Intersectin-1s: an important regulator of cellular and molecular pathways in lung injury

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Abstract: Acute lung injury (ALI) and acute respiratory distress syndrome (ARDS) are severe syndromes resulting from the diffuse damage of the pulmonary parenchyma. ALI and ARDS are induced by a plethora of local or systemic insults, leading to the activation of multiple pathways responsible for injury, resolution, and repair or scarring of the lungs. Despite the large efforts aimed at exploring the roles of different pathways in humans and animal models and the great strides made in understanding the pathogenesis of ALI/ARDS, the only viable treatment options are still dependent on ventilator and cardiovascular support. Investigation of the pathophysiological mechanisms responsible for initiation and resolution or advancement toward lung scarring in ALI/ARDS animal models led to a better understanding of the disease's complexity and helped in elucidating the links between ALI and systemic multiorgan failure. Although animal models of ALI/ARDS have pointed out a variety of new ideas for study, there are still limited data regarding the initiating factors, the critical steps in the progression of the disease, and the central mechanisms dictating its resolution or progression to lung scarring. Recent studies link deficiency of intersectin-1s (ITSN-1s), a prosurvival protein of lung endothelial cells, to endothelial barrier dysfunction and pulmonary edema as well as to the repair/recovery from ALI. This review discusses the effects of ITSN-1s deficiency on pulmonary endothelium and its significance in the pathology of ALI/ARDS.

Keywords: intersectin-1s, endothelium, lung injury, apoptosis, permeability.

INTRODUCTION

Acute lung injury (ALI) and its severe form, acute respiratory distress syndrome (ARDS), are syndromes of acute hypoxemic respiratory failure resulting from a variety of direct and/or indirect injuries to the gas exchange unit of the lungs.[1] Clinically, ALI is characterized by (1) significant arterial hypoxemia (partial pressure of arterial O_2/fraction of inspired O_2 [PaO_2/FiO_2]; 300 mmHg in ALI and <200 mmHg in ARDS), which is refractory to oxygen supplementation, (2) patchy bilateral lung infiltrates suggestive of edema, (3) decreased pulmonary compliance, (4) the absence of measurable evidence of cardiac failure, and (5) lack of an alternative justification for these findings.[2,3] ALI/ARDS is the result of a variety of causes that trigger stereotyped physiopathological responses, making the modeling of the illness difficult.

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A variety of insults can initiate and induce ALI/ARDS; the American-European Consensus Conference in 1994 divided these insults into (1) direct (pulmonary or primary) injuries of lung parenchyma, such as aspiration of gastric content and smoke inhalation, and (2) indirect (extrapulmonary or secondary) injuries, such as sepsis, trauma, and blood transfusions, mainly a result of an acute systemic inflammatory response.[4] Recently, some studies have shown differences in clinical sequelae between the two modes of injury, because others have not demonstrated any significant differences in outcomes and response to therapy in ARDS due to direct versus indirect causes. Direct lung injury was associated with higher mortality rate, increased ventilator requirements, and pulmonary consolidation, whereas indirect lung injury was associated with interstitial edema and alveolar collapse.[5] Regardless of the initial cause of the injury, the mortality is high. Incidence of ALI is reported as 17–34 to 78.9 per 100,000 people per year.[6,7] More recent estimates indicate around 190,000 of ALI cases per year in the United States, with 75,000 associated deaths per year.[8] Unfortunately, the incidence figures are not very consistent, likely because of problems with reliability of diagnosis and also because ALI is a critical-care illness, making its epidemiology directly linked to availability of intensive care unit resources.[9] The main treatment option remains ventilator and cardiovascular support, based on recognition of clinical signs.[3] To facilitate case recognition and more consistent treatment strategies based on severity, the American-European Consensus Conference used epidemiological, physiological, and clinical data to revise and update the ARDS definition.[10] The new "Berlin" definition of ARDS includes (1) a criterion of less than 7 days to define acute onset, (2) clinical judgment for characterizing hydrostatic pulmonary edema, unless there is no apparent ARDS risk factor, (3) three categories of ARDS severity—mild (201–300 mmHg), moderate (101–200 mmHg), and severe (≤100 mmHg)—based on the PaO_2/FiO_2 ratio, and (4) a continuous positive airway pressure value of at least 5 cm H_2O. The requirement of pulmonary artery wedge pressure and the category of ALI were removed. Even if this new definition is valuable as a clinical prediction tool, it still does not resolve the heterogeneity, the complex pathology, and the cellular

