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DANCE in developing and injured lung

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Jean, Jyh-Chang, Ifeanyi Eruchalu, Yu Xia Cao, and Martin Joyce-Brady. *DANCE* in developing and injured lung. *Am J Physiol Lung Cell Mol Physiol* 282: L75–L82, 2002.—We identified rat developing arteries and neural crest derivatives with multiple epidermal growth factor-like domains (*DANCE*) as a developmentally regulated gene using suppression-subtractive hybridization. Northern analysis confirmed a fivefold induction of this mRNA transcript between *fetal day 18* and *20* that persisted through *postnatal day 17*. The level was declining at *postnatal day 21* and was similar in adult lung to that at *fetal day 18*. In adults *DANCE* mRNA abundance was highest in lung, kidney, and spleen, lower in heart, skeletal muscle, and brain, but absent from liver and thymus. It was abundant in pulmonary artery endothelium and a lung epithelial type 2 cell line, barely detectable in vascular smooth muscle, and absent in fibroblasts. In situ hybridization revealed a regulated pattern of expression in endothelial cells of fetal, postnatal, and adult lung. Because *DANCE* mRNA was inducible in systemic arteries during recovery from injury, we searched for induction in lung injured by hyperoxia. Mouse *DANCE* mRNA abundance was unchanged during an acute 3-day exposure period, induced threefold 5 days into the recovery phase, and returned to baseline at *days 8*, *11*, and *14*. In situ hybridization at *day 5* suggested a diffuse pattern of induction. *DANCE* may play a role in lung endothelial cell biology during development repair after injury.

developing arteries and neural crest derivatives with multiple epidermal growth-like factor domains; development; endothelium

NEAR THE END OF GESTATION the lung is transformed from an organ of secretion to one of gas exchange. This transformation is a complex process that extends into the postnatal period. The genes that regulate this change in lung architecture are poorly understood. We used suppression-subtractive hybridization to screen newborn vs. late fetal lung RNA for novel genes that are associated with this transition. Partial sequencing and analysis of 65 clones from this screen identified 10 that could encode a potentially novel gene product. We performed a series of Northern blots using total RNA from several stages of lung development and selected one of these clones for further study based on the abundance and the size of the mRNA transcript and the pattern of developmental regulation. The clone that we selected was very similar to a novel human gene of unknown function, *UP50* (GeneBank accession no. AF093118). It was represented by 3 independent clones from the original 65. During the course of our work, a full-length clone of human *UP50* together with the homolog in the rat and the mouse were characterized by two independent groups, and the gene was shown to be regulated during blood vessel development in the embryo and vascular remodeling in the adult. One group characterized the gene by the acronym *EVEC* for cDNA expressed in embryonic vasculature and the presence of Ca^{2+}-binding epidermal growth factor (EGF)-like repeats contained in the predicted protein structure (6). The other group named the gene by the acronym *DANCE* for developmental arteries and neural crest EGF-like (9). As a result of these studies, we hypothesized that *DANCE* expression in the lung would be regulated during perinatal blood vessel development and reactivated in the adult lung after injury.

MATERIALS AND METHODS

Animals and reagents. Timed-pregnant females and mature male rats and mice were obtained from Charles River Laboratories (Wilmington, MA). Animals were housed at the Animal Science Center at Boston University School of Medicine according to approved guidelines. Female rats were killed by pentobarbital sodium overdose at *days 18* and *20* postcoitus, and the fetuses were retrieved by hysterotomy. Rats at postnatal ages 1, 1.5, 4, 6, 14, 17, and 21 days and adults age 4–6 wk were killed in a similar fashion. Cell lines were obtained as follows. Murine lung alveolar type 2-derived MLE cells were provided by Dr. Amos Charles, primary bovine pulmonary artery endothelial cells by Dr. Robin Aguilar, and pulmonary plus aortic smooth muscle cells by Dr. Alan Fine, all at The Pulmonary Center, Boston University School of Medicine. Materials for agarose gel electrophoresis were obtained from International Biotechnologies (New Haven, CT). Probes for β-actin and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) were described previously (10).

RNA analysis. RNA was isolated from freshly harvested rat lung or confluent cultures of cells. Total RNA was prepared using TRIreagent (Molecular Research Center, Cincinnati, OH) according to the manufacturer's protocol and was quantitated by spectrophotometry. Ten micrograms were separated by electrophoresis in a 1% agarose gel, transferred to a Hybond membrane (Stratagene, Cedar Creek, TX) by capillary electrophoresis, and immobilized with a Stratagene ultraviolet light cross-linker according to standard protocols

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(12). A 930-bp *DANCE* DNA probe was available to us from our subtractive hybridization screen and had been cloned into the TA vector from InVitrogen (San Diego, CA; see Ref. 5). The insert was excised with *Eco*R I, purified by agarose gel electrophoresis, eluted, and quantitated spectrophotometrically. The probe was labeled with [^{32}P]dCTP using the Prime-A-Gene system from Promega (Madison, WI) according to the manufacturer's exact protocol, purified with Nuc-trap columns (Stratagene), and then hybridized to a nylon membrane with QuickHyb, again according to the manufacturer's protocol (Stratagene). Analysis of RNA integrity was performed by probing in an identical fashion for β-actin or GAPDH. In each case, the membrane was finally washed as directed, dried, exposed to Kodak X-OMAT AR film at −70°C, and developed. Autoradiographic signal intensity was quantitated by densitometry using a Molecular Devices instrument and was normalized to the signal for β-actin or rRNA.

