管理を継続した。ICU 帰室後も出血は持続し，赤血球濃厚液計 8 単位を輸血した。ICU で人工呼吸管理を継続した後，第 7 病日に左咽頭側切開アプローチで下咽頭腫瘍切除術を施行した。甲状軟骨後面より咽頭腔に入り，血管腫の茎を確認して切除した。手術時間は 2 時間 42 分，術中出血は 5 g であった。術後喉頭ファイバーによる観察と頻繁にリークテストを行い気道の開通を確認し，喉頭浮腫予防にデキサメタゾン 8 mg を投与した後，第 9 病日に抜管した。抜管後気道狭窄音を認めたが，残存する前頸部血管腫のために気管切開および輪状甲状間膜切開は不可能と考えられたため，非侵襲的陽圧換気法（non-invasive positive pressure ventilation：NPPV）を導入した。NPPV 導入後気道狭窄音は減弱し，喉頭浮腫が改善したため第 13 病日で NPPV を離脱した。NPPV 離脱後，経鼻カニューレでの酸素 $3\,l \cdot \mathrm{min}^{-1}$投与下で Pa_{O_2} 180 mmHg と経過良好であったため，第 14 病日に一般病床へ転床となった。

2. 考　察

本症例は下咽頭に有茎性の血管腫を認め，術前から CICV が予測された。また喉頭・気管腹側には舌骨の下方 1 cm より尾側に病変が連続しているため，気管切開・輪状甲状間膜切開などの侵襲的気道確保も困難と考えられた。CICV が予測される症例では，米国麻酔科学会（American Society

of Anesthesiologists：ASA）difficult airway algorithm[2]で意識下挿管が推奨されている。本症例では，麻酔導入時の気道確保方法として自発呼吸温存下での意識下ファイバー挿管を選択したが，その主な理由には，① 意識下であれば全身麻酔導入後よりも上気道の開通が維持でき下咽頭の視野が良好である，② 直達喉頭鏡を使用した気管挿管と比べてファイバー挿管のほうが腫瘍への接触が少ないことから出血が少なく気道の観察も行いやすい，という2点が挙げられる。ファイバー操作時およびガイド下にチューブを進める際に下咽頭血管腫を傷つけて出血させてしまう可能性はあったが，無理をせずに抵抗があればチューブを回転させながらゆっくりと進めることでリスクは最小限になると考えた。

意識下ファイバー挿管に際してレミフェンタニル持続投与による鎮痛は患者の苦痛除去と喉頭の防御反射抑制に有用であると報告されており[3]，リドカインの鼻腔粘膜局所噴霧とレミフェンタニル持続投与の併用で意識下ファイバー挿管時に安定した呼吸・循環動態が得られたことが報告されている[4]。意識下ファイバー挿管に併用される上喉頭神経ブロックや経喉頭ブロックは，前頸部血管腫からの出血や薬液の血管内投与の危険性が高く本症例では禁忌と考えられた。また，本症例ではリドカイン噴霧とレミフェンタニル持続静注を併用し，気管支ファイバーの操作中も適度な鎮痛・鎮静が維持できた。仰臥位で声門部視認不可能であった場合は，患者に深呼吸を促すとともに下顎を挙上することで下咽頭のスペースが広がり視野が改善することが報告されている[5]。

ASA difficult airway algorithm[2]では，意識下挿管で気道確保が不成功に終わった場合，① 他のオプションを考慮する，② 侵襲的気道確保，③ 手術中止，の3つが選択肢として挙げられている。① 他のオプションにはラリンジアルマスク（laryngeal mask airway：LMA）による気道確保，局所麻酔下での手術が挙げられる。本症例ではLMAは手術操作の妨げになるだけでなく，挿入時に血管腫を傷つけて出血し，挿入できても腫瘍が声門部を覆っていれば換気不可能になることも考えられた。局所麻酔下での手術は気道が術野であり不可能であった。また代替の挿管手段であるintubating LMA，光源付きスタイレット，エラスティックブジー，エアウェイスコープなどは，ファイバーによる声門部視認が困難な本症例では有効とはいい難い。② 侵襲的気道確保については，コントロール不可能な大量出血の危険性を考えると躊躇せざるをえない。よって本症例で挿管不可能と判断した場合には，③ 手術中止の選択が残される。その際にはレミフェンタニルの投与を中止すればすみやかな全覚醒が期待できる。なお，レミフェンタニル投与中に筋硬直や自発呼吸停止が起こった場合にも，ナロキソン静注により対処可能であると考えた。気管支ファイバーとの接触により下咽頭血管腫から出血させて挿管不可能となった場合には，前頸部出血のリスクがあっても緊急で輪状甲状間膜切開を施行し，ただちに経皮的心肺補助装置（PCPS）を導入することも念頭におく必要があった。

換気・挿管・気管切開困難が予測された頸部海綿状血管腫の症例を経験した。本症例の麻酔導入に際して，リドカイン噴霧とレミフェンタニル持続静注を併用した意識下ファイバー挿管により安全な気道確保を実施できた。

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ABSTRACT

Airway Management in a Patient with Cavernous Hemangioma of the Hypopharynx and Larynx

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A 48-year-old woman was diagnosed with cavernous hemangioma of hypopharynx and larynx, which extended to the trachea and mediastinum. She was scheduled for tracheostomy and open surgical excision of hypopharynx hemangioma under general anesthesia. On induction of anesthesia, we planned awake fiberoptic intubation according to the difficult airway algorithm of the American Society of Anesthesiologists. Under continuous infusion of remifentanil at 0.1-0.2 $\mu g \cdot kg^{-1} \cdot min^{-1}$, the patient became sedated while spontaneously breathing, and her pain and laryngeal reflexes were reduced. Although tracheal intubation was successfully accomplished without injuring the hypopharynx hemangioma, tracheostomy was difficult because of bleeding from the surgical site. After 3hr of surgery with 1,880 g of blood loss, the surgeons quitted tracheostomy and the patient was transferred to the intensive care unit. Her airway was managed with endotracheal tube for 7 days, and open surgical excision of hypopharynx hemangioma was performed on day 7. The patient was successfully extubated on day 9 with the support of non-invansive positive pressure ventilation. Awake fiberoptic intubation under remifentanil infusion is safe and useful approach for patients with airway hemangioma.

key words：airway hemangioma, awake fiberoptic intubation, remifentanil

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Laudanosine has No Effects on Respiratory Activity but Induces Non-Respiratory Excitement Activity in Isolated Brainstem-Spinal Cord Preparation of Neonatal Rats

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Abstract Laudanosine, a degradation of neuomuscular blocking agent atracurium, crosses the blood-brain barrier and is indicted to trigger seizures at high concentration. In *Xenopus Oocytes* expressing nicotinic acetylcholine receptors (nAChRs), laudanosine has activating and inhibiting effects on nAChRs depending on its concentration. nAChRs is related to respiratory activities and thus, in the present study, we analyzed effects of laudanosine on central respiratory activities using isolated brainstem-spinal cord preparation of neonatal rats. The rhythmic inspiratory burst activity of the C4 spinal ventral root was recorded using a glass suction electrode as an index of respiratory rate. After superfusion with mock cerebrospinal fluid (CSF), the preparation was superfused with mock CSF containing laudanosine 1, 10 or 100 μM for 60 minutes. Laudanosine 1, 10 and 100 μM (n = 10 in each) did not induce any effects on C4 respiratory rate. In all 10 preparations, laudnosine 100 μM induced non-respiratory excitement activities that are possibly same as seizure observed in vivo study.

1 Introduction

Laudanosine is a metabolite of the neuromuscular blocking agent atracurium (Fodale and Santamaria 2002). It crosses the blood-brain barrier and accumulates in the cerebrospinal fluid (CSF) (Eddleston et al. 1989; Tassonyi et al. 2002), although neuromuscular blocking agents do not cross the blood-brain barrier. In the in vivo, laudanosine penetrated into CSF induces excitement (Lanier et al. 1985; Beemer et al. 1989) and seizure (Chapple et al. 1987).

Although laudanosine has no muscle relaxation effects via muscular nicotinic acetylcholine receptor (nAChR), laudanosine induces the dual mode of action on neuronal nAChR; it inhibits α4β2 and α7 neuronal nAChRs expressed in *Xenopus Oocytes* at high concentration, whereas it activates α4β2 neuronal nAChRs at low concentrations (Chiodini et al. 2001). nAChR subunits α4β2 and α7 expressed in ventrolateral medulla modulate respiratory activities (Hatori et al. 2006).

Therefore, we investigated the effects of laudanosine on respiratory activities using isolated brainstem-spinal cord preparation of neonatal rats.

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2 Methods

This study was approved by the Animal Ethical Committee of Teikyo University Experiments were performed on the brainstem-spinal cord preparation of neonatal Wistar rat (0–4 days old; $n = 30$). The surgical procedure used to make these preparations has been described in detail elsewhere (Sakuraba et al. 2003). Briefly, the rats were deeply anesthetized with diethyl ether, and the brainstem and cervical spinal cord were isolated in a chamber filled with oxygenated mock CSF. Then, the cerebellum and pons were ablated. The isolated preparation was continuously superfused at the rate of 3.5–4.5 mL/min in a 2-ml recording chamber with the ventral side upwards. The preparation was superfused at 26°C with control mock CSF equilibrated with a 95% O_2 and 5% CO_2 (pH = 7.5). The composition of the mock CSF was (in mM) 118 NaCl, 3 KCl, 1.5 $CaCl_2$, 1 $MgCl_2$, 25 $NaHCO_3$, 1.2 NaH_2PO_4, and 30 glucose.

Inspiratory discharges of respiratory motor neurons were monitored by extracellular recording with glass suction electrodes applied to the proximal cut end of C4 ventral roots of spinal nerves, and amplified with a differential AC amplifier (Model 1700, A-M systems, Carlsborg, WA, USA) and integrated (time constant: 100 ms). Axoscope software and Digidata 1200B interface (Axon Instruments, Foster, CA, USA) were used to collect data for off-line analysis.