and molecular mechanism of ARDS. There is an unmet need for groundbreaking research to help reduce mortality and morbidity from ARDS.[11] Availability of human lung tissue for scientific investigation is limited by the lack of surgical biopsies of ALI/ARDS in clinical practice. Thus, modeling the disease in animals has been the basis for many crucial advances in this field. Animal models of ALI provide an excellent tool to test in a controlled environment the data collected from critically ill patients and to investigate new pathways, fundamental for improving clinical outcomes. Widely used experimental models are based on reproducing injury to epithelial and endothelial lung barriers and acute parenchyma inflammation. Animal studies are hierarchical, from mice to primates, and differ in mimicking the complex features of human ALI; most experimental models, such as mechanical ventilation, lipopolysaccharide (LPS) injection, and cecal ligation and puncture (considered "the gold standard" for animal models of sepsis), mimic sepsis-induced ALI; oleic acid injection mimics ALI caused by lipid embolization, while acid aspiration, pulmonary ischemia/reperfusion, and surfactant abnormalities simulate different conditions (disruption of the alveolocapillary interface with neutrophilic infiltration, increased vascular permeability, etc.) associated with ALI.[12] To this date, no single animal model reproduces all of the characteristics seen in patients. Although with limitations, each model is relevant for specific ALI features and helps answer mechanistic and therapeutic questions we still have about the disease. The focus of this review is to underline some of the latest attempts at modeling the disease and to understand the specific mechanisms triggered by defined factors involved in ALI/ARDS initiation, progression, and resolution.

ANIMAL MODELS OF ALI

Animal models of ALI/ARDS have been notably useful for exploring pathophysiological mechanisms and for evaluating novel therapeutic agents while contributing significantly to the development of treatment plans for these complex syndromes. As the scientific community began to realize that humans are inherently variable in their response to causative stimuli of ALI and that pharmacologic treatments have shown little or no benefit,[13] it was claimed that one of the

reasons for this unfortunate condition, at least for sepsis-induced ALI/ARDS, is the redundancy in the pathways driving the systemic inflammatory response syndrome.[14] Clinical studies revealed significant variability among individuals in the expression of blood cytokines in response to LPS;[15] however, detailed investigations revealed that this variability in inflammatory responses to LPS is caused by polymorphisms affecting the Toll-like receptor-1, leading to excessive inflammation and increased risk for poor outcomes in patients with sepsis.[15,16] Against this background, modeling the acute and chronic changes of ALI in animals, in order to understand and establish the molecular mechanism of disease pathogenesis, has been a major challenge. Over time, many animal models of ALI have been developed (Table 1), the advantages and disadvantages in each case meticulously characterized and broadly reviewed.[1,12] When the limitations are recognized and the necessary caution is used, studies performed on animal models are very useful for identifying, evaluating, and establishing key physiological mechanisms, the extent of involvement of different molecular pathways, and their subtle individual adaptation, while allowing the development of new hypotheses and therapies for treatment of human disease. The animal models highlighted in Table 1 have been extensively reviewed

over time;[1-3,12,31] thus, we discuss here briefly the emerging human models.