In situ hybridization. A 276-bp RNA probe was generated by PCR amplification of rat *DANCE* cDNA using GCGGGGTACCACATCCACACACTGGTTGCC and GTCCCCGCGGACAGTGTTTAGATATTGATG as 5′- and 3′-primers, respectively. The PCR product was subcloned into pBluescript KS$^+$ using the *Sac* II and *Kpn* I restriction sites that were incorporated in the primers. Radiolabeled sense and antisense RNA probes were transcribed in vitro using T3 or T7 promoters, respectively, and [^{35}S]dUTP (800 Ci/mmol; Amersham, Arlington Heights, IL). Paraffin-embedded sections of rat lung at *fetal days 18* and *21.5*, *postnatal day 2*, and adult age 4 wk were rehydrated and subjected to the following sequential prehybridization steps. There was an initial treatment for 20 min with 4% paraformaldehyde (Polysciences, Warrington, PA), followed by a 3-min exposure to 26.7 μg/ml of proteinase K in buffer solution (Qiagen, Valencia, CA) and then another 5-min exposure to 4% paraformaldehyde, a 10-min incubation with a 1:400 dilution of acetic anhydride in 1 M triethanolamine, and then a 5-min wash each in PBS and 0.15 M NaCl. Slides were then dehydrated and air-dried. For hybridization, the tissue sections were incubated with 15 μl of hybridization solution that had been preheated to 80°C. The solution contained 30,000 counts·min^{-1}·μl^{-1} [^{35}S]RNA probe, 100 mM dithiothreitol (DTT), 50% formamide, 0.3 M NaCl, 20 mM Tris·HCl, pH 7.4, 5 mM EDTA, 10 mM $NaH_2PO_4 \cdot H_2O$, pH 8, 10% dextran sulfate, 1× Denhardt's solution, and 0.5 mg/ml yeast tRNA. The slides were covered with coverslips and incubated at 50°C for 16 h. Thereafter, they were washed sequentially with 10 mM DTT-5× saline-sodium citrate (SSC) at 50°C for 30 min, 10 mM DTT-50% formamide-2× SSC at 65°C for 20 min, 0.4 M NaCl-0.1 M Tris (pH 7.5)-50 mM EDTA (pH 8) at 37°C for 10 min, RNase A at 20 μg/ml and 37°C for 60 min, and 10 mM DTT-50% formamide-2× SSC at 37°C for 15 min. Last, they were incubated in 0.3 M ammonium acetate in 30, 60, 80, and 95% ethanol and finally 100% ethanol. The slides were then dipped in Kodak NTB-2 emulsion (Eastman Kodak, Rochester, NY) at 45°C, air-dried, exposed at 4°C for 3 wk, developed with Kodak D-19 developer, and visualized on a Leitz orthoplan microscope. A mouse embryo section at *day 12* of gestation was used as a positive control. Photographs were obtained using the Improvision Open-Lab Users Software program (Quincy, MA). Similar experiments and controls were performed on adult mouse lung on the 5th day of the recovery phase after a 3-day exposure to >95% O_2.

Exposure to hyperoxia. Mice were exposed to an atmosphere of >95% O_2 and balance nitrogen (Wesco Gases, Billerica, MA) for 72 h by enclosing their cages in an air-tight glove bag. O_2 concentration was monitored continuously by oximetry. Crystalit absorption medium (Pharmacal, Naugatuck, CT) was added to bedding in the cage at 10 g/ft^2. Drierite (Vacumed, Ventura, CA) and Sodasorb (Intertech Resources, Lincolnshire, IL) were dispersed throughout the glove bags to absorb water and CO_2. Thereafter, they were returned to room air, and lung tissue was harvested at 0, 1, 5, and 14 days. Controls were maintained in room air at all times. Additional studies were done in hyperoxia to examine the pattern of *DANCE* expression at *days 0*, *3*, *5*, *8*, *11*, and *14* in the recovery phase.

Statistics. To assess the degree of change in the level of mRNA expression, the results of three independent filter hybridization experiments were analyzed by densitometry, and the result was expressed as the mean ± SE (n = 3 experiments). The mean *DANCE* mRNA levels observed in the rat at *fetal days 18* and *20* were compared in a t-test for independent samples. The means of *DANCE* mRNA expression in the mouse lung after exposure to hyperoxia were compared by ANOVA and a Tukey's honest significance difference test using the Statistica software package of StatSoft (Tulsa, OK). A P value of <0.05 was considered significant.