C4 respiratory rate were calculated from the total number of bursts within a 4-min period before switching the superfusate. After the preparation was superfused with control mock CSF for 20 min and C4 activity reached a steady state, the control superfusate was replaced by a test solution: mock CSF containing laudanosine at 1, 10 and 100 μM (Sigma, St. Louis, MO, USA) for 20 min, followed by a washout period using the mock CSF for 40 min. C4 respiratory rate was counted at 0 min (control), 10 min and 20 min after superfusion with mock CSF containing laudanosine.

Changes in C4 respiratory rate were compared by using one-way analysis variance followed by Dunnet test. $P < 0.05$ was considered significant. Data are expressed as mean ± SD.

3 Results

Laudanosine 1, 10 and 100 μM ($n = 10$ in each) did not induce any effects on C4 respiratory rate (Fig. 1). Laudanosine 100 μM induced non-respiratory excitement activities in all 10 preparations (Fig. 2).

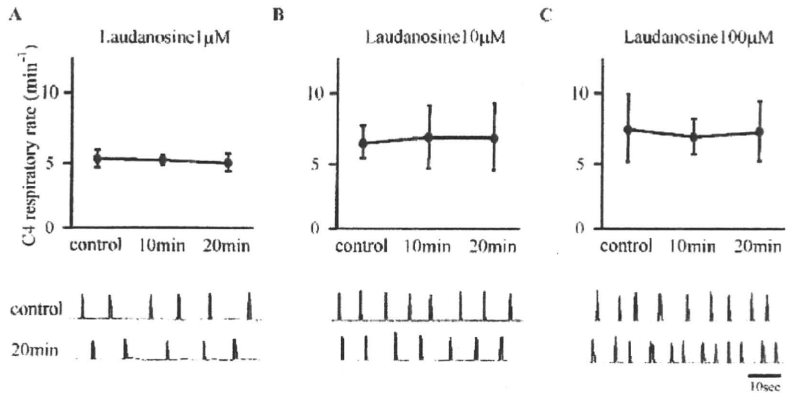

Fig. 1 Effects of laudanosine 1 μM (**A**), 10 μM (**B**) and 100 μM (**C**) on C4 respiratory rate

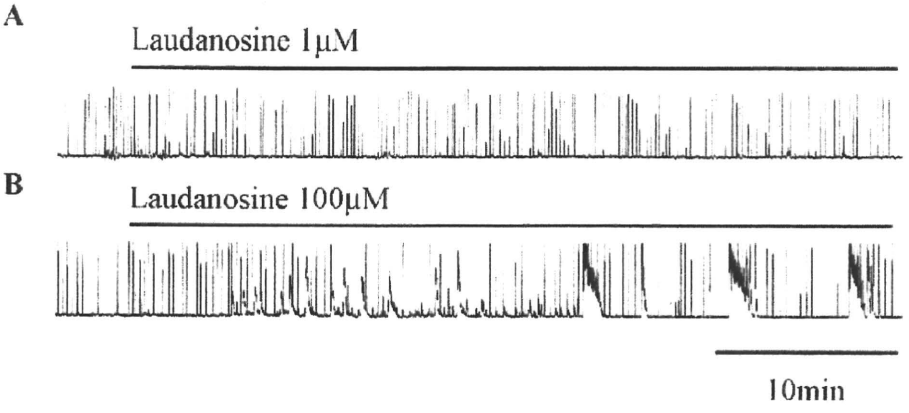

Fig. 2 Representative recording of non-respiratory excitement activities induced by laudanosine 100 μM (**B**). Laudanosine 1 μM (**A**) and 10 μM do not induce such activities

4 Discussion

Laudanosine does not induce any changes in respiratory activities. However, it is unclear that it is due to its no effects on central respiratory control or due to its interactive effects on several kinds of receptors because laudanosine is indicated to have effects not only on nAChRs but also on SK channel, opioid receptors and so on. Further pharmacological studies to prevent its potential interactive effects on several receptors are needed.

On the other hand, high concentration of laudanosine induces non-respiratory excitement activities like vecuronium bromide and apamin, SK channel antagonist, reported in the previous studies using the same preparation (Onimaru et al. 1996;

Sakuraba et al. 2003). Therefore, laudanosine may induce non-respiratory excitement activities through neuronal nAChRs or SK channel. Although vecuronium bromide suppresses respiratory activities (Sakuraba et al. 2003), apamin induces no effects on respiratory activities (Onimaru et al. 1996). SK channel expresses on many neurons and inhibits neuron activities by hyperpolarization. Thus, laudanosine-induced non-respiratory excitement activity in the present study is more possibly through SK channel.

In conclusion, laudanosine has no effects on respiratory activities but high concentration of laudanosine induces non-respiratory excitement activities.

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Effect of JM-1232(-), a New Sedative on Central Respiratory Activity in Newborn Rats

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Abstract JM-1232(-), a newly manufactured isoindole derivative, shows sedative effect at a lower concentration compared with propofol. In the present study, we analyzed the response of the central respiratory activity to JM-1232(-). The brainstem-spinal cord of a newborn rat was isolated and was continuously superfused with oxygenated artificial cerebrospinal fluid (ACSF). Rhythmic inspiratory burst activity was recorded from C4 spinal ventral root using a glass suction electrode. We measured C4 burst rate and amplitude of integrated C4 activity. After obtaining a control recording, the preparation was superfused with ACSF containing JM-1232(-) at 10, 100 or 500 μM for 10 min. The application of both 10 and 100 μM JM-1232(-) did not decrease C4 burst rate significantly. However, 500 μM JM-1232(-) reduced C4 burst rate. On the contrary, C4 burst amplitude was not affected by the application of JM-1232(-) for 10 min at any concentrations. In conclusion, JM-1232(-) at a low concentration (but presumably higher than hypnotic dose), did not depress the central respiratory activity, whereas at a high concentration depression was seen.

1 Introduction

JM-1232(-), a newly manufactured isoindole derivative, shows sedative effect at a low concentration and possesses a wide therapeutic index compared with propofol (Kanamitsu et al. 2007). The drug can be used without emulsion because of its water-soluble property (Kanamitsu et al. 2007).

Recently, several studies have showed the effect of JM-1232(-) on nociceptive stimuli (Nishiyama et al. 2008; Chiba et al. 2009) and shivering (Masamune et al. 2009). However, there has been no thorough investigation of JM-1232(-)-induced respiratory depression. In the present study, we analyzed the response of the central respiratory activity to JM-1232(-).

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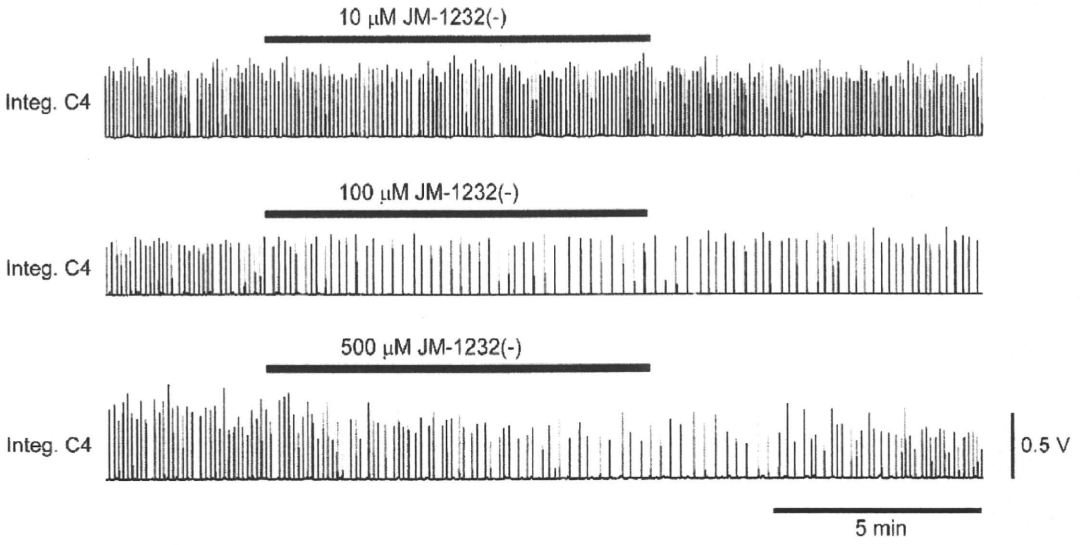

Fig. 1 Representative recordings of the integrated C4 activity (Integ. C4) before, during, and after superfusion with 10, 100 or 500 μM JM-1232(-) The horizontal bars indicate the duration of superfusion with JM-1232(-)

2 Methods

All procedures were conducted in accordance with the institutional guidelines regarding the care of the animals.

Data were obtained from 20 newborn Wistar rats (0–4 days old). Generation of the isolated brainstem-spinal cord preparation has been described in detail elsewhere (Kuwana et al. 1998). In brief, the rats were anesthetized with diethyl ether and the brainstem caudal to the caudal cerebellar artery and cervical spinal cord were isolated in a chamber filled with oxygenated artificial cerebrospinal fluid (ACSF). The cerebellum and pons were ablated. Each preparation was placed ventral side up in a recording chamber (volume, 2 ml) and superfused (flow 4 ml · min^{-1}) with control ACSF equilibrated with a control gas mixture (5% CO_2 in oxygen; pH7.4). Its temperature was maintained at 25–26°C. The composition of the ACSF was (in mM): 126 NaCl, 5 KCl, 1.25 NaH_2PO_4, 1.5 $CaCl_2$, 1.3 $MgSO_4$, 26 $NaHCO_3$ and 30 glucose. C4 ventral root activity was recorded using a glass suction electrode, amplified with a conventional alternating current amplifier (AVH 11, Nihon Kohden, okyo, Japan), and integrated (time constant: 100 m sec). We measured C4 burst rate as an index of the inspiratory rate (Murakoshi et al. 1985) and the integrated amplitude as an index of the tidal volume (Eldridge 1971).

