Embryonic Overexpression of Receptors for Advanced Glycation End-Products by Alveolar Epithelium Induces an Imbalance between Proliferation and Apoptosis

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Receptors for advanced glycation end-products (RAGEs) are multi-ligand cell surface receptors highly expressed in the lung that contribute to alveolar epithelial cell differentiation during embryogenesis and the modulation of pulmonary inflammation during disease. When RAGEs are overexpressed throughout embryogenesis, severe lung hypoplasia ensues, culminating in perinatal lethality. However, the possible mechanisms that lead to the disappearance of pulmonary tissue remain unclear. A time course of lung organogenesis, commencing on Embryonic Day (E) 12.5, demonstrated that increased RAGE expression primarily alters lung morphogenesis beginning on E16.5. Terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) immunohistochemistry and immunoblotting for active caspase-3 confirmed a shift toward apoptosis in lungs from RAGE-overexpressing mice, compared with wild-type control mice. This observation supports previous work where electron microscopy identified the cellular blebbing of alveolar epithelium in embryonic RAGE-overexpressing mice. Assaying for NF-κB also revealed elevated nuclear translocation in lungs from transgenic mice compared with control mice. An RT-PCR assessment of genes regulated by NF-κB demonstrated the elevated expression of Fas ligand, suggesting increased activity of the Fas-mediated signal transduction pathway in which ligand–receptor interactions trigger cell death. These data provide evidence that the expression of RAGEs must be tightly regulated during homeostatic organogenesis. Further elucidations of the RAGE signaling potentially involved in cell-cycle abnormalities may provide insights into the progression of RAGE-mediated lung diseases.

Keywords: RAGE; apoptosis; lung; transgenic

CLINICAL RELEVANCE

Understanding the role of the receptor for advanced glycation end-products (RAGE) in the context of lung development has been enigmatic. This investigation further clarifies severe abnormalities in lung formation when the availability of RAGE is increased. Insights related to the RAGE-mediated apoptosis of pulmonary epithelium and impaired branching morphogenesis may reveal details in the pathology of premature lung disease such as bronchopulmonary dysplasia and the persistent airspace enlargement coincident with respiratory distress.

The vertebrate lung is a complex branching organ comprised of numerous specialized cell types, programmed by a host of intricate signaling and transcriptional control mechanisms. During the pseudoglandular stage, undifferentiated cells become ciliated columnar epithelial cells, nonciliated Clara cells, goblet cells, and neuroendocrine cells that line the conducting airways. Distally, parenchymal cells in the eventual respiratory compartment differentiate to become alveolar Type I (ATI) and II (ATII) cells. The tight regulation of complex, interrelated processes that control cell growth and differentiation is therefore critical to forming a viable lung (1).

The receptor for advanced glycation end-products (RAGE) is a member of the immunoglobulin superfamily of cell-surface receptors. The receptor contains a V-region–like domain crucial for ligand binding and two C-region–like domains, a single-pass hydrophobic transmembrane domain, and a short, 43-amino-acid, highly charged cytoplasmic domain essential for intracellular signaling (2–4). RAGE is a dynamic receptor capable of binding ligands with variable yet related tertiary structures (5). Initially characterized and named for its ability to bind nonenzymatically glycoxidized macromolecules, or advanced glycation end-products, RAGE also binds a myriad of other molecules, including proinflammatory cytokine-like mediators of the S100/calgranulin family, amyloid β-fibrils, and the high-mobility group box 1 (HMGB-1) (2, 6, 7).

RAGE is expressed in many cell types, including endothelium, smooth muscle, macrophages, and epithelium. However, it is most abundantly expressed by alveolar epithelium (8). Although some evidence suggests minimal expression by ATII cells (5, 9), differentiated ATI cells are the predominant RAGE-expressing cells in the lung (10). RAGE is clearly detected in pulmonary cells during lung neonatal development (11), suggesting possible contributions to organogenesis and homeostasis. RAGE was shown to promote epithelial cell adherence and dynamically influence cell spreading, implying a possible role in the ATII to ATI cell transition (12). Moreover, recent findings suggest a role for RAGE in cytoskeletal disruption, revealing possible functions related to cell plasticity and survivability (13). The expression of RAGE is also up-regulated in cases of injury and disease. For example, full-length, membrane-bound RAGE is increased in oxidative asthma (14), smoke-related chronic obstructive pulmonary disease (15–18), acute respiratory distress syndrome (19), and acute lung injury induced by radon, cytokines, hyperoxia, and lipopolysaccharide (20, 21). Soluble RAGE, capable of binding ligand but unable to transduce intracellular signaling, is also increased in a subset of these disease conditions (19, 21–23). However, precise roles for RAGE during lung development remain enigmatic.