RESULTS

Regulation of DANCE in the developing rat lung. Total lung RNA was analyzed by filter hybridization at the seven developmental time points shown in Fig. 1*A*. The 2.4-kb *DANCE* mRNA transcript is upregulated 4.96 ± 0.51-fold between *fetal day 18* and *day 20* of gestation ($P < 0.001$, $n = 3$). This level of expression is maintained at *postnatal days 1*, *1.5*, *4*, and *6*. In the adult lung, the mRNA level declines to or below that at *fetal day 18*. To further define the profile of postnatal *DANCE* expression, lung RNA from rats at 6, 14, 17, and 21 days of age was compared with that from adult lung in Fig. 1*B*. The level of *DANCE* mRNA expression at *postnatal day 6* is maintained through *postnatal days 14* and *17*. A decline is evident at *day 21* and again in the adult. Both membranes were probed for β-actin to control for mRNA integrity and loading. The *DANCE* autoradiogram in Fig. 1*A* was developed after a 2-h exposure of the nylon filter to film.

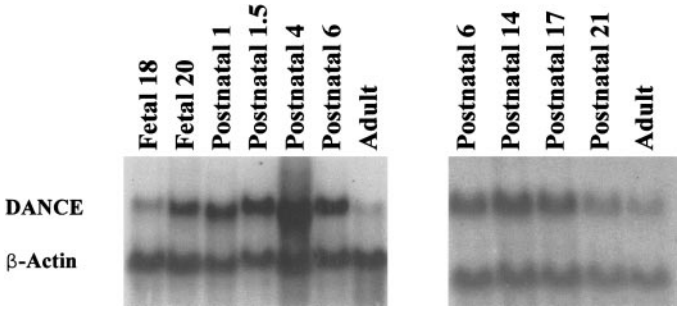

Fig. 1. Regulation of developmental arteries and neural crest epidermal growth factor-like (*DANCE*) mRNA in the lung. Total RNA was obtained from rat lung at several developmental time points and analyzed for *DANCE* and β-actin mRNA expression as described in MATERIALS AND METHODS. *A*: comparison of total lung RNA from 18 and 20 days of gestation, *postnatal days 1*, *1.5*, *4*, and *6*, and adult rats. Adult lung is at 6 wk of age. The *DANCE* autoradiogram in *A* was developed after a 2-h exposure of the nylon filter to film. *B*: comparison of total lung RNA from *postnatal day 6*, *14*, *17*, and *21* and adult rats.

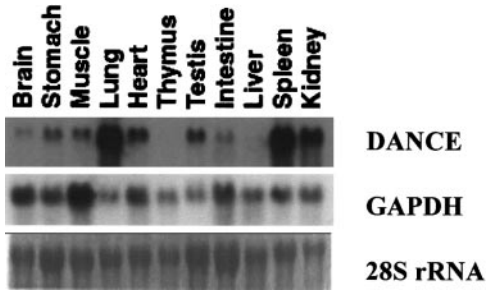

Fig. 2. Tissue-specific expression of *DANCE* mRNA in the rat. Total RNA was prepared and analyzed for *DANCE* mRNA expression from several tissues of adult rats as described in MATERIALS AND METHODS. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA expression was analyzed as a control for mRNA integrity and the ethidium bromide-stained 28S rRNA band as a loading control. The *DANCE* autoradiogram was developed after a 6-h exposure of the nylon filter to film.

Tissue-specific expression of DANCE in the adult rat. The distribution of *DANCE* mRNA expression in the adult rat was investigated by similar analysis with total RNA obtained from different tissues in Fig. 2. *DANCE* mRNA abundance is highest in the lung, the kidney, and the spleen followed by the heart, skeletal muscle, and the brain. No signal was detected in the liver or the thymus. The membrane was probed with GAPDH to control for RNA integrity. The ethidium bromide-stained 28S rRNA band is shown to compare RNA loading.

Cell-specific expression of DANCE. To determine the potential cell of origin of lung *DANCE* expression, we examined mRNA derived from several lung cell types by filter hybridization in Fig. 3. The signal was most abundant in primary pulmonary artery endothelial cells and a lung alveolar epithelial type 2 cell line (MLE cells). A much lower level of expression was evident in vascular smooth muscle cells derived from the pulmonary artery and the aorta. There was no detectable signal in human embryonic lung fibroblasts (IMR-90).