After obtaining the recording with control ACSF, the preparation was superfused with ACSF containing JM-1232(-) (Maruishi Pharmaceutical Co. Ltd., Osaka, Japan) at 10, 100 or 500 μM for 10 min followed by washout for 20–30 min using control ACSF.

Table 1 **Effect of JM-1232(-) on C4 burst activity**

Concentration (μM)	n		Control	JM-1232(-)	Washout
10	6	C4 burst rate (min^{-1})	8.1 ± 1.4	7.9 ± 1.5	7.9 ± 1.4
		C4 burst amplitude (V)	0.50 ± 0.13	0.53 ± 0.13	0.51 ± 0.12
100	8	C4 burst rate (min^{-1})	8.6 ± 2.9	6.4 ± 2.1	9.2 ± 4.1
		C4 burst amplitude (V)	0.55 ± 0.09	0.55 ± 0.09	0.51 ± 0.10
500	6	C4 burst rate (min^{-1})	9.4 ± 1.4	5.4 ± 1.9*	6.8 ± 3.2
		C4 burst amplitude (V)	0.50 ± 0.11	0.47 ± 0.14	0.46 ± 0.14

*$P < 0.01$

All recorded signals were fed into a personal computer after analog/digital conversion (Power Lab/4sp, ADInstruments, Castle Hill, Australia) for subsequent analysis (Chart version 5, ADInstruments, Castle Hill, Australia). Analysis of the respiratory parameters was performed off-line. Respiratory parameters obtained before the superfusion of the ACSF containing drugs were defined as control values. C4 burst rate and amplitude were compared using a one-way analysis of variance, followed by a Dunnett test. All statistical analyses were conducted using Graph-Pad Prism 3.0 software (Graph-Pad Software Inc., San Diego, CA). All values were reported as the mean ± SE and all P values < 0.05 were considered significant.

3 Results

Representative recordings of integrated C4 activity before, during and after superfusion with JM-1232(-) -containing ACSF are shown in Fig. 1. The application of 10 μM JM-1232(-) for 10 min did not decrease C4 burst rate significantly (98 ± 5% of control rate) (Table 1). C4 burst rate slightly decreased by superfusion of ACSF containing 100 μM JM-1232(-) for 10 min (75 ± 8% of control rate), but this decrease was not significant (Table 1). However, the application of 500 μM JM-1232(-) for 10 min significantly reduced C4 burst rate (56 ± 5% of control rate) (Table 1). C4 burst amplitude was not changed by the application of JM-1232(-) for 10 min at any concentrations (Table 1).

4 Discussion

We have demonstrated that the threshold of respiratory depression in JM-1232(-) should lie between 100 or 500 μM.

The peak blood concentration in in vivo rats was 0.78 μM when 0.76 mg JM-1232(-), which corresponds to the hypnotic dose, was given intravenously. Therefore, the peak brain concentration should be lower than 0.78 μM. On the other hand, propofol concentration in the brain tissue of rat in vivo was reported to be approximately 80–200 μM when an anesthetic dose of propofol was given intravenously (Shyr et al. 1995). Thus, the concentration of JM-1232(-) in the brain tissue after the injection of an anesthetic dose may be lower than that of propofol. Conversely, in the

brainstem-spinal cord preparations, only 5 μM propofol induced respiratory depression (Kashiwagi et al. 2004), whereas 500 μM JM-1232(-) only 44% reduction in respiratory rate. Moreover, the application of flumazenil, a benzodiazepine receptor antagonist may reverse JM-1232(-)-induced respiratory depression by the following reasons. First, JM-1232(-) was reported to act through the benzodiazepine site of γ-amino butyric acid type A receptors (Masamune et al. 2009). Second, flumazenil reverse the antinociceptive effect of JM1232(-) (Nishiyama et al. 2008; Chiba et al. 2009). Considering previous report and our results, JM-1232(-) seems to have a wider safety margin in respiratory depression than propofol.

In summary, JM-1232(-) at a low concentration (but presumably higher than hypnotic dose), did not depress the central respiratory activity, whereas at a high concentration depression was seen.

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Thyroid Transcription Factor-1 Influences the Early Phase of Compensatory Lung Growth in Adult Mice

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Rationale: **Compensatory lung growth has been well described as a phenomenon in many animal models, but still little is known about the nature, extent, and modulation of such growth. We hypothesized that compensatory lung growth may at least in part recapitulate developmental lung growth, and factors known to be important during normal lung development, such as thyroid transcription factor 1 (TTF-1), may be reactivated during compensatory lung growth.**

Objectives: **To investigate the role of TTF-1 in correlation with the morphological changes during compensatory lung growth.**

Methods: **Sequential changes in TTF-1 expression and morphology were examined in the residual right lung after left pneumonectomy in 9-week-old mice. The effect of temporary knockdown of TTF-1 on compensatory lung growth was also evaluated.**

Measurements and Main Results: **TTF-1 was transiently but significantly elevated at an early stage in compensatory lung growth. Morphologically, a process resembling septation in lung development may have been initiated during this period in the vicinity of the alveolar duct. Furthermore, temporary knockdown of TTF-1 transiently but significantly delayed the early phase of compensatory lung growth.**

Conclusions: **These results indicate the influential role of TTF-1 in modulating, and possibly initiating, the early phase of compensatory lung growth. Morphologically, compensatory lung growth may at least in part resemble developmental growth.**

Keywords: thyroid transcription factor 1; septation; alveolar duct

Lung resection continues to be the primary treatment for many types of lung diseases, including cancer and inflammatory lung diseases. One of the most important factors that determine the level of resectability is the residual lung function. We know clinically that after lung resection in adults the residual lung increases in volume to some extent, but this is considered to be primarily hyperinflation with minimal recovery and possibly even deterioration in lung function (1). On the other hand, in children, recovery in lung function after lung resection has been reported (2). It is well established that alveoli multiply after birth up until about 8 years of age (3), and in addition, adult lungs transplanted into immature recipients have been reported to show hyperplastic growth (4). These results suggest that it may be possible, at least in part, to restore or augment compensatory growth capability even in adult lungs.

Compensatory lung growth after lung resection has been reported in many animal models, including mice (5, 6). Compensatory lung growth has been well described as a phenomenon, but still little is known about the nature, extent, and modulation of such growth. The involvement of multiple factors, such as epidermal growth factor (7), hepatocyte growth factor (8), keratinocyte growth factor (9), and vascular endothelial growth factor (10), has been implicated in compensatory lung growth, but what triggers and what drives compensatory lung growth is still not clear. Although still controversial, it has been postulated that compensatory lung growth may at least in part recapitulate developmental lung growth (11). If so, compensatory lung growth may occur via partial reactivation of normal developmental pathways, and factors known to be important during normal lung development, such as thyroid transcription factor 1 (TTF-1), may be reactivated during compensatory lung growth. The reported reappearance of TTF-1 in regions of regenerating lung after lung injury supports the possibility that TTF-1 plays a role in alveolar cell growth and differentiation and that TTF-1 may be a critical factor in the restoration of alveolar structures that accompanies recovery from functional loss after lung diseases or lung injury (12).

To our knowledge, the role of TTF-1 in compensatory lung growth has not been closely investigated. In the present study, we show that TTF-1 expression was transiently elevated at an early stage in compensatory lung growth and that morphologically, a process resembling septation during lung development may have been initiated during this period in the vicinity of the alveolar duct. Furthermore, temporary knockdown of TTF-1 delayed the early phase of compensatory lung growth, indicating its influential role in modulating and possibly initiating the early phase of compensatory lung growth.

AT A GLANCE COMMENTARY

Scientific Knowledge on the Subject

Compensatory lung growth has been well described in many animal models as a phenomenon, but still little is known about the nature, extent, and modulation of such growth.

What This Study Adds to the Field

Here we show that expression of thyroid transcription factor 1, a factor known to be indispensable in normal lung development, significantly influences the early phase of compensatory lung growth in adult mice, and that morphologically, a process resembling septation in lung development may be initiated during this period in the vicinity of the alveolar duct.

METHODS

Animal Experiments

Specific pathogen-free, 9-week-old, inbred male C57BL/6 mice, weighing approximately 20 g, were purchased from CLEA Japan, Inc. (Tokyo,

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Japan). The mice were kept in a 12-hour light/12-hour dark cycle with free access to food and water. The mice were randomly assigned to three experimental groups: left thoracotomy under mechanical ventilation (THX group), left pneumonectomy under mechanical ventilation (PNX group), or 9-week-old male mice without any interventions (CON group).

The mice were anesthetized with 100 mg/kg of ketamine and 10 mg/kg of xylazine administered subcutaneously. They were intubated with an 18-gauge catheter and connected to a rodent ventilator, adjusted to maintain a respiratory rate of 100 breaths/minute, 10-ml/kg tidal volume, 2 cm H_2O positive end-expiratory pressure, and 0.21 inspired oxygen. A 20-mm long posterolateral skin incision was made, followed by thoracotomy in the fifth intercostal space with dissection of the serratus anterior and a latissimus dorsi muscles. In the THX group, thoracotomy was closed without pneumonectomy. In the PNX group, the whole left lung was resected from the pleural cavity. The left main bronchus with left pulmonary artery and vein were ligated at the hilum with 5–0 silk before removal of the lung. The tidal volume was reduced from 10 to 6 ml/kg after the lung was removed. The fifth intercostal space was closed with a single surgical suture, and the skin and muscle incisions were closed with two sutures to avoid excessive tension on the muscles. The duration of mechanical ventilation for the whole surgical procedure was approximately 10 min in both groups. All mice recovered quickly after termination of mechanical ventilation and were promptly extubated. The mice were weighed and were observed daily for any signs of distress or changes in behavior. The mice were killed at respective time points by injection of 100 mg/kg of ketamine and 10 mg/kg of xylazine, followed by exsanguination from the inferior vena cava.