Because RAGE binds specific ligands, it is linked to several downstream signaling pathways. For example, the activation of RAGE can occur in response to damage-associated molecular patterns (DAMPs) via the NF-κB pathway (24). Research also identifies proinflammatory RAGE signaling involving NF-κB after Ras activation in cells and tissues exposed to tobacco smoke (25). Key DAMP molecules include members of the S100/calgranulin family and HMGB-1, both of which efficiently

bind RAGE (26, 27). These molecules, normally secreted after apoptotic or necrotic events, can serve as trophic factors in low concentrations, or enhance inflammatory/cell death responses at higher concentrations. Downstream gene products produced through RAGE signaling include NF-κB, cyclooxygenase (COX)-2, IL-1β, and TNF-α (28). Because the promoter for the RAGE gene contains NF-κB binding sites (29) and is regulated by early growth response gene (Egr)-1 in cases of tobacco-related disease (30), a possible autoinflammatory loop may be triggered, suggesting a central role for RAGE in the tissue loss observed in chronic disease states.

In the present study, lung developmental progression was evaluated in lungs that used the promoter for surfactant protein–C (SP-C) to overexpress RAGE conditionally in the respiratory compartment. Previous research revealed that the SP-C promoter is active as early as Embryonic Day (E10) in primitive pulmonary epithelial cells in both the proximal and distal lung (31) (see Figure E1 in the online supplement). However, the use of the SP-C promoter to drive the expression of RAGE did not induce anomalies until E16.5, and those anomalies progressively worsened, resulting in severely diminished lung formation on E18.5. Previous data indicate that distal lung structures exhibit marked signs of nuclear fragmentation and cellular blebbing when viewed by electron microscopy (31), both of which are typical hallmarks of apoptosis. Our work involving immunohistochemical terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) procedures validated the apoptotic characteristics evident in electron microscopy, and revealed significant increases in cells that express blunt DNA fragments. The immunoblotting of lung lysates also confirmed active apoptosis via a caspase-3–mediated pathway. NF-κB and a potent proinflammatory downstream product, Fas ligand (FasL), were both up-regulated in the lungs of RAGE transgenic (TG) mice, indicating that cellular apoptosis was likely activated via extrinsic pathways. Taken together, these data suggest a homeostatic role for RAGE in normal physiological environments, and provide mechanistic insights into deleterious processes that are activated when RAGE is overexpressed during development and disease.

MATERIALS AND METHODS

Mice

Mice were derived from a C57Bl/6 background (Jackson Laboratories, Bar Harbor, ME). Two transgenic lines (hSP-C-rtTA and TetO-RAGE) were crossed to create doxycycline (Dox, 625 mg/kg; Harlan Teklad, Madison, WI)–inducible mice that up-regulated RAGE in the alveolar epithelium. RAGE TG mice had both transgenes, and control mice were age-matched single or nontransgenic littermates. Dams were fed Dox before conception until they were killed on E12.5–E18.5. Tail biopsies were genotyped as previously described (32). Mice were used in accordance with protocols approved by the Institutional Animal Care and Use Committee at Brigham Young University.

Histology and Immunostaining

Lungs from E15.5–E18.5 RAGE TG and wild-type mice ($n = 3$ per group) were processed, embedded, and sectioned (33). Lungs were stained with hematoxylin and eosin for general lung morphology at 24-hour intervals, beginning on E12.5. Immunohistochemistry involved antibodies for RAGE (AF1145, 1:500; R&D Systems, Minneapolis, MN) and proliferating cell nuclear antigen (PCNA, SC-7907, 1:500; Santa Cruz Biotechnology, Santa Cruz, CA). The TdT-FragEL DNA Fragmentation Detection Kit (Calbiochem, Rockland, MA) was used to immunohistochemically evaluate apoptosis.