Localization of DANCE in developing rat lung by in situ hybridization. To determine the exact cellular site of *DANCE* expression in developing and adult rat lung, we used in situ hybridization to probe for *DANCE* mRNA in fetal lung at *days 18* and *21.5* of gestation in Fig. 4 and in postnatal lung 2 days after birth and 4 wk of age in Fig. 5. Lung tissue sections from these four developmental periods were examined at the same time using the same radiolabeled probe and processing materials so that the results are directly comparable. In all instances, *DANCE* expression was prominent in vascular cells, absent from epithelial cells, and detectable at a low level in the interstitium. Comparing the signal in *fetal day 18* lung with that of *fetal day 20* and *postnatal day 2*, we determined that the increase in *DANCE* mRNA expression reflects an increase in the level of expression per vascular cell and an increase in the number of blood vessels in the lung. Last, in contrast to that described in the mouse lung (6), a *DANCE* signal was still evident in virtually all of the blood vessels of the normal adult rat lung. These are distal branches of the pulmonary artery. A low level of expression is also detectable in the interstitium. This pattern of expression is very uniform. The specific signal was evident in sections of mouse embryo, which were used as a positive control, but not in those of rat lung exposed to the sense probe as the negative control (data not shown).

To examine the cellular site of *DANCE* expression in the distal branch of the pulmonary artery in greater detail, a higher magnification view from *postnatal day 2* is shown in Fig. 6, *A* and *C*. The signal is prominent in the endothelial cells but less detectable in the smooth muscle cells. Signal is absent in the adjacent airway epithelium but is still detectable within the interstitium. Figure 6*B* shows the phase-contrast image corresponding to the dark-field image in Fig. 6*A*.

DANCE induction in adult mouse lung after exposure to hyperoxia. In animal models of balloon-induced vascular injury, *DANCE* expression is upregulated only during the late phase of repair (6, 9). Therefore, we sought to determine if *DANCE* expression in the lung is altered in response to injury as well. We chose O_2 as the injury agent since a brief exposure to a toxic level of this gas induces an injury from which the animal can recover over time. Because we used mice for these experiments, we first confirmed that *DANCE* mRNA expression is developmentally regulated in newborn and adult mouse lung as in rat lung (Fig. 7*A*). We then performed the hyperoxia exposure experiment and analyzed total lung RNA at 0, 1, 5, and 14 days as described in MATERIALS AND METHODS and shown in Fig. 7*B*. No detectable changes in lung *DANCE* mRNA expression were observed in the control mice exposed to normal O_2. However, compared with the *day 0* control, *DANCE* mRNA was induced 3.2 ± 0.92-fold at *day 5* of the recovery phase ($P < 0.05$) in the mice that were exposed to hyperoxia. The mRNA level returned to near baseline at *day 14*. The filter was probed for 28S rRNA as a control for RNA loading. To further define the profile of *DANCE* induction in the posthyperoxia recovery period, lung RNA was examined at 0, 3, 5, 8, 11, and 14 days. Again the peak of induction occurred

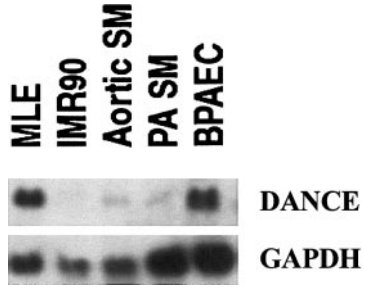

Fig. 3. Cell-specific expression of *DANCE*. Total RNA was prepared and analyzed for *DANCE* and GAPDH mRNA expression from different lung- and aorta-derived cells as described in MATERIALS AND METHODS. This *DANCE* autoradiogram was developed after a 24-h exposure of the nylon filter to film. SM, smooth muscle, PA SM, pulmonary artery SM; BPAEC, bovine pulmonary artery endothelial cells.