All experimental protocols were reviewed by the Committee on the Ethics of Animal Experiments at the School of Medicine, Keio University, and were performed in accordance with Guidelines for Animal Experiments issued by the School of Medicine, Keio University Experimental Animal Center.

Cell Line

A mouse lung epithelial cell line, MLE12 (CRL-2110, American Type Culture Collection, Manassas,VA), was used to evaluate the efficacy of TTF-1 silencing oligonucleotides. This cell line is known to express TTF-1 (13). The cells were maintained in Dulbecco's medium: Ham's F12, 50:50 mix, supplemented with insulin (0.005 mg/ml), transferrin (0.01 mg/ml), sodium selenite (30 nM), hydrocortisone (10 nM), β-estradiol (10 nM), *N*-2-hydroxyethylpiperazine-*N'*-ethane sulfonic acid (10 mM), l-glutamine (2 mM), and 2% fetal bovine serum, under 5% CO_2.

Western Blot Analysis for TTF-1

For TTF-1 Western blot analysis, the right lung was resected at respective time points, blotted dry, immediately snap frozen in liquid nitrogen, and stored at −80°C. Western blot analysis for TTF-1 protein was performed according to a standard protocol. Briefly, lung tissue was lysed with a denaturing RIPA buffer (Sigma, Stockholm, Sweden), the lysate was centrifuged at 14,000 rpm for 15 minutes at 4°C, and the supernatant was mixed with Laemmli buffer and applied to sodium dodecyl sulfate–polyacrylamide gel electrophoresis gels. The proteins were separated by 12.5% sodium dodecyl sulfate–polyacrylamide gel electrophoresis under reducing conditions and then transferred to poly vinylidene di fluoride (PVDF) membrane for 90 minutes at 90 V using HorizBlot system (ATTO, Tokyo, Japan). After blocking nonspecific reactions with Block Ace (Dainippon Pharmaceutical, Osaka, Japan), the primary antibody for TTF-1 (H-190; Santa Cruz Biotechnology Inc., Santa Cruz, CA) or antibody for β-actin (Abcam, Cambridge, UK) were incubated with the blot overnight at 4°C. The secondary anti-rabbit IgG, ECL anti-rabbit IgG horseradish peroxidase linked with whole antibody (GE Healthcare, UK), was incubated with the blots for 1 hour at room temperature. Bands were detected by enhanced chemiluminescence using ECL Western blotting detection reagents (Amersham Bioscience Corp Buckinghamshire, UK). Band densitometry was quantified using Image J (NIH, Bethesda, MD). Values were normalized to β-actin.

Histological Analyses

For histological analyses, the right lung was inflated with intratracheal instillation of 10% buffered formalin at a pressure of 20 cm H_2O. The trachea was tied under pressure, and the lung was fixed in the chest cavity for 48 hours before removal. Total right lung volume was measured from the fixed specimen by volume displacement as described by Scherle (14), and was normalized to the body weight as lung volume index (LVI). The lung tissue was then embedded in paraffin, and cut sagittally in 4-μm sections. Hematoxylin and eosin staining was done for morphological analyses. Light microscopic morphometric techniques were applied, and the alveolar surface area per unit of lung volume (SVw) was measured as previously described by Weibel (15) and Kawakami and colleagues (16). Briefly, a standard line of the same length (LT) was drawn on the field, and intersections with this line were counted (Iw). SVw was calculated as SVw = 2 Iw/LT. Alveolar duct area was traced and calculated using Image J. Morphologically, alveoli were identified as polyhedral, cup, or wedge-shaped terminal air spaces with discrete septae, whereas terminal, somewhat elongated air spaces from which alveoli emerged were considered as alveolar ducts (17). Five fields were analyzed per animal in four animals.

Immunohistochemistry for TTF-1 and Ki-67 was performed as follows. The primary antibodies used were: anti–TTF-1 rabbit polyclonal antibody (5 μg/ml: clone H-190) and anti–Ki-67 rabbit monoclonal antibody (10 μg/ml: clone SP6; LabVision, Fremont, CA). Secondary antibodies used were: anti-rabbit Ig Immpress (Vector Laboratories, Burlingame, CA) for both. Then they were visualized with 3, 3′-diaminobenzidine (DAB) (Sigma). Nuclei staining positive were counted and expressed in proportion to the number of nuclei in alveolar septal cells. For further analysis, we subgrouped the alveolar septal cells into cells associated with the alveolus (AL), the alveolar duct (AD), or the septal structure protruding into the alveolar duct (ADS), respectively (Figure 2A). Double-staining for TTF-1 and prosurfactant protein C (proSPC) was performed as follows. Anti-proSPC rabbit polyclonal antibody (5 μg/ml: ab28744; Abcam, Cambridge, MA) was used as the primary antibody. Anti-rabbit Ig Immpress and DAB were also used as described above. Thereafter, the sections were rinsed in 1 M glycine-hydrochloric buffered solution for 2 hours. Then the incubation with anti–TTF-1 antibody and was done by ALP-ABC system: biotinylated goat anti-rabbit IgG (Nichirei Bioscience, Tokyo, Japan) and ALP conjugated Strept ABC complex (Dako, Glostrup, Denmark). The final product was visualized Fast Red Substrate Kit (Nichirei).

For each analysis, one section was randomly selected per animal, and five 200-fold magnification fields were randomly selected per section. The slides were coded and masked for identity and were examined by Y.T. and E.I.

Lung Dry Weight Measurements

The lung dry weight in proportion to body weight, lung dry weight index (LDWI), was measured as a gross assessment of compensatory lung growth. The resected lungs were completely dried in a vacuum drying oven (DP22; Yamato Scientific, Tokyo, Japan) at 95°C, and at −270 cm H_2O for 48 hours, and then were weighed.

Knockdown of TTF-1 by Small Inhibitory RNA

Five TTF-1–silencing small inhibitory RNA oligonucleotides, si#1 through si#5, were synthesized and purified by Invitrogen (Carlsbad, CA). Briefly, single-strand RNA was synthesized by the phosphoramidite method. After synthesis of single-strand RNA, RNA was deprotected in two steps from base and phosphate protecting group and 2′-hydroxyl function protecting. Single-strand RNA was desalted or purified after deprotection. The single-strand RNA was annealed as siRNA. By Western blot analysis using MLE 12 cells, oligonucleotides si#2, and si#4 were found to be effective (data not shown). Sequences of si#2 and si#4 were (5′-UUGAAACGUCGCUCGAGCUCGUACA-3′) and (5′-GCUACAAGAUGAAGCGCCGGCUAA-3′), respectively. As control nonsilencing oligonucleotide (nonsi), stealth RNAi negative control duplex (Invitrogen) was used. Each inhibitory RNA was administered intranasally as previously reported (18) using surface active material, cationic cardiolipin analog (CCLA)-based liposome (NeoPharm, Inc., Waukegan, IL). Thirty-five milligrams per kilogram of si#2, si#4, or nonsi were administered into the nasal orifices using a microliter pipetter mixed with CCLA as a total of approximately 25 μl. The administration was done approximately 30 minutes after extubation in both thoracotomy (THXsi#2, THXsi#4, and THXnonsi groups) and pneumonectomy (PNXsi#2, PNXsi#4, and PNXnonsi groups), at which time the mice had sufficiently recovered breathing but were still im-

mobilized. The right lung was resected for histology and protein analyses as described.

RNA Isolation and Reverse Transcription-Polymerase Chain Reaction

Total RNA was isolated by using Isogen (Nippon Gene, Tokyo, Japan) from the residual right lung 12 hours after left pneumonectomy and subsequent administration of TTF-1 siRNAs (#2, #4) or nonsilencing oligonucleotide. Reverse transcriptase–polymerase chain reaction (PCR) for mouse TTF-1 mRNA was performed as described previously (19). The PCR oligonucleotide primers used were 5′-GAGCTGCCTGACGGCC AGGT-3′ (forward) and 5′-TACTCCTGCTTGCTGATCCA-3′ (reverse) for β-actin, and 5′-AACAGCGGCCATGCAGCAGCAC-3′ (forward) and 5′-CCATGTTCTTGCTCACGTCC-3′ (reverse) for TTF-1.

The PCR was performed in 50 mM KCl, 10 mM TRIS, pH 8.3, 1 mM dNTP, 0.5 mM each primer, 5% dimethyl sulfoxide, 2 units Taq DNA polymerase, and $MgCl_2$ (1.5 mM for β-actin, and 1.0 mM for TTF-1). The PCR conditions were 94°C for 5 minutes for 1 cycle followed by 30 to 40 cycles of 94°C for 1 minute, 56°C for 1 minute, 72°C for 1 minute, with a final extension cycle of 72°C for 7 minutes. The products of these reactions were resolved by gel electrophoresis on 2% agarose gels and stained with ethidium bromide.

Statistical Analysis

Data are expressed as mean ± SD. Comparisons between groups were done using Mann-Whitney *U* test (StatView; Abacus, Berkeley, CA). Body weight was compared within groups using paired *t* test (StatView). Other comparisons within groups were done using Mann-Whitney *U* test because the animals were killed for respective time point measurements. *P* values less than 0.05 were considered to be significant.

RESULTS

TTF-1 Protein Expression was Increased Promptly and Transiently in the Right Lung after Left Pneumonectomy

Western blot analysis showed that TTF-1 protein expression in the right lung was increased in the PNX group in comparison with the THX group. This tendency was observed as early as 1 hour, was most significant at 12 hours, and then was diminished beyond 24 hours (Figures 1A and 1B). TTF-1 protein expression in the THX group was not significantly different in comparison with the CON group. Analysis by immunohistochemistry showed that within the PNX group, TTF-1–positive alveolar septal cells were increased significantly at 12 hours (Figure 2A). Double staining for TTF-1 and proSPC at 12 hours in the PNX group indicated that TTF-1–positive cells were also proSPC positive (Figure 2B). The overall proportion of TTF-1–positive alveolar septal cells gradually decreased after 12 hours to the level close to the CON group by 48 hours, although statistical significance was still present (Figure 2C). This tendency was similar in AL, AD, and ADS cells, but the magnitude of increase in comparison with the CON group seemed to be most prominent in the AD cells (Figures 2D–2F). These results indicated that there was a prompt and temporary up-regulation of TTF-1, presumably in the type II alveolar cells in the right lung after left pneumonectomy, and that this up-regulation appeared to be predominant in the AD cells.