Immunoblotting

Lungs from E15.5–E18.5 RAGE TG mice and wild-type control mice were homogenized in radioimmunoprecipitation assay (RIPA) buffer supplemented with protease inhibitors (Thermo Scientific, Pittsburg, PA). To ensure equal loading of protein samples, a quantification of protein concentrations was performed using the BCA Protein Assay Kit (Thermo Scientific). Protein samples (10 μg each) were subjected to immunoblotting, using anti-PCNA (1:1,000, Santa Cruz Biotechnology), total caspase-3 (catalogue number 9662, 1:1,000; Cell Signaling, Beverly, MA), RAGE (AF1145), and glyceraldehyde 3-phosphate dehydrogenase (11). Membranes were incubated with appropriate secondary antibodies, detected with ECL-plus (Amersham, Piscataway, NJ), and developed. Band densitometry involved digitized images and Un-Scan-It software (Silk Scientific, Orem, UT).

Assessment of Nuclear NF-κB, FasL, and B-Cell Lymphoma-2

Nuclear lysates were isolated from lungs of E16.5 RAGE TG and control mice ($n = 3$ per group), using the NE-PER Extraction Kit (Thermo Scientific). Total nuclear protein was quantified using the bicinchoninic acid assay (BCA) technique (32), and total nuclear NF-κB was assessed in nuclear lysates (3 μg each), using the ELISA-based TransAM p65 NF-κB Transcription Factor Assay Kit (Active Motif, Carlsbad, CA). NF-κB activity in lysates from transgenic mice was reported after normalizing activity in control mice to 1. Total RNA from RAGE TG and wild-type lungs ($n = 3$ per group) was isolated from E16.5 animals, using the Absolutely RNA Kit (Stratagene, Santa Clara, CA). RNA was quantified, and 1 μg of each sample was converted to cDNA and assessed using the Mouse NF-κB–Regulated cDNA Plate Array (Signosis, Sunnyvale, CA).

Statistical Analysis

For immunohistochemistry, at least eight high-power fields ($\times 400$) per slide ($n = 3$ per group) were randomly imaged, and blindly counted PCNA or TUNEL-positive cells were statistically evaluated at each time point, using a Student t test of the means. At least three samples were used for immunoblots and cDNA screens per group, and the Student t test was used to assess the level of significance at $\alpha = 0.05$. All values are presented as means $\pm$ SD, and $P \leq 0.05$ was considered significant.

RESULTS

Lung Hypoplasia Was Temporally Affected by RAGE Overexpression during Lung Organogenesis

Immunostaining for RAGE revealed epithelial-specific patterns of RAGE augmentation in the RAGE TG mouse compared with wild-type control mice (Figure 1). Immunoblotting also confirmed expression of RAGE in wild-type mice as early as E15.5, and significant increases in expression in lung lysates from TG animals (Figure 2I). Previous work demonstrated that the embryonic overexpression of RAGE adversely affected lung development, leading to an embryonic lethal phenotype. However, the timing and mechanisms of RAGE-mediated underdevelopment of the lung remained unclear. A progressive time-course evaluation of lungs from RAGE TG and wild-type control mice indicated that the elevated expression of RAGE adversely impaired lung morphogenesis beginning on E16.5 (Figures 2I and 2J), and culminating in severely underdeveloped lungs on E18.5 (Figures 2M and 2N). Histological evaluation revealed that lung development transpired normally from E12.5–E15.5 in RAGE TG mice (Figures 2B, 2D, 2F, and 2H) compared with wild-type littermates (Figures 2A, 2C, 2E, and 2G). After E15.5, a period coinciding with the canalicular stage of pulmonary development, notable tissue loss occurred, and the degree of tissue loss increased as embryogenesis progressed. Wild-type and RAGE TG mice in the absence of Dox administration appeared histologically similar to Dox-fed wild-type mice (32), suggesting that no adverse effects to pulmonary organogenesis were attributable to the transgenes alone or to Dox administration.
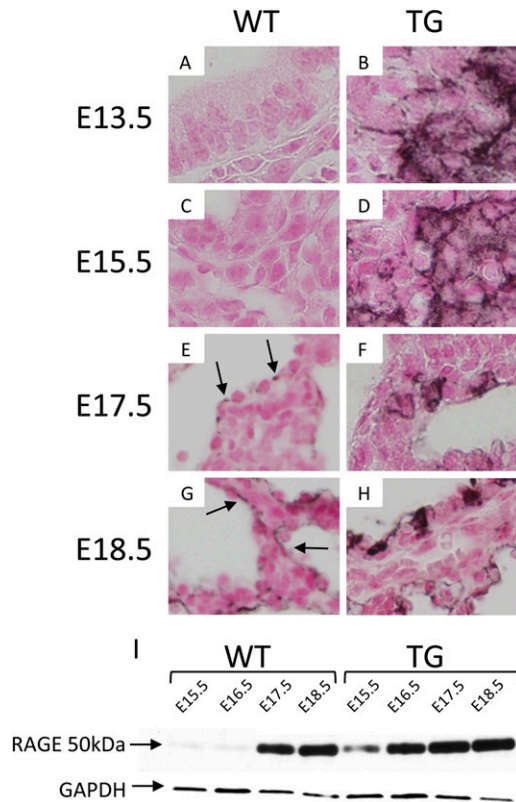
Figure 1. The expression of the receptors for advanced glycation end-products (RAGE) was elevated in embryonic RAGE transgenic (TG) murine lungs. Compared with wild-type (WT) murine lungs (*A, C, E, G*) with basal RAGE expression (*E* and *G*, *arrows*), immunohistochemistry revealed increased RAGE expression in RAGE TG murine lungs on Embryonic Day (E) 13.5 (*B*), E15.5 (*D*), E17.5 (*F*), and E18.5 (*H*). Original magnification of representative images, ×400. Immunoblotting for RAGE identified detectible expression as early as E15.5, and elevated expression in lungs from TG mice from E15.5–E18.5. GAPDH, glyceraldehyde 3-phosphate dehydrogenase.