HUMAN MODELS OF ALI

An emerging field for ALI studies is the development of human models of the disease. The foremost impact of this type of ALI model is the development and testing of specific therapies and therapeutic targets. These models could be divided into three groups: in vivo, ex vivo, and inhaled bacterial products (specifically LPS). The in vivo models include the one-lung ventilation technique, in which one lung is collapsed during thoracic anesthesia while the other one is ventilated. The method has clinical significance, since 3% of patients with lobectomy and 8% of patients with pneumonectomy develop ALI and the rate of mortality in both cases reaches 40%–60%.[32,33] Two other pathological states associated with high incidence of ALI are esophagectomy, a condition in which ALI was found in almost 24% of cases,[34] and cardiopulmonary bypass, in which the disease developed in 2% of cases.[35] One-lung ventilation is associated with pulmonary inflammation, endothelial and epithelial dysfunction, neutrophil infiltration, and vascular activation, signs that belong to ALI of different etiologies, while some cardiopulmonary bypass causative mechanisms include endothelial dysfunction, ischemia-

Table 1. Animal models of ALI

Model	General/technical considerations
LPS[17]	High reproducibility, some resemblance to the human disease, strain specific
Oleic acid[18]	Reproducible, mechanisms of action not clear
Bacterial administration[19, 20]	Significant biological and individual variations, resemblance to toxemia
Cecal ligation and puncture[21]	Similarities to human disease, biological variability
Acid aspiration[22]	Reproducible, similarities to exudative phase of human disease
Bleomycin[23]	Reproducible, strain variability, pathophysiology still not well known
Hyperoxia[24]	Reproducible, scant inflammatory response, direct relationship with human disease questionable
Lavage of surfactant[25]	Some similarities to the human disease, technologically demanding
Ischemia/reperfusion[26]	Surgery needed, elevated interstitial participation
Mechanical stretch injury[27-30,a]	Challenging, mainly in small animals; ventilation strategy determines the severity of lung injury; type of ventilator and physiological monitoring are important; main advantage: clinical relevance

[a] High tidal volume type of ventilation injury.

reperfusion, inflammation, and atelectasis.[35,36] These models hold a lot of practical promise, but at the same time, like all pathologies for which the mechanisms are not well understood, they hold a lot of challenges. Because of the high incidence of ALI in esophagectomy, cohorts of patients undergoing the procedure are enrolled in a clinical trial in which inhibitors of hydroxymethylglutaryl-coenzyme A (CoA) reductase (simvastatin) are tested as potential drugs for reducing the incidence of ALI (http://www.controlledtrials.com/ISRCTN5643987).

The ex vivo models usually use donor lungs that, according to the International Society for Heart and Lung Transplant, do not meet the standards for transplantation and therefore have a modified basal physiology usually associated with comorbidities and large variations in ischemic times. For ex vivo research, the lungs are kept in a viable status with well-controlled ventilation and perfusion systems, which are demanding with respect to the surgical and perfusion expertise needed, not to mention the insufficient available data regarding the effect of organ cooling, required for lung transportation. Of the two methods used for ex vivo maintenance of the lungs—isolated perfused lungs and ex vivo lung perfusion—only the first was used to study ALI.[37,38] Ex vivo lung perfusion is a technically demanding methodology used to assess the quality of donor lungs harvested in special circumstances and to recondition them in order to qualify for transplant. These methods have tremendous potential for study of different lung pathologies, ALI/ARDS included, but the cost, technicality, and competition with the transplant option make it less accessible; thus, modeling ALI in an ex vivo lung perfusion setting should be initiated.

The inhaled-LPS model induces an inflammatory response with neutrophil recruitment and mild enhancement of cytokine production,[39] associated with fibrinolytic and coagulopathic defects similar to the ones described in human ALI.[40] On the basis of this evidence, the model was used to assess the effect of simvastatin, a hydroxymethylglutaryl-CoA reductase inhibitor, on the inflammatory component of ALI. The treatment reduced the number and activation of neutrophils in the alveolar space, as well as the level of most cytokines in the bronchoalveolar lavage fluid.[41] All in vivo human models are plagued by limitations, such as ethical considerations, clinical priorities, and mandated patient safety, that sometimes make their interpretation more complicated than expected.[42]

Regardless of their limitations, the animal and human models of ALI will continue to be used and refined, since there are no alternatives available for analysis and therapeutic testing in the setting of a complex biological system. In parallel, it is expected that the refinement of human models (specifically, ex vivo models) of ALI will spawn new, forceful, and relevant data to be used before clinical trials on patients, while allowing for definition of more realistic primary and secondary end points.