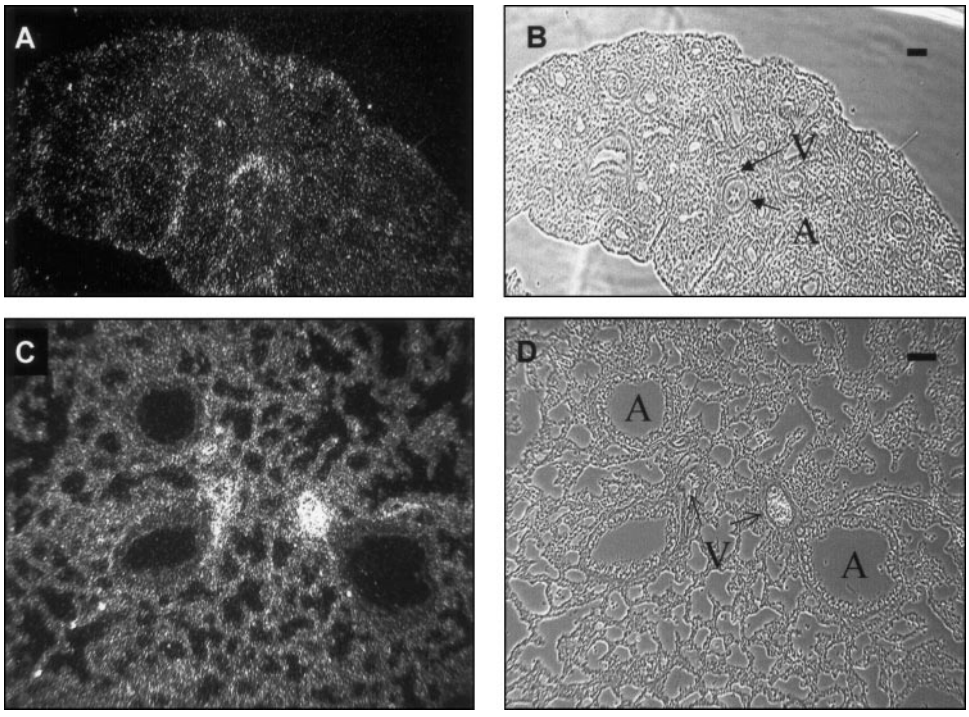

Fig. 4. *DANCE* mRNA localization in blood vessels of fetal rat lung by in situ hybridization. Tissue sections were obtained from fetal rat lung and processed for in situ hybridization as described in MATERIALS AND METHODS. Dark-field (*A* and *C*) and phase-contrast (*B* and *D*) images for fetal lung at *gestational day 18* (*A* and *B*) and *day 21.5* (*C* and *D*). A, airway; V, blood vessel. Magnification bar indicates 58 μm in *B* and 60 μm in *D*.

at *day 5* and declined by *day 8*. An in situ hybridization study was performed at the *day 5* time point in the control and the hyperoxia-exposed lung. No signal could be localized to a specific lung cell population, indicating that *DANCE* was upregulated in a diffuse pattern (data not shown).

DISCUSSION

DANCE is a mnemonic for a newly described gene that is expressed largely in cells of developing arteries and neural crest derivatives and whose protein product contains multiple EGF domains (9). This gene has also been characterized independently by another group as *EVEC* or a cDNA that is expressed in embryonic vasculature with multiple Ca^{2+}-binding EGF-like (cbEGF) repeats contained in the protein (6). In this study, we used *DANCE* to denote this gene. Both groups demonstrated that this mRNA transcript is expressed in vascular smooth muscle cells and endothelial cells of developing arteries in the embryo. The encoded product is a secreted protein that contains six cbEGF domains. This motif is also found in other proteins, and it

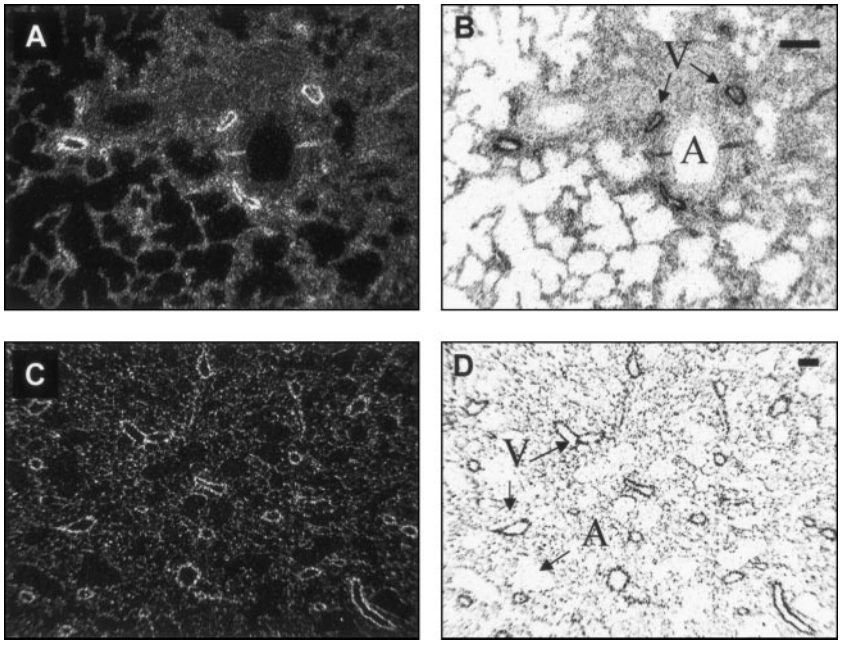

Fig. 5. *DANCE* mRNA localization in blood vessels of postnatal and adult rat lung by in situ hybridization. Tissue sections were obtained from postnatal rat lung and processed for in situ hybridization together with those of Fig. 4 as described in MATERIALS AND METHODS. Therefore, the results are directly comparable. Dark-field (*A* and *C*) and phase-contrast (*B* and *D*) images are shown for postnatal lung at 2 days of age (*A* and *B*) and for adult lung at 6 wk of age (*C* and *D*). Magnification bar indicates 125 μm in *B* and 140 μm in *D*.