Proliferation Was Unchanged in the RAGE-Overexpressing Lung

Because diminished cellular proliferation may contribute to lung hypoplasia, cellular propagation was studied in the context of RAGE overexpression. The immunohistochemistry of lung samples from E15.5–E18.5 was performed, using antibodies against PCNA, a marker of cell proliferation that detects cells during the S-phase of the cell cycle. As anticipated, numerous proliferating cells were positively stained for PCNA at each time point in both wild-type and RAGE TG lungs (Figure 3A). After the percentage of PCNA-positive cells at each time point was obtained and evaluated, differences between groups were not statistically significant (Figure 3B), although the sum of all PNCA-positive cells trended downward as pulmonary development progressed (*see* Table E1 in the online supplement).

To assess qualitative PCNA expression further (Figure 3A), immunoblotting for PCNA was performed. Lysates from E15.5–E18.5 lungs resected from RAGE TG mice revealed diminishing PCNA expression, compared with lungs from age-matched control mice (Figures 3C and 3D). However, the expression of PCNA was not significantly different between the two groups of mice (Figures 3C and 3D).
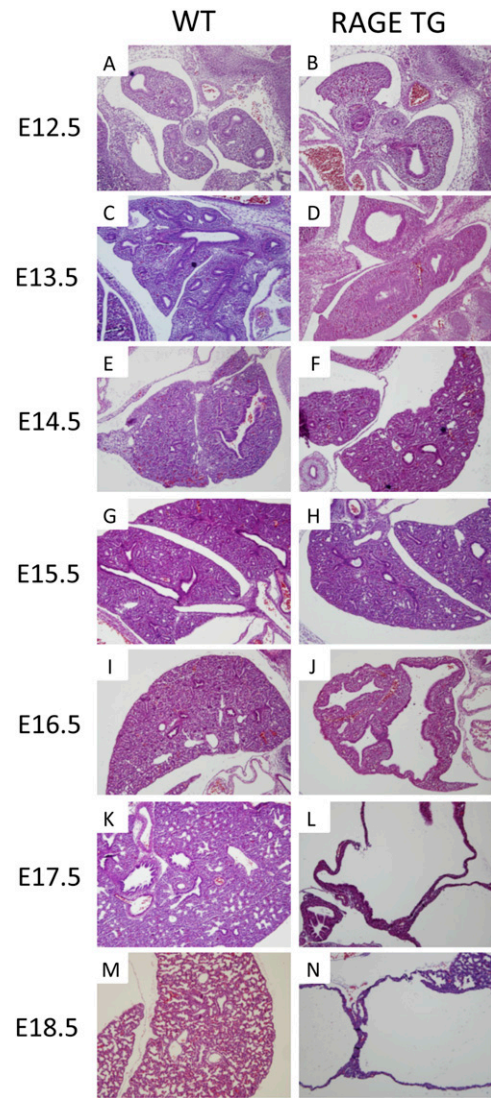

Figure 2. RAGE overexpression altered lung organogenesis, beginning on E16.5 and continuing through E18.5. A representative time course of hematoxylin-and-eosin–stained sections revealed standard lung formation through E15.5 in doxycycline (Dox)–fed RAGE TG mice (*B, D, F,* and *H*), compared with Dox-exposed wild-type counterparts (*A, C, E,* and G). Histology on E16.5 (*J*) revealed the initial stages of aberrant lung structures, culminating in profound tissue loss on E17.5 and E18.5 in the RAGE TG murine model (*L* and *N*) compared to Dox-exposed wild-type lungs (*I, K,* and *M*). All images are representative. Original magnification, ×100.