ETIOLOGY, RISK, AND CELLULAR AND MOLECULAR PATHOPHYSIOLOGIC MECHANISMS OF ALI

ALI/ARDS has many causes, with sepsis of pulmonary or nonpulmonary origin being the most common.[7] The lung response to injuries starts with disruption of the alveolar-capillary interface (clinically known as the exudative phase), which transitions to a remodeling phase (clinically known as the fibroproliferative phase) independent of the initial cause;[43] the separation in time of the two phases was proven to be an unrealistic criterion as long as the fibrosing alveolitis begins[43] almost at the same time as the alveolocapillary damage.[44,45] ALI/ARDS is an overwhelming inflammatory process that could be divided into two phases: the initial (acute) phase—characterized by epithelial and endothelial injury recorded histopathologically as diffuse alveolar damage, type I pneumocytes necrosis, neutrophil infiltration, alveolar hemorrhage, and hyaline membrane deposition[46]—and a fibroproliferative phase with various degrees of fibrosis, neovascularization, and, afterward, resolution.[47] The fibroproliferative phase is seen as a process of lung repair caused by type II pneumocyte and fibroblast propagation that may restore morphofunctional characteristics of the lung. Thus, the pathological model of ALI includes, besides the exudative and proliferative stages, a phase of fibrosis that usually evolves toward resolution, but if persistent, it may progress to a point of no return, when

the destruction of alveolocapillary basement membrane cannot be restored. The factors controlling progression to fibroproliferation instead of resolution of inflammatory process and restoration of normal pulmonary architecture are poorly understood.[48] Currently, it is not possible to correlate the clinical path of ALI/ARDS patients with mechanisms controlling the cellular and molecular basis of lung repair.[49] Broadly defined, the disease includes processes related to direct lung responses to microbial and other environmental insults as well as those related to indirect pulmonary insults such as extrapulmonary sepsis and trauma, shock, burn injury, and mass transfusions. On this background, a wide variety of cell types, proteins, and inflammatory mediators participate in the inflammation, immunity, and tissue restoration or fibroproliferation.

The lung consists of three main types of cell: endothelial cells, which represent up to 50% of all cells in the lung,[50] the epithelium, which is the first line of defense against particulate material, and fibroblasts. Under inflammatory conditions there is a significant increase in lymphocytes and macrophages in the lung parenchyma, which together with the above cells participate in the pathogenesis and resolution of ALI.

ENDOTHELIAL CELLS AND ALI

The vascular component of the alveolocapillary barrier, formed by a tiny endothelial cell layer, is essential for maintaining adequate pulmonary homeostasis.[51] Strategically located at the interface between blood and the surrounding tissues, the endothelium is endowed with constitutive and inducible features that allow it to adapt to local conditions and to respond to a plethora of injuries from blood and tissue (Table 2). The main function of the endothelial layer is to assemble a functional, semipermeable barrier that separates the blood from the surrounding tissues while allowing a bidirectional flux of water and small and large solutes, as well as blood cells.[52] Specific structural features of the endothelial phenotype, such as the extremely rich vesicular population, tight and adherens junctions, focal adhesions, a particularly structured cytoskeleton, and local adaptations to meet permeability needs (caveolar diaphragms, fenestrae), have been the subject of excellent reviews.[51,52,88,89,91,92] While the participation of some