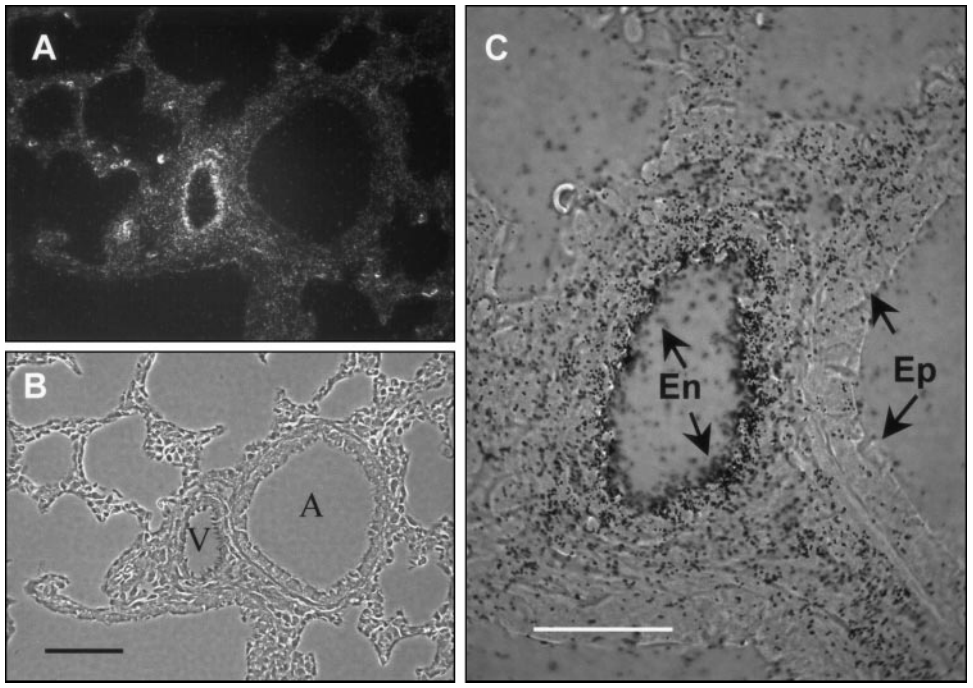

Fig. 6. *DANCE* mRNA expression predominates in lung endothelial cells. Dark-field (*A*) and corresponding phase-contrast (*B*) images of *postnatal day 2*. Magnification bar indicates 160 μm. *C*: higher-power view of the blood vessel in *A* showing that the in situ hybridization signal is intense in the endothelial cells (En), declines as one moves through the wall of the blood vessel and into the lung interstitium, and is absent in epithelial cells (Ep). Magnification bar indicates 30 μm.

can function as a stabilizer of tertiary protein structure or as a mediator of protein-protein interactions. The exact role of the multiple cbEGF motifs in *DANCE* protein is not yet known. However, an RGD sequence is embedded within the first cbEGF domain located at the NH_2 terminus. This sequence as been shown to mediate endothelial cell adhesion in vitro in an integrin-dependent fashion. Hence, *DANCE* protein functions as a ligand for integrins, and the RGD motif is recognized by many integrins, including $\alpha_5\beta_1$. Its multiple-domain structure suggests an ability to bind to other cell surface receptors as well. This gene is largely downregulated in most adult tissues but is inducible after blood vessel injury to systemic arteries. The late time course of this induction after injury has lead investigators to postulate a potential role for *DANCE* as a negative regulator of proliferation. *DANCE* and two other homologous genes, *UPH1* for *UP50* homolog 1 (GeneBank assession no. AF093119), and *S1–5*, a gene product in the EGF-like repeat family that stimulates DNA synthesis (7), are believed to comprise a new EGF-like protein family denoted as the "*DUS*" family (9).

DANCE expression in the embryonic mouse lung (E16.5) has been shown to be abundant in cells of the developing pulmonary outflow tract and the distal branches of the pulmonary artery by in situ hybridization. No lung cells exhibited an in situ signal of this intensity in the adult mouse lung, suggesting that a downregulation of *DANCE* gene expression after vessel