Apoptosis Was Elevated in the RAGE-Overexpressing Lung

To assess whether increased apoptosis contributed to the severe lung hypoplasia evident in RAGE-overexpressing mice, a quantitative TUNEL staining approach was used. The number of positively identified apoptosing cells via TUNEL staining was significantly increased in lungs from RAGE TG mice, compared with wild-type control mice, on each day from E15.5–E18.5 (Figures 4A and 4B). As revealed by representative immunostaining (Figure 4A), cells undergoing apoptosis were sporadically detected in lung parenchyma. In addition to immunohistochemistry, immunoblotting for caspase-3 was performed to detect both extrinsic and intrinsic apoptotic pathways. RAGE TG lungs demonstrated a significant increase in the amount of total caspase-3 (35 kD) and active caspase-3 (17-kD and 19-kD forms), compared with wild-type littermates (Figure 4C). Densitometry of
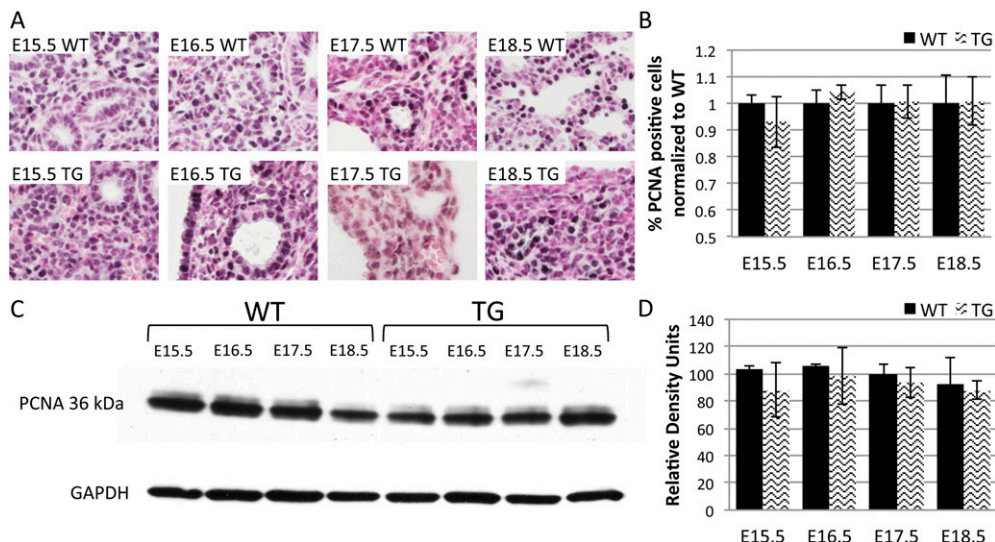
Figure 3. Proliferation was not diminished in RAGE TG lungs. (*A*) Immunostaining for proliferating cell nuclear antigen (PCNA) from E15.5–E18.5 demonstrated widespread proliferation in both wild-type and RAGE TG lungs. Cell counts by blinded individuals were performed to view potential differences in the quantity of proliferating cells. One hundred cells were identified and counted in clusters from at least eight random ×400 viewing areas. The percentage of positively stained (encompassing both punctate and heavy nuclear staining) cells was determined. (*B*) The percentage of PCNA-positive cells in RAGE TG lungs was insignificant, compared with the normalized percentage from wild-type lungs. (*C* and *D*) Immunoblotting for PCNA from whole-lung lysates suggested no significant change in PCNA expression from E15.5 onward in the distal lung. A minimum of three animals were evaluated in each experimental group. *$P \leqslant 0.05$.

the bands showed a significant increase in the amount of active caspase-3 in RAGE TG mice on E15.5, compared with wild-type E15.5 lungs (Figure 4D). Although the amount of active caspase-3 in RAGE TG murine lungs declined as E18.5 approached, each time point was characterized by significantly increased active caspase-3 expression when compared with control mice (Figure 4D).