Table 2. Major functions of pulmonary endothelium

Function
1.   Barrier function[52-55]
2.   Regulation of coagulation and thrombolysis[56,57]
3.   Drug removal and biotransformation[58-61]
4.   Active interactions with cellular components of blood, with bacteria, viruses, or any particulate compounds found in the blood or in the tissue[62-65]
5.   Expression of a variety of receptors and signaling molecules[66-70]
6.   Expression of definite enzymes such as NO synthase, ACE, endothelin converting enzyme, lipoprotein lipase[71-73]
7.   Synthesis of vasoactive autacoids involved in establishing and controlling the vascular tone as well as the blood flow[74-76]
8.   Binding and interacting with immune complexes and immunocompetent cells upon activation[77-79]
9.   Production of ROS and RNS, cytokines, growth factors[80-83]
10. Expression of adhesion molecules[84-87]
11. Expression of specific structural elements (fenestrations, diaphragms, interendothelial sealing, supramolecular complexes)[88-90]

Note: ACE: angiotensin-converting enzyme; RNS: reactive nitrogen species; ROS: reactive oxygen species.

of these endothelial characteristic structures in the inflammatory process is well described and functionally characterized (e.g., endothelial adherens junctions), the involvement of other elements, such as the endothelial tight junctions, is less known and often controversial, in regard to both function and structural/morphological organization.[55,93-95] This is significant because opening of interendothelial space, comprising both tight and adherens junctions, is the first structural element that changes in ALI, causing pulmonary edema. There will be no increase in permeability without the rearrangement of the tight junctional complex, located most apically in all endothelial cells and in some parts of the vascular tree (arteries and capillaries); in some specific vascular beds (brain, testis), tight junctional complexes can be found in more than one location along the inter-

endothelial space.[53,96-98] Endothelial participation and the mechanisms involved in exacerbated vascular permeability in ALI have been extensively reviewed.[84,94]

In pathological conditions, dysfunctional endothelial cells show impaired caveolae and clathrin-coated vesicle endocytosis and altered intracellular trafficking and signaling of cell surface receptors such as transforming growth factor beta receptor (TGFβR) and bone morphogenetic protein receptor-2 (BMPR2), both implicated in the pathogenesis of ALI/ARDS.[99-102] However, the consequences of endothelial cell endocytic dysfunction in the context of human lung disease in general, and in ALI/ARDS in particular, are understudied. Endocytic dysfunction and nonproductive assembly of endocytic machinery may alter canonical signaling pathways, with detrimental consequences for endothelial cell function.[103,104] Frequently, dysfunctional endothelial cells show increased occurrence of pleomorphic endocytic/transcytotic structures that function as morphological intermediates of alternative transport pathways to compensate for deficient vesicular trafficking.[11,105] Our recent work indicates that in mouse lung endothelial cells deficient of intersectin 1-s (ITSN-1s), a general endocytic protein required for endothelial cell survival, these enlarged endocytic structures and impaired endocytosis alter the signaling of Alk5, a broadly expressed type 1 TGFβR;[106] as result, downstream signaling of Alk5 is switched from the canonical Smad2/3 signaling to Erk1/2 MAPK, resulting in proliferation of endothelial cells and microvascular and lung remodeling.[107] Endothelial cells are not only a target but also an active player in ALI/ARDS. While endothelial cells alone are insufficient to cause ALI,[17] their injury/dysfunction and activation, as well as interaction with alveolar epithelium, are critical not only for the onset of ALI/ARDS but also for repair and remodeling of the injured lung. Endothelial cell heterogeneity, the complexity of these cells' interaction with other pulmonary cells, and the lack of complete understanding of the molecular mechanisms involved in endothelial response in ALI/ARDS make lung endothelium an attractive field for exploration, with high chances of improving detection, amelioration, and therapy for these severe syndromes.