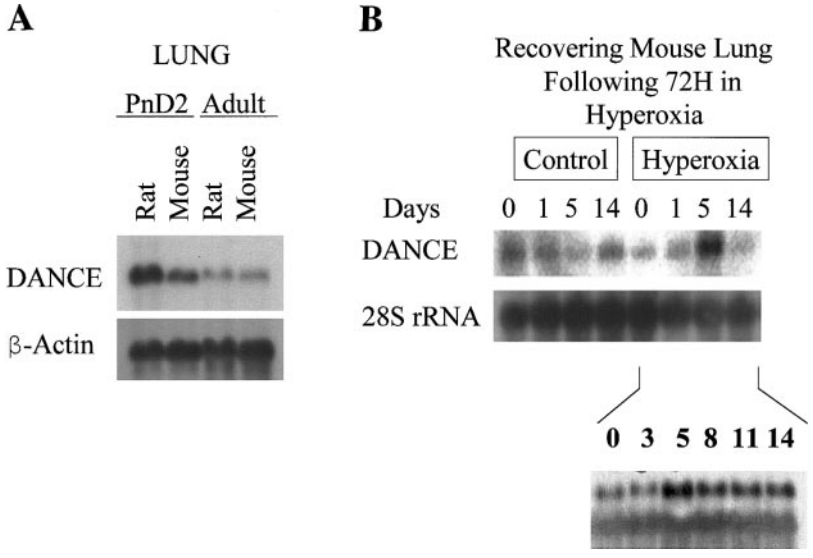

Fig. 7. *DANCE* expression in rat vs. mouse lung and induction after sublethal exposure of adult mice to hyperoxia. *A*: total RNA was obtained from *postnatal day 2* (PnD2) and adult rat and mouse lung and was analyzed for *DANCE* and β-actin mRNA expression as described in MATERIALS AND METHODS. *B*: mice were exposed to a normal control level or >95% O_2 (hyperoxia) for 3 days, and then total RNA was isolated from the lung at *day 0*, *1*, *5*, and *14* of the recovery period and analyzed for *DANCE* mRNA and 28S rRNA expression. This *DANCE* autoradiogram was produced after a 5-day exposure of the nylon membrane to film. To further define the temporal profile of *DANCE* induction after exposure to hyperoxia, total lung RNA was examined at six time points, including *days 0*, *3*, *5*, *8*, *11*, and *14*, and the results are shown in *B*, *bottom*.

development was complete (6). Using suppression-subtractive hybridization, we identified *DANCE* as a candidate gene that was developmentally regulated in the late fetal rat lung. Herein, we have confirmed this regulation and made three important observations about *DANCE* expression. First, *DANCE* exhibits a dramatic pattern of developmental regulation in blood vessels of the rat lung. This begins in the fetus late in gestation and extends after birth well into the late postnatal period. Lung *DANCE* is expressed predominately in the endothelial cell and much less so in the vascular smooth muscle cell. The temporal profile of *DANCE* expression correlates well with the anatomic profile of late fetal and postnatal lung vascular development. Second, the level of *DANCE* mRNA expression is downregulated in the adult rat lung and the adult mouse lung. However, *DANCE* mRNA expression in the adult rat lung persists largely in all of the distal branches of the pulmonary artery. This contrasts with the adult mouse lung (6). Third, *DANCE* expression is inducible in the adult mouse lung after injury by hyperoxia as it is in systemic arteries of the rat after injury by mechanical agents or the mouse by atherosclerosis. Taken together, our data suggest that *DANCE* may function in the lung, as in the systemic circulation, as a regulator of blood vessel development and cellular repair after injury.

Blood vessel ontogeny in the lung is a topic of active investigation, and it appears to involve both vasculogenic and angiogenic mechanisms (4). A very recent study used the *LacZ* gene under the control of an endothelial cell-specific promoter to analyze the ontogeny of blood vessels in the lung. The authors concluded that blood vessels form in a continuous fashion throughout lung development in proportion to overall lung growth. There is no accentuation of this process in the late phase of fetal lung development as suggested in earlier anatomic studies (13). *DANCE* mRNA is certainly expressed continually in the late fetal and postnatal lung, but this mRNA is upregulated about fivefold in the pseudoglandular period between 18 and 20 days of gestation. This induction correlates with a period when the endothelium exhibits its highest growth rate and achieves its largest absolute mass of cellular volume according to those same anatomic studies (8). Also during this time there is a progressive accumulation of endothelial, smooth muscle, and precursor cells around branches of the developing pulmonary arteries (1, 8). The process appears to be related to maturation of the blood vessel wall and involves an expanding proportion of the lung mass in the later phases of fetal lung development. Little is yet known about the genes that control this process, but the $\alpha_5\beta_1$-integrin, a receptor for the RGD motif found in *DANCE*, is also expressed in vessel walls at this stage of lung development as it was in the outflow tract of the developing heart (11). *DANCE* has been shown to mediate endothelial cell adhesion through binding to integrins (9). Hence *DANCE* could be functioning as a regulator of endothelial cell migration related to maturation of the blood vessel wall during lung development in the late fetal and the postnatal periods. *DANCE* mRNA expression extends for 21 days into the postnatal period. Detailed morphometric studies in the rat have delineated a period of extensive cellular proliferation from *day 4* to *7* that is associated with a wave of alveolar formation up to *day 13*. Beyond *day 13*, the major events are an expansion and thinning of the alveolar septa that continues up to *day 21*. Therefore, *DANCE* expression correlates well with the temporal course of lung vascularization and remodeling involving the endothelium (1).

Our data using filter hybridization analysis with total RNA from the developing rat lung confirm previous literature (6), which suggested that the relative level of *DANCE* mRNA expression is downregulated in the adult, and show that the relative level declines to at least that seen in the fetal lung at 18 days of gestation. However, our in situ hybridization data contrast with that of the adult mouse lung in that *DANCE* mRNA expression in the adult rat lung is clearly still evident in all of the distal branches of the pulmonary artery although little signal is detectable in the microvasculature. Hence, *DANCE* expression must decline as the vessel extends into the periphery of the lung. In addition, the in situ hybridization study also shows that the *DANCE* mRNA level has declined in intensity in the cells of the distal branches of the pulmonary artery, presumably as a result of normal postnatal vascular remodeling. This species-specific difference in adult lung *DANCE* expression between the mouse and the rat may be linked to the proposed function of *DANCE* as a regulator of cell proliferation. The rat continues to grow throughout its lifetime, in contrast to the mouse. Therefore, the function of *DANCE* in the rat lung may be required because of the continual nature of vascular growth.