Nuclear Translocation of NF-κB and Expression of Its Targets Were Misregulated in the RAGE-Overexpressing Lung

Total nuclear protein was isolated from whole-lung lysates procured from E16.5 mice and assessed for NF-κB activity because of the severity of hypoplasia observed at that time (Figure 2J). After normalizing nuclear NF-κB expression in the wild-type animals to 1, ELISA revealed a significant increase in the activation and nuclear translocation of NF-κB in lungs from RAGE TG mice (Figure 5A). Because NF-κB activation was elevated

in RAGE TG mice, two NF-κB–regulated genes that function in apoptotic pathways were screened. Compared with control lungs, RAGE TG lungs expressed FasL at concentrations approximately twofold higher on E16.5 (Figure 5B). The significantly increased FasL, known to promote apoptosis after interactions with its receptor (FasR), suggests that extrinsic apoptotic pathways were activated in RAGE TG mice. Interestingly, the expression of B-cell lymphoma (Bcl)-2, an antiapoptotic factor, was significantly diminished in RAGE TG murine lungs on E16.5 (Figure 5C). Additional data further suggest that abnormal levels of NF-κB, FasL, and Bcl-2 expression in the RAGE TG mouse after E16.5 continued to affect lung development adversely (not shown).

DISCUSSION

In the present study, we used a gain-of-function methodology to determine the mechanisms that underlie lung hypoplasia
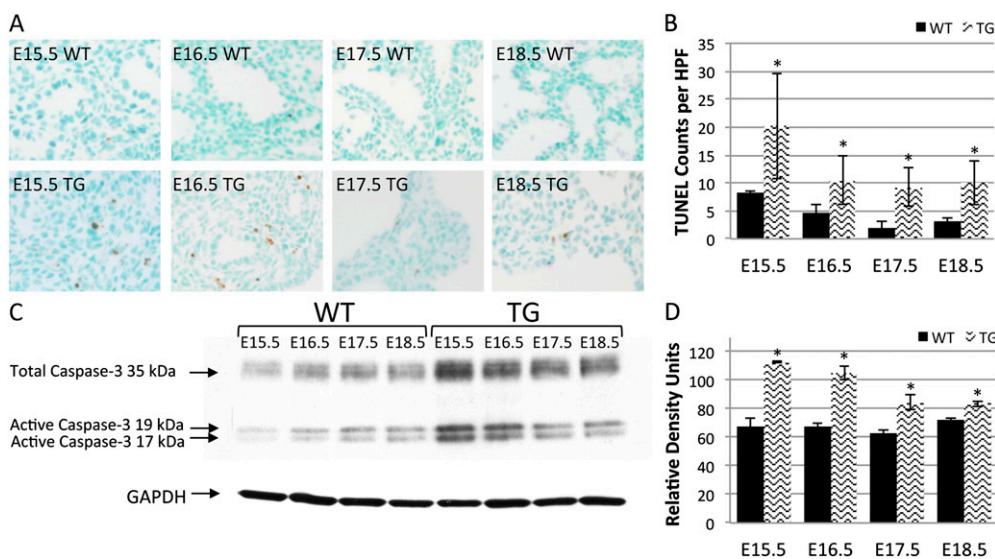

Figure 4. Apoptosis was significantly increased in the developing RAGE TG lung. (*A*) A detectable increase in the number of actively apoptosing cells by terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) staining was observed in RAGE TG lungs compared with wild-type lungs at all time points tested. TUNEL-positive cells in ×400 images were sporadic throughout the distal lung, with occasional staining found in cuboidal/columnar cells in the airways of RAGE TG mice. (*B*) Cell counts performed by blinded individuals demonstrated an increase in the number of positively stained cells at all time points. (*C*) Immunoblotting confirmed that active apoptosis was occurring in a caspase-3–mediated manner, indicated by a significant increase in the amount of total and cleaved caspase-3 products (17 kD and 19 kD) within the lung lysates of RAGE TG lungs. (*D*) Density of the cleaved caspase-3 bands was significantly increased in RAGE TG murine lungs at all time points. A minimum of three animals were evaluated in each experimental group. *$P \leqslant 0.05$.
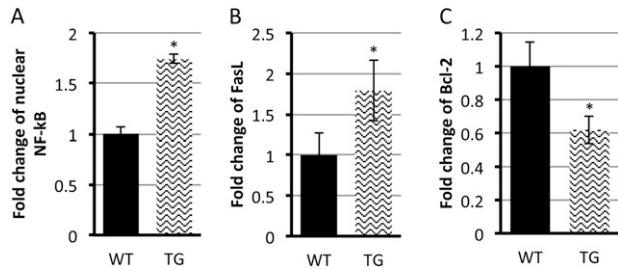
Figure 5. NF-κB and its targets, the Fas ligand (FasL) and B-cell lymphoma (Bcl)-2, were misregulated in RAGE TG lungs compared with control lungs. (*A*) ELISA experiments showed that active NF-κB protein was elevated in nuclear lysates from E16.5 RAGE TG mice, compared with age-matched, wild-type mice. Messenger RNA concentrations of the proapoptotic factor FasL were increased in RAGE TG mice on E16.5 (*B*), and antiapoptotic Bcl-2 mRNA was diminished (*C*) when compared with control mice. Measurements for FasL and Bcl-2 were standardized to glyceraldehyde 3-phosphate dehydrogenase. A minimum of three animals were evaluated in each experimental group and *$P \leq 0.05$.