In the Residual Right Lung after Left Pneumonectomy, LVI Increased Immediately, after an Increase in LDWI

The body weight of the mice was reduced in the PNX and THX groups, in comparison with the CON group at 24 and 48 hours. Although statistically significant, the difference was 7% at most (Figure 3A). The decreased body weight in the PNX and THX groups was regained from 3 days. The body weight of the mice did not differ significantly between the PNX group and the THX group throughout the experiment period. Therefore, it was considered feasible to compare weight-based indices between these groups.

On gross appearances of the fixed specimens, the residual right lung in the PNX group seemed to be larger than the right lung in the CON group at 48 hours (Figure 3B). Based on the TTF-1 expression data, we examined the macroscopic changes in the right lung after left pneumonectomy focusing primarily on the early phase of compensatory lung growth. Residual right LVI was increased significantly in the PNX group in comparison with the THX group as early as 1 hour after left pneumonectomy (Figure 3C). Within the PNX group, this increase in residual right LVI continued until 7 days but leveled off beyond 7 days. There was no significant change within the THX group during this period, and no significant differences between the THX group and the CON group. In contrast to the changes in LVI, residual right LDWI did not increase significantly in the PNX group in comparison with the THX group until 48 hours (Figure 3D). Beyond 48 hours, this increase continued within the PNX group until 7 days and then leveled off beyond 7 days. Residual right LDWI in the PNX group at 7 days was not statistically different from the total LDWI (right plus left) in the CON group (Figure 3E). There were no significant changes in right LDWI within the THX group during this period, and no significant differences in the right LDWI between the THX group and the CON group. These results suggested that initially there was right lung expansion as early as 1 hour after left pneumonectomy, followed by compensatory lung growth, which became apparent by 48 hours and progressed until approximately

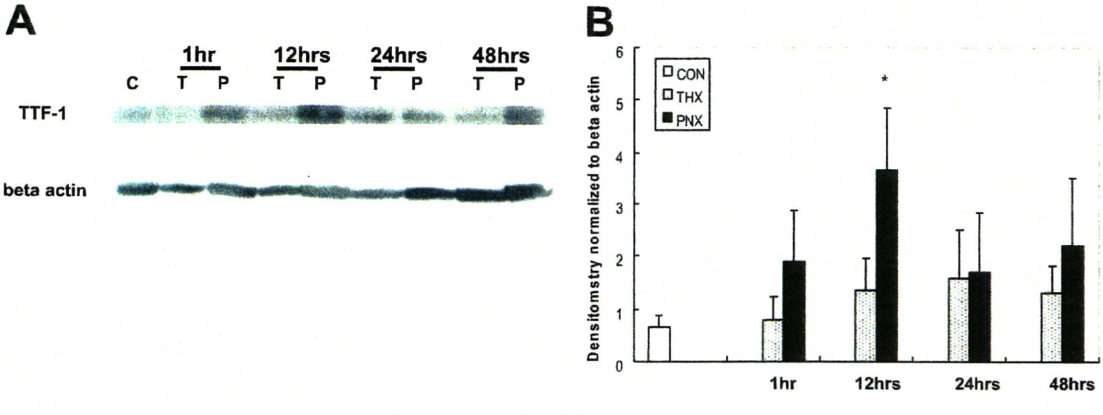

Figure 1. Thyroid transcription factor 1 (TTF-1) protein expression by Western blot analysis in the right lung after left pneumonectomy (PNX group), left thoracotomy (THX group), or no intervention (CON group). (*A*) TTF-1 expression was increased in the PNX group (P) in comparison with the THX group (T) and the CON group (C). (*B*) Densitometry values were normalized to β-actin. TTF-1 expression was increased in the PNX group (n = 4 for each time point) in comparison with the THX group (n = 4 for each time point), and the CON group (n = 4). Statistical significance was seen at 12 hours. *P = 0.02 versus CON and THX at 12 hours.

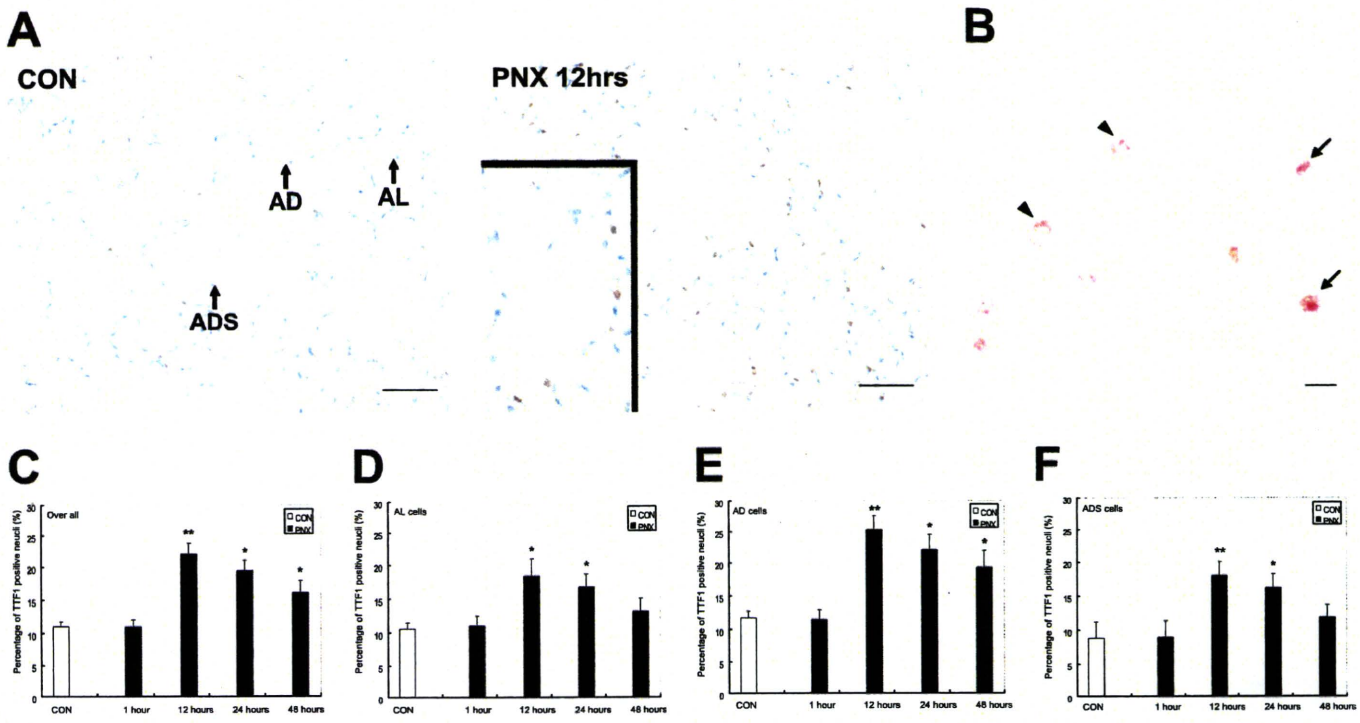

Figure 2. The presence and proportion of thyroid transcription factor 1 (TTF-1)-positive nuclei was evaluated by immunostaining in the right lung after left pneumonectomy (PNX group), or no intervention (CON group). (*A*) TTF-1–positive cells were prominent at 12 hours in the PNX group by immunohistochemistry. For further analysis, the alveolar septal cells were subgrouped into cells associated with the alveolus (AL), the alveolar duct (AD), or the septal structure protruding into the alveolar duct (ADS), as represented. Inset shows TTF-1–positive nuclei in the PNX group, primarily in AD cells (scale bars, 100 μm). (*B*) Double staining for TTF-1 and prosurfactant protein C (proSPC) at 12 hours in the PNX group indicated that TTF-1–positive cells were also proSPC positive. TTF-1 was identified by red-colored nuclei, and proSPC by brown-colored granules in the cytoplasm. Cells positive for both TTF-1 and proSPC are indicated by *arrows*, whereas TTF-1–negative but proSPC-positive cells are indicated by *arrowheads* (scale bar 10 μm). (*C*) Within the PNX group (n = 4, 20 fields for each time point), the overall proportion of TTF-1–positive alveolar septal cells was increased significantly at 12 hours, after which it was gradually decreased to the level close to the CON group (n = 4, 20 fields) by 48 hours. $*P < 0.0001$ versus CON and PNX 1 hour; $**P < 0.0001$ versus all other measurements. This tendency was similar in (*D*) AL cells, $*P < 0.0001$ versus CON and PNX 1 hour; (*E*) AD cells, $*P < 0.0001$ versus CON and PNX 1 hour; $**P < 0.0006$ versus all other measurements; and (*F*) ADS cells, $*P < 0.01$ versus CON and PNX 1 hour. $**P < 0.01$ versus all other measurements. The magnitude of increase in comparison with the CON group seemed to be most prominent in the AD cells.

7 days, at which time the residual right LDWI in the PNX group matched the total (right plus left) LDWI in the CON group.