Compared with developing tissues, *DANCE* mRNA abundance clearly declines in the lung of adult mice and rats and in many arteries as well (6, 9). However, *DANCE* expression in the adult rat still remains relatively abundant in the lung, the kidney, and the spleen. This pattern is similar to that reported for the adult mouse (9). In contrast, *DANCE* expression in human adult is more abundant in the heart than the lung (6). *DANCE* mRNA expression also remains relatively abundant at intercostal branch sites of the adult mouse aorta despite its overall downregulation in this vessel as a whole. This persistence was postulated to be the result of ongoing cellular injury at these sites because of hemodynamic stress (9). The lung, the kidney, and the spleen are highly vascular organs, but it is difficult to postulate ongoing hemodynamic injury as the explanation for *DANCE* mRNA expression, particularly in the lung. Alternatively, *DANCE* may serve a function related to the differentiated state of vascular cells in these organs. The uniform absence of *DANCE* mRNA expression in the human (6), the mouse (9), and the rat liver, another highly vascular organ, suggests tissue-specific and species-specific roles for *DANCE*.

DANCE expression can be upregulated in the adult mouse lung after injury induced by a transient exposure to hyperoxia. These studies were performed to determine if *DANCE* expression could be induced after

injury in the lung as it as been described in the systemic arterial wall after injury in the rat and atherosclerosis in the mouse (6, 9). We chose a transient exposure to hyperoxia as the injury agent because the sequential changes in lung cell morphology were already well characterized for both the acute and the recovery phases in the rat (3, 14). During the acute phase of the 3-day exposure to hyperoxia, signs of severe structural injury are most evident within capillary endothelial cells of the lung. The interstitium shows an increase in cellularity and edema fluid, whereas the epithelium remains more normal in appearance (3). Upon a return to normoxia, a repair process is evident within 3 days of recovery (14) and is marked by a dramatic decrease in the number of neutrophils in the interstitium and an increase in the number of parenchymal lung cells, such as endothelial, type 2 epithelial, myofibroblast, and alveolar macrophage cells. Thereafter, the numbers of all of these cell types appear to decline except for the endothelium, which continues to increase up to the 7th day. Between 14 and 28 days into the recovery period, the cellular architecture of the lung appears almost normal.

This sequence of morphological changes is similar in different animal species (2), and because baseline lung *DANCE* expression in the mouse is lower by in situ hybridization than in the rat, we used mice for these experiments. There was little evidence of *DANCE* induction immediately after the acute phase of exposure to hyperoxia nor at 1 or 3 days into the recovery period. *DANCE* was upregulated about threefold at *day 5* of recovery, a time when the proliferative response in the lung parenchyma is already complete in most cells except for the endothelium. The result of our in situ hybridization study suggests a diffuse pattern of *DANCE* mRNA induction. This is compatible with the diffuse nature of the injury in the capillary endothelium, whereas little proliferation or differentiation is reported in distal branches of the pulmonary artery. However, we were unable to show this directly in vivo, but *DANCE* did not appear to be induced in a new cell population either. *DANCE* mRNA abundance has returned to baseline by *day 8* of the recovery phase and remained at this level at *day 11* and *day 14*. The relatively late time course of *DANCE* induction in the injured lung is similar to that previously described in cells of the systemic arterial wall after mechanical injury and supports the hypothesis that *DANCE* could function in an autocrine or paracrine fashion as a negative regulator of cell proliferation. The association of *DANCE* expression with the cellular state of proliferation may be relevant to our observation that this mRNA is expressed in the lung epithelial MLE cell line, but no signal was evident in primary lung epithelial cells by in situ hybridization. A proliferative state is maintained in the MLE cell line through immortalization with SV40 large-T antigen (15). Further studies with endothelial and epithelial cells in culture will be required to investigate this hypothesis.

In summary, *DANCE* is a newly described gene that appears to play a role in vascular cell biology during blood vessel development and repair after injury (6, 9). Our study in the developing rat lung suggests that *DANCE* expression in this organ predominates in the endothelial cell. Also, the rat is unique in that a high level of *DANCE* expression is maintained in the endothelial cells of the distal branches of the pulmonary artery in the adult lung. *DANCE* may serve a species-specific role in the rat to maintain lung blood vessel integrity. Future studies may be aimed at examination of *DANCE* expression during the repair of different forms of lung injury and during the remodeling that is seen in pulmonary vascular disease. In addition, studies may be undertaken to link *DANCE* expression with cellular processes such as proliferation, migration, and differentiation in primary cultures of pulmonary artery endothelial cells. These studies should provide new insight into a new gene family that can regulate endothelial cell function and blood vessel formation during late fetal and neonatal lung development and cellular repair after lung injury.

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