resulting from RAGE signaling. This research is a natural extension of our previous work, where we demonstrated that RAGE up-regulation caused pulmonary underdevelopment via hindered cytodifferentiation and a possible impairment of branching morphogenesis (32). Through the use of a tetracycline-inducible respiratory epithelial cell-specific RAGE overexpressing mouse, we discovered the precise timing of lung hypoplasia and airspace enlargement directly attributable to RAGE augmentation in the alveolar compartment during embryonic development. Furthermore, despite possible compensation via the proliferation of respiratory epithelium during the pseudoglandular period of development, the elevated apoptosis of differentiating epithelial cells during the late pseudoglandular and canalicular periods was a significant cause of irreversible lung simplification.

Our finding that general lung histology in RAGE TG mice was indistinguishable compared with age-matched control mice through E15.5 (Figure 2) suggests that early signaling events transpiring between the foregut endoderm and surrounding splanchnic mesoderm were unaffected. In particular, mesenchymal fibroblast growth factor–10 (34), endodermally derived fibroblast growth factor receptor–2 (35), sonic hedgehog (SHH)/GLI 2,3 (36), and retinoic acid receptors (37) each play important roles during early tracheal–pulmonary morphogenesis. Because lung organogenesis appeared normal through E15.5, our data indicate that these early signaling molecules are likely unaffected by RAGE overexpression, although RAGE was detected as early as E13.5 (Figure 1). Despite the lack of an early phenotype, the expression of thyroid transcription factor 1 (TTF-1), a critical early lung transcriptional regulator, is decreased in RAGE TG mice (32). TTF-1 is known to interact with a host of factors necessary to lung maturation (1), so later prenatal events, such as surfactant homeostasis, vasculogenesis, host defense, fluid homeostasis, and inflammation mediated by TTF-1, may have contributed to impaired lung formation in the RAGE TG mouse.

A combination of TUNEL staining and immunoblotting for caspase-3 led to the discovery that apoptosis was significantly elevated in RAGE TG mice in the late pseudoglandular (E15.5), canalicular (E16.5–E17.5), and early saccular (E18.5) periods of lung development. In regard to caspase expression, our data support previous research that focused on lung apoptosis and the correspondence between elevated total and active caspase-3 expression (38). The detection of apoptosis during these periods also corroborated findings by Kresch and colleagues (39), who

studied the ontogeny of apoptosis in embryonic rat lungs. Specifically, epithelial cell apoptosis was detected from the canalicular stage and beyond, suggesting the need to control respiratory epithelial cell deletions precisely during normal lung organogenesis (40). Despite the notion that the deletion of pulmonary epithelium by apoptosis may be a physiologically significant event in lung remodeling during late stages of gestation, excessive cell death, as observed here (Figure 4), likely contributed to the deleterious loss of lung tissue.