Alveolar Duct Area in the Right Lung Increased Promptly and Temporarily after Left Pneumonectomy

Histological appearance showed that there was initially a significant increase in the right lung alveolar duct size in the PNX group in comparison with the THX group. This increase was not so apparent beyond 48 hours (Figure 4A). The calculated alveolar duct area showed a similar trend, showing a significant increase in the PNX groups in comparison with the CON group at 1 hour, followed by a gradual decline over time in the PNX group to a similar level as the CON group (Figure 4B). Statistical significance between CON group and PNX group was lost at 3 days. As for the calculated surface area of the alveoli per volume of lung, in the PNX group it tended to decrease from 1 hour to 24 hours in comparison with the CON group, which was recovered by 48 hours (Figure 4C). The number of alveoli per field also showed a similar trend (Figure 4D), indicating that the decrease in the calculated surface area of the alveoli per volume of lung in the PNX group was primarily due to the increase in the alveolar duct area, and that the sizes of the alveoli were not altered during this period. These results suggested that the immediate increase in LVI was managed primarily through the expansion of the alveolar duct, which was restored, possibly by a subsequent increase in the number of surrounding alveolar septal cells.

Alveolar Septal Cell Proliferation after Left Pneumonectomy Was Most Prominent in the ADS Cells

Further analysis by immunohistochemistry showed that overall, the proportion of alveolar septal cells with Ki-67–positive nuclei was significantly increased beyond 12 hours in the PNX group until 7 days in comparison with the CON group (Figure 5A). The difference was not significant at 14 days. This tendency was similar in AL, AD, and ADS cells, but the magnitude of increase seemed to be most prominent in the ADS cells particularly at 48 hours (Figures 5B–5E). Furthermore, the number of ADS cell nuclei per field increased transiently and significantly at 24 and 48 hours in the PNX group (Figure 6), suggesting that these cells have proliferated and that they may have been incorporated into the newly formed alveolar septal cells.

Transient TTF-1 Knockdown Temporarily Delayed Compensatory Lung Growth

In the PNXsi#2 and PNXsi#4 groups, expression of TTF-1 mRNA was reduced at 12 hours in comparison with the PNXnonsi group (Figure 7A). TTF-1 expression by Western analysis was significantly reduced in comparison with the PNXnonsi group at 48

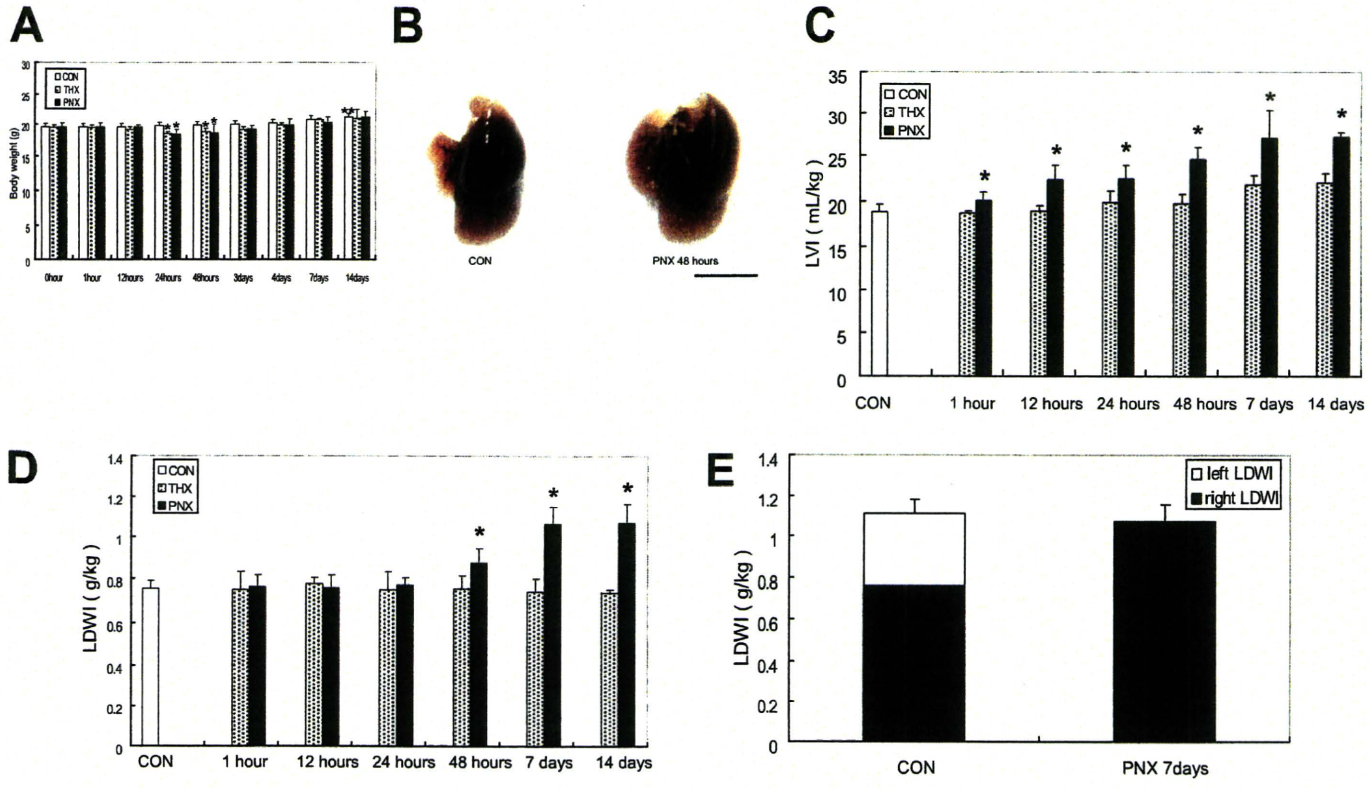

Figure 3. Compensatory lung growth was evaluated in the right lung after left pneumonectomy (PNX group), left thoracotomy (THX group), or no intervention (CON group). (*A*) The body weight of the mice was reduced in the PNX (n = 4) and THX (n = 4) groups in comparison with the CON (n = 4) group at 24 and 48 hours, but was regained from Day 3. The body weight of the mice did not differ significantly between the PNX group and the THX group throughout the observation period; $*P = 0.02$ versus respective CON. In the CON group, body weight was significantly increased at 14 days in comparison with 0, 1, 12, and 24 hours, indicating animal growth, $**P < 0.05$ versus CON group at 0, 1, 12, and 24 hours. (*B*) On gross appearances of the fixed specimens, the residual right lung seemed to be larger in the PNX group in comparison with the CON group at 48 hours (scale bar, 5 mm). (*C*) Lung volume index (LVI) in the right lung increased significantly in the PNX (n = 4 for each time point) group in comparison with the THX (n = 4 for each time point) group beyond 1 hour. Within the PNX group, this increase in LVI continued until 7 days but leveled off at 14 days. There was no significant change within the THX group during this period, and no significant differences between the THX group and the CON (n = 4) group, $*P < 0.05$ versus CON and THX at corresponding time points. (*D*) Lung dry weight index (LDWI) in the right lung did not increase significantly in the PNX (n = 4 for each time point) group in comparison with the THX (n = 4 for each time point) group until 48 hours. Within the PNX group, this increase leveled off beyond 7 days. There were no significant changes in LDWI within the THX group during this period and no significant differences between the THX group and the CON (n = 5) group, $*P < 0.05$ versus CON and THX at corresponding time points. (*E*) LDWI of the right lung in the PNX (n = 4) group at 7 days was not statistically different from the total LDWI (right plus left) in the CON group (n = 5).

hours (Figure 7B). The effect of TTF-1 knockdown was transient, as this difference was not apparent at 7 days (Figure 7C). LDWI was also significantly decreased in the PNXsi#2 and PNXsi#4 groups in comparison with the PNXnonsi group at 48 hours, but was restored to a similar level as the PNXnonsi group by 7 days (Figure 7D). TTF-1 expression was not significantly different between the THXsi#2 and the THXsi#4 group in comparison with the THXnonsi group at 48 hours (Figure 7E). Histological studies showed no apparent indications of lung injury after TTF-1siRNA administration (data not shown). In the PNXsi#2 and PNXsi#4 groups, the alveolar duct area remained significantly increased at 48 hours in comparison with the PNXnonsi group (Figure 7F). This difference was not significant in the THXsi#2, THXsi#4, and THXnonsi groups at 48 hours (Figure 7G), indicating that the increase in alveolar duct area in the right lung after left pneumonectomy was prolonged by TTF-1 knockdown. These results suggested that TTF-1siRNA administration transiently suppressed the induction of TTF-1 in the right lung after left pneumonectomy without significantly affecting the baseline expression of TTF-1. Furthermore, compensatory growth in alveolar septal cells was temporarily delayed as a result of transient TTF-1 knockdown.

DISCUSSION

TTF-1 is a member of the Nkx2 family of homeodomain-containing transcription factors and is selectively expressed in the forebrain, the thyroid, and the lung. TTF-1 is required for lung morphogenesis, and is precisely regulated throughout lung development (20, 21). In the postnatal lung, TTF-1 is expressed primarily in type II epithelial cells and is required for the transcription of surfactant protein–associated genes (22).

In the present study, TTF-1 protein expression in the residual right lung was increased in the PNX group in comparison with the THX group. This increase was most significant in the PNX group at 12 hours, suggesting that TTF-1 may play a role in regulating the early phase of compensatory lung growth. From previous reports in rats, after a rapid phase of overinflation, compensatory lung growth progresses within 4 days followed later by a remodeling phase beyond 2 weeks up to 1 month (23).