Clear and predictable patterns of lung and respiratory epithelial cell apoptosis during fetal development exist (38, 41). Such patterns provide opportunities for the elucidation of molecular regulatory pathways that control the mechanisms of cell death. One such well-studied pathway involves the recruitment of activated NF-κB. NF-κB was initially identified as a transcription factor in B cells, and has since been detected ubiquitously in the cytoplasm of all cell types (42). When stimulated, NF-κB translocates to the nucleus, where it regulates the expression of more than 200 genes that influence cell growth, survival, and inflammation (43). Because a significant increase in nuclear NF-κB activity was evident in RAGE TG mice compared with control mice (Figure 5A), NF-κB appears to be an important signaling intermediate downstream of RAGE. This discovery led to the proposal that the Fas/FasL pathway, a cascade directly influenced by NF-κB signaling, may be a modulator of the late-gestational apoptosis mediated by the augmentation of RAGE. The Fas/FasL pathway is a widely distributed apoptosis signal transduction pathway in which ligand–receptor interactions trigger cell death (44, 45). FasL is a Type II transmembrane protein belonging to the tumor necrosis factor family (46, 47) that is cleaved by matrix metalloproteinase (MMP)-7 to elaborate ligand. The binding of FasL to FasR activates intracellular caspases 3 and 8, and culminates in apoptosis (47, 48). Previous studies showed that ATII cells and ATII-like cell lines express Fas (49), and that apoptosis can be induced in these cells by crosslinking agonistic anti-Fas antibodies both *in vitro* and *in vivo* (50–52). Our discovery that FasL was significantly upregulated in RAGE TG murine lungs suggests that the Fas/FasL pathway may be a pivotal mediator of apoptosis in the airway epithelial compartment during the pericanalicular period. Furthermore, because the normal expression of RAGE is elevated during this pericanalicular period, our data may suggest that a remodeling of pulmonary airspaces via ATII apoptosis may occur at least in part because of RAGE-mediated mechanisms.

Moreover, we observed that Bcl-2 was down-regulated in the lungs of RAGE TG mice (Figure 5C). The Bcl-2 gene was originally discovered in a follicular B-cell lymphoma, where a chromosomal translocation moves the gene into juxtaposition with transcriptional enhancer elements of the immunoglobulin heavy chain locus (53). Bcl-2 is situated upstream of the apoptotic pathway, and it provides an important decisional checkpoint against irreversible cellular damage after the delivery of a death stimulus (54, 55). Specifically, Bcl-2 is an antiapoptotic mitochondrial outer membrane permeabilization protein that functions by extending cellular survival via the inhibition of a variety of apoptotic deaths (53–59). We observed that Bcl-2 was decreased in RAGE TG murine lungs on E16.5, a period that coincided with the apoptosis potentially mediated by FasL.

In conclusion, susceptibility to impaired branching morphogenesis and elevated apoptosis are features of premature lung diseases such as neonatal respiratory distress and bronchopulmonary dysplasia (BPD). Because RAGE signaling increases the secretion of proinflammatory cytokines (IL-1β and IL-6) and chemokines (MCP-1) known to be involved in the pathogenesis of BPD (22, 25, 60, 61), the possibility exists that RAGE, at least in part, contributes to a premature BPD
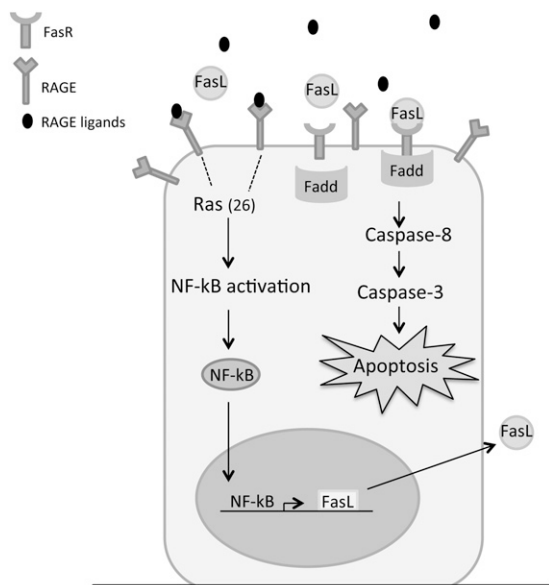
Figure 6. Working model demonstrates RAGE-mediated proapoptotic signaling. RAGE signals through rat sarcoma (Ras), and causes the nuclear translocation of active NF-κB. In the lungs of RAGE TG mice, active caspase-3 and elevated FasL suggest that RAGE influences the proapoptotic signaling involving these molecules during lung formation. Fadd = Fas-associated protein with death domain.

phenotype. Our ability to imitate and extend postcanalicular apoptosis via the up-regulation of FasL, a target of NF-κB, through RAGE signaling reveals important RAGE-mediated functions in normal lung development (Figure 6). Abnormal RAGE signaling that influences Fas/FasL pathways should be studied further, so that specific contributions to pulmonary architecture and cellular remodeling in the developing lung can be clarified. Although the present results relate to the context of RAGE up-regulation during developmental milestones, the apoptotic index stemming from increased RAGE expression in the adult must also be characterized. Therefore additional studies should continue, to identify the downstream targets of increased RAGE expression and possible endogenous ligands responsible for orchestrating RAGE-mediated alterations in lung formation and physiology.

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