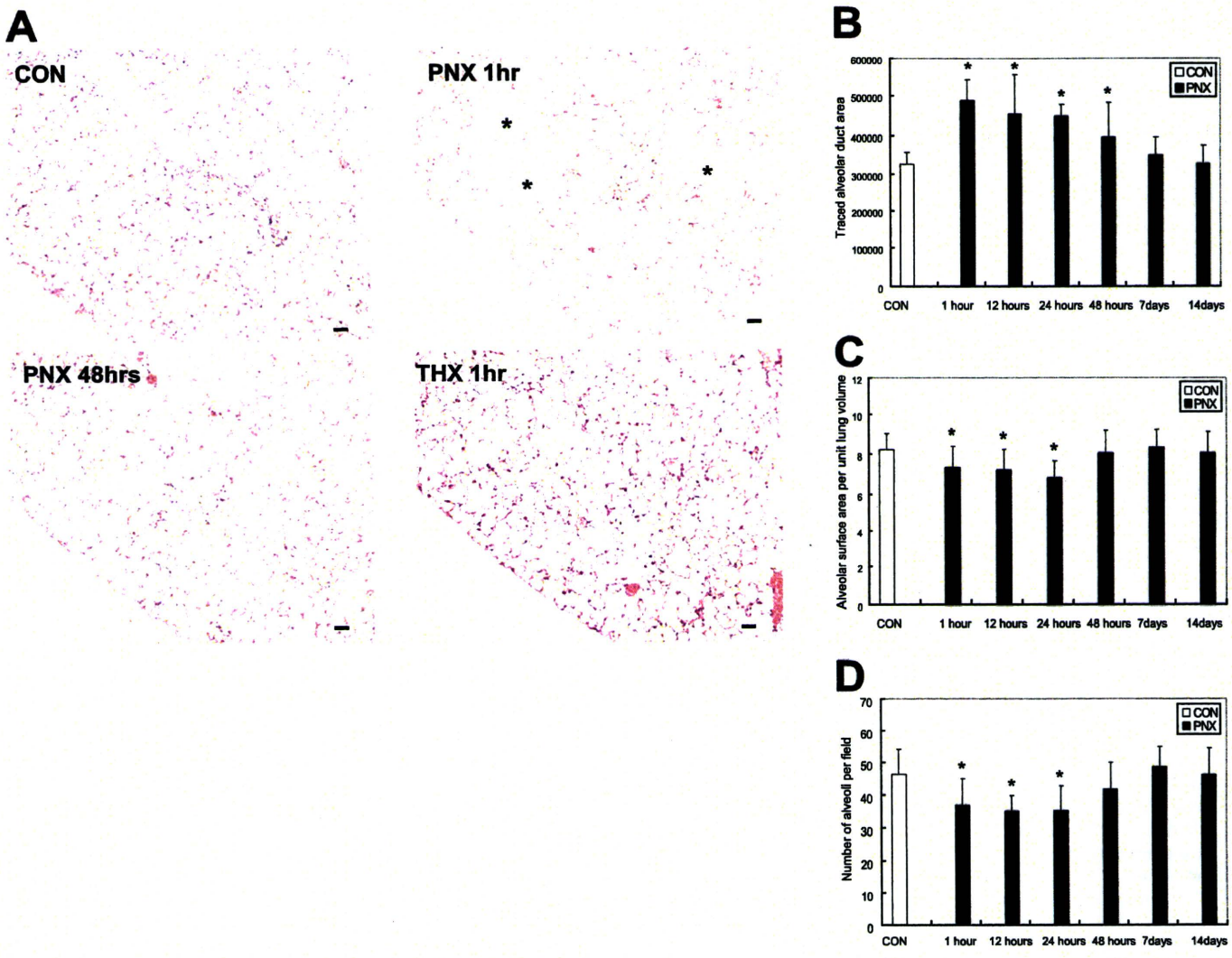

Figure 4. Morphological changes during compensatory lung growth in the right lung after left pneumonectomy (PNX group) or no intervention (CON group). (*A*)There was initially a significant increase in the right lung alveolar duct size in the PNX group (asterisks indicate representative expanded alveolar ducts) in comparison with the THX group. This increase was not so apparent beyond 48 hours (scale bars, 100 μm). (*B*) The alveolar duct area was traced using Image J. Within the PNX group (n = 4, 20 fields for each time point), the calculated alveolar duct area at 1 hour showed a significant increase in comparison with the CON (n = 4, 20 fields) group, followed by a gradual decline over time to a similar level as the CON group. Statistical significance between CON group and PNX group was lost beyond 3 days, $*P < 0.0001$ versus CON. (*C*) The surface area of the alveoli per unit lung volume was calculated as described in the methods. The calculated surface area of the alveoli per unit lung volume in the PNX (n = 4, 20 fields for each time point) group tended to decrease from 1 hour to 24 hours in comparison with the CON (n = 4, 20 fields) group, which was recovered by 48 hours, $*P < 0.03$ versus CON. (*D*) The change in number of alveoli per field in the PNX (n = 4, 20 fields for each time point) group also showed a similar trend in comparison with the CON (n = 4, 20 fields) group as the surface area of the alveoli per unit lung volume, $*P < 0.0001$ versus CON.

But morphological studies looking at the earlier phase of compensatory lung growth are still few. Based on the TTF-1 expression data, we examined the morphological changes focusing primarily on the early phase of compensatory lung growth after left pneumonectomy in mice. In the present study, compensatory lung growth was apparent beyond 48 hours and leveled off at approximately 7 days, as shown by the changes in residual right LDWI. The increase in the residual right LDWI was preceded by the swift increase in the residual right LVI after left pneumonectomy. Morphological analyses showed that the alveolus size did not change significantly during compensatory lung growth, and suggested that the initial increase in lung volume was primarily due to the increase in alveolar duct size, which was evident as early as 1 hour, corresponding with the increase in residual right LVI. The increase in alveolar duct area was alleviated beyond 48 hours presumably due to compensatory growth of the surrounding alveolar septal cells.

It is known that lung distention, or mechanical stretch, has an important role in the initiation of compensatory lung growth and is possibly more influential than hypoxia or changes in pulmonary blood flow (24). The present results suggested that distention was initially most prominent in the right lung alveolar duct region after left pneumonectomy. Analysis of proliferation by regional subgrouping of alveolar septal cells into AL, AD, and ADS cells suggested that initial compensatory lung growth may have occurred primarily through the proliferation of ADS cells, which exist in the vicinity of the extended alveolar duct. Increase in ADS cells may represent septation, possibly from AD cells or the

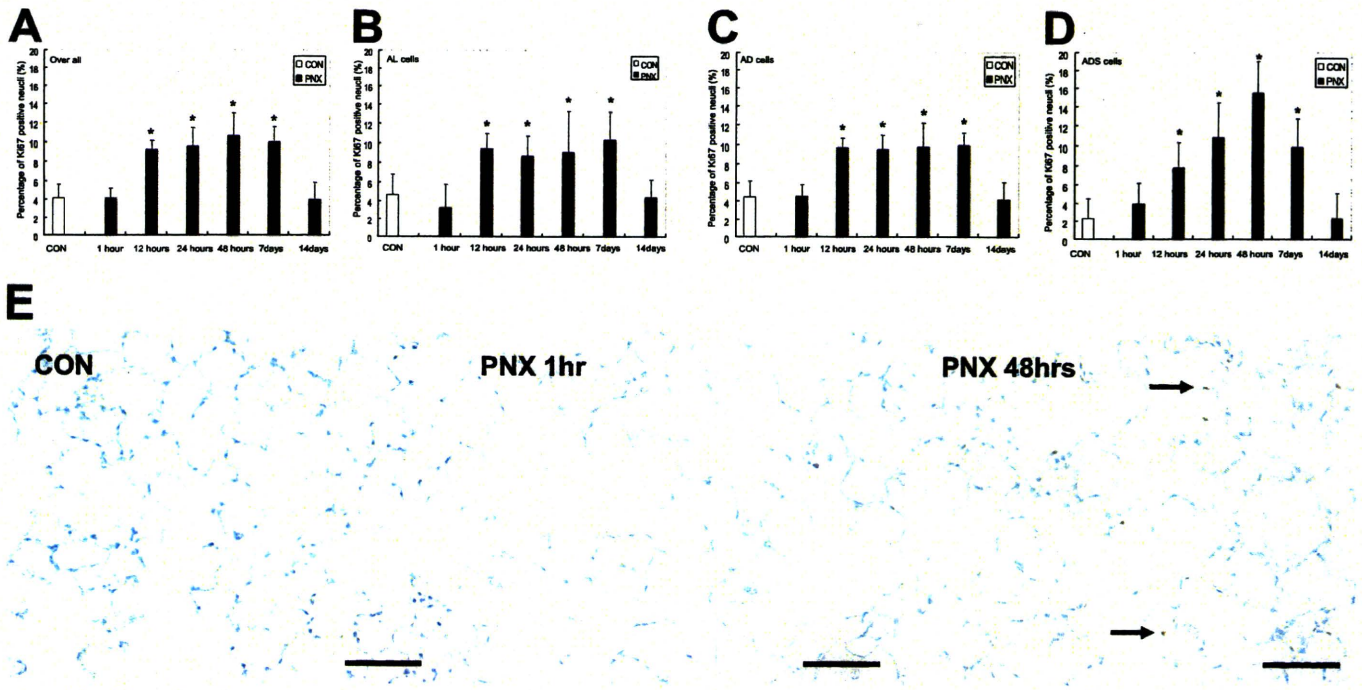

Figure 5. Cell proliferation was evaluated sequentially in the right lung after left pneumonectomy (PNX group), or no intervention (CON group) by Ki-67 immunostaining. (*A*) By immunohistochemistry, the overall proportion of alveolar septal cells with Ki-67–positive nuclei was significantly increased beyond 12 hours in the PNX group (n = 4, 20 fields at each time point) until 7 days in comparison with the CON group (n = 4, 20 fields). The difference was not significant at 14 days, $*P < 0.01$ versus CON, PNX 1 hour, and PNX 14 days. This tendency was similar in (*B*) alveolus (AL) cells, $*P < 0.03$ versus CON, 1 hour, and 14 days; (*C*) alveolar duct (AD) cells, $*P < 0.001$ versus CON, 1 hour, and 14 days; and (*D*) septal structure protruding into the alveolar duct (ADS) cells, $*P < 0.003$ versus CON, 1 hour, and 14 days. (*D* and *E*) The magnitude of increase seemed to be most prominent in the ADS cells, particularly at 48 hours (*arrows* indicate Ki-67–positive ADS cells, scale bars, 100 μm).

cells in the vicinity of the alveolar duct, and at least on qualitative morphology the process appeared to be resembling septation in lung development. Furthermore, preceding the increase in Ki-67–positive cells, the proportion of TTF-1–positive cells was most prominently increased in the AD cells where presumably the magnitude of stretching was greatest. These results suggest that a process resembling septation may have occurred through the proliferation of ADS cells, which was correlated with the increase in TTF-1 expression in the stretched AD cells. The TTF-1–positive cells were also proSPC-positive, indicating that these are type II alveolar cells. Furthermore, transient TTF-1 knockdown temporarily but significantly delayed the alleviation of alveolar duct expansion and compensatory lung growth at 48 hours. The results of this study correlatively suggest that TTF-1 influences the early phase of compensatory lung growth. Compensatory lung growth may be initiated by mechanical stretch in the alveolar duct region leading to a process resembling septation, which presumably occurs at least in part via reactivation of normal developmental pathways. What triggers compensatory lung growth is still not clear, but factors known to be important during normal lung development, such as TTF-1, may be transiently reactivated during the early phase of compensatory lung growth and possibly initiate the process. Lung-specific TTF-1 overexpression after pneumonectomy as well as TTF-1 knockdown may provide additional evidence, but because pneumonectomy by itself significantly elevates TTF-1 expression, the results of such experiments may be difficult to interpret.

Other possibilities remain, such as the transient elevation of TTF-1 in the present study being a part of an injury-repair response (25). However, at least morphologically, we found no apparent indications of lung injury in the residual lung after pneumonectomy. We consider that even if TTF-1 elevation is a repair response, it can still be considered as an important factor in compensatory lung growth, because compensatory lung growth may itself require, at least in part, an injury-repair response. Notwithstanding, the role of TTF-1 in lung development and compensatory lung may need to be distinguished. Recent reports

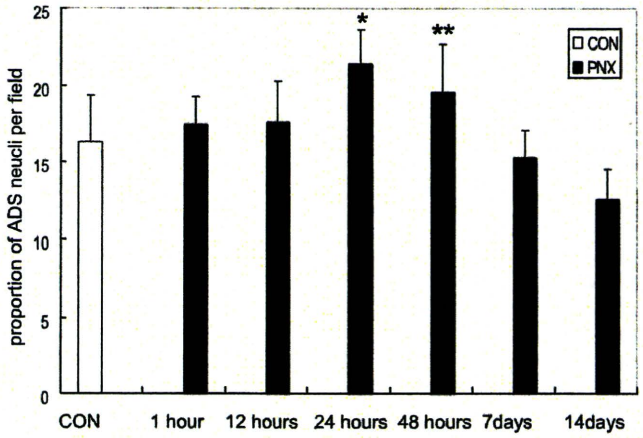

Figure 6. The proportion of the nuclei of the cells in the septal structure protruding into the alveolar duct (ADS) per field was increased transiently but significantly at 24 and 48 hours in the left pneumonectomy (PNX; n = 4, 20 fields for each time point) group; $*P < 0.02$ versus CON group (n = 4, 20 fields) and all other time points; $**P < 0.02$ versus CON group and other time points except PNX 12 hours.

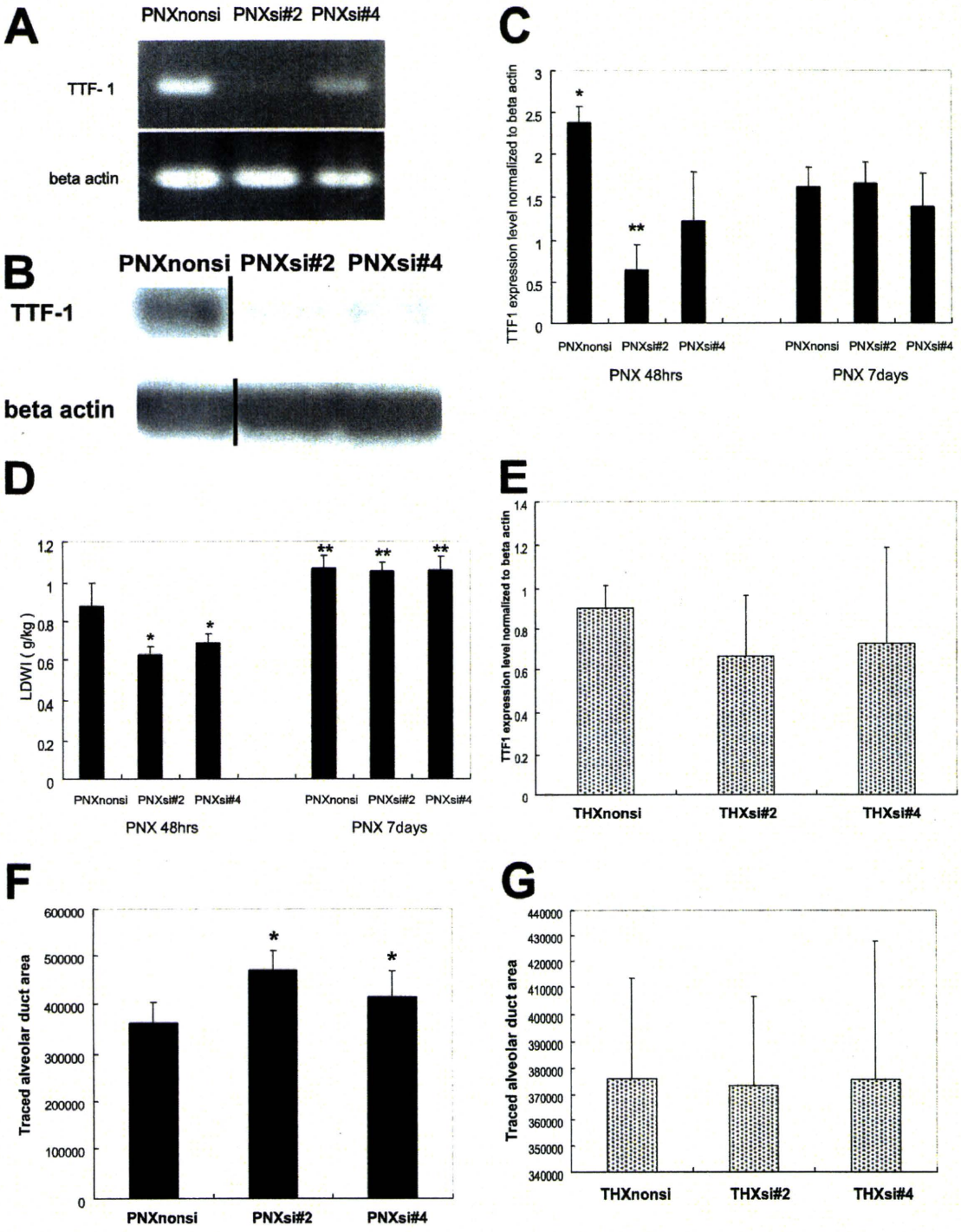

Figure 7. After left pneumonectomy and subsequent intranasal administration of small inhibitory RNAs (siRNAs) (PNXsi#2, PNXsi#4, and PNXnonsi groups) or thoracotomy and subsequent intranasal administration of siRNAs (THXsi#2, THXsi#4, and THXnonsi groups), thyroid transcription factor 1 (TTF-1) expression level in the right lung was measured. Densitometry values were normalized to β-actin for Western blot analyses. (*A*) Mouse TTF-1 mRNA expression was analyzed by reverse transcriptase–polymerase chain reaction at 12 hours. In the PNX group, the TTF-1 mRNA expression level was decreased by the administration of siRNAs. (*B*) In the PNX group, administration of siRNAs significantly reduced TTF-1 expression level at 48 hours. TTF-1 and β-actin for the PNXnonsi group was taken from a separate gel. (*C*) In the PNXsi#2 (n = 5) and PNXsi#4 (n = 5) groups, TTF-1 expression by Western blot analysis was significantly reduced in comparison with the PNXnonsi (n = 5) group at 48 hours. At 7 days, the level of TTF-1 expression was significantly reduced in all groups in comparison with the PNXnonsi group at 48 hours. The effect of administration of siRNAs was no longer apparent at 7 days. $*P < 0.03$ versus all other measurements; $**P < 0.03$ versus all other measurements except PNXsi#2 at 48 hours. (*D*) Right lung dry weight index (LDWI) was significantly decreased in the PNXsi#2 (n = 4) and PNXsi#4 (n = 4) groups in comparison with the PNXnonsi (n = 4) group 48 hours, but was restored to a similar level as the PNXnonsi (n = 4) group by 7 days, $*P < 0.05$ versus PNXnonsi at 48 hours; $**P < 0.05$ versus all measurements at 48 hours. (*E*) TTF-1 expression was not significantly different between the THXsi#2 (n = 3) and the THXsi#4 (n = 3) group in comparison with the THXnonsi (n = 3) group at 48 hours. (*F*) Alveolar duct area was traced using Image J. In the PNXsi#2 (n = 3), and PNXsi#4 (n = 3) groups, alveolar duct area remained significantly increased at 48 hours in comparison with the PNXnonsi (n = 3) group, $*P < 0.01$ versus PNXnonsi. (*G*) The difference in alveolar duct area was not significant in the THXsi#2 (n = 3), THXsi#4 (n = 3), and THXnonsi (n = 3) groups at 48 hours.

indicate that TTF-1 works in concert with multiple coactivators to achieve normal alveolarization in lung development (26, 27). Observations into the changes in the potential downstream effectors of TTF-1 will be necessary to further dissect the potential mechanisms involved in compensatory lung growth.

In the present study, it is likely that we observed primarily the changes in alveolar cells rather than the endothelial cells or the interstitial cells, which also play important roles in compensatory alveolar septal tissue growth (28). TTF-1 was considered to be expressed predominantly in the type II alveolar cells, but further