APOPTOSIS IN ALI/ARDS: ITSN-1s REGULATION OF ENDOTHELIAL CELL APOPTOSIS

Apoptosis has been documented in the lung during pathogenesis of ALI/ARDS.[108] Two hypotheses—"neutrophilic apoptosis" and "epithelial apoptosis"—connect apoptosis to the pathogenesis of ALI in both experimental models of ALI/ARDS and humans.[109] The loss of cells from the alveolocapillary unit may result from apoptosis of epithelia, endothelia, and neutrophils, and this is an important event for the initiation and the development of ALI/ARDS.[110] Apoptosis and the clearance of apoptotic cells can be detrimental or beneficial, depending on cell type, circumstances, and timing.[111] For example, inhibition of neutrophil apoptosis has been shown to contribute to basement membrane destruction and increased permeability of the alveolocapillary barrier.[112] In contrast, phagocytosis of dead neutrophils inhibits proinflammatory cytokine production and leads to the resolution of ALI/ARDS.[113] However, ALI/ARDS can occur without neutrophil activation as well.[114] Early apoptosis of epithelial and endothelial cells, leading to loss of alveolocapillary barrier integrity, with consequent pulmonary edema and acute hypoxemia, is pivotal for human ALI/ARDS.[22] Accumulation of apoptotic cells in the lung due to deficient clearance, combined with enhanced endothelial/epithelial cell death at the same site, is even more deleterious to ALI/ARDS progression.[115] Growing evidence indicates, however, that apoptosis may be helpful during ALI resolution; apoptotic/activated cells may release growth factors and circulating microparticles that contribute to upregulation of proliferative signaling pathways, apoptosis resistance, or replacement by progenitor cells.[116-121] The replacement of dead cells may lead to neovascularization, repair of the injured lungs, and microvascular/lung tissue remodeling.[122] Lung epithelial and endothelial cell apoptosis and microvessel loss, followed by endothelial cell proliferation and abnormal neovascularization, are pathological traits seen in the lungs of ALI/ARDS patients.[123] Circulating microparticles can transfer genetic material and proteins from the donor cells (cells generating the microparticles) to a wide range of target cells, by several mechanisms: (1) internalization and lysosomal processing of

microparticles, (2) fusion-mediated transfer of surface receptors, proteins, and lipids, (3) outside-in signaling via ligand-receptor internalization, and (4) temporary fusion with the target cell, followed by complete or selective transfer of microparticle content.[116] The microparticles are heterogeneous, in terms of cellular origin, biochemical makeup, and size; elevated levels of microparticles have been associated with sepsis and ALI.[124] They may have both positive and harmful effects in ALI/ARDS, with the potential of ameliorating or worsening several processes with well-documented contributions to the pathology of ALI/ARDS, such as vascular permeability, coagulation, and inflammation.[116]

Studies on animal models have shown that ALI triggers growth factor–mediated interactions between the alveolar, endothelial, and mesenchymal cells, aimed at restoring lung integrity.[125] Vascular endothelial growth factor (VEGF), transforming growth factor beta (TGFβ), epithelial growth factor (EGF), and basic fibroblast growth factor (bFGF) are known mediators of cell proliferation and differentiation, angiogenesis, apoptosis, vascular permeability, and tissue repair[126-130] Studies have shown that plasma VEGF levels rise and intrapulmonary levels fall in the early stages of lung injury, with normalization of both during patients' recovery.[131] The actions of TGFβ in ALI/ARDS have been evaluated mostly during the late phases mediating mesenchymal cells hyperproliferation and synthesis of collagen fibers, lung remodeling, and fibrosis.[130]

In vivo long-term ITSN-1s knockdown (KD_{ITSN}), via repeated delivery of $siRNA_{ITSN}$ (small interfering RNA)/cationic liposome complexes, induced extensive apoptosis of mouse lung epithelial and endothelial cells in a process that involved downregulation of Erk1/2 MAPK signaling.[107] Death of endothelial cells resulted in destruction of the mouse pulmonary microvessels and loss of alveolar septa, pathophysiological hallmarks of ALI/ARDS.[132] After only 7 days of KD_{ITSN}, the expression of several growth factors (i.e., TGFβ, VEGF) whose downstream signaling includes Erk1/2 MAPK was increased. As result, Erk1/2 signaling was restored, and the remaining endothelial cells exhibited phenotypic changes including hyperproliferation and apoptosis resistance against KD_{ITSN}, leading to increased microvessel density, repair, and remodeling of the injured lungs.[107]

The literature is limited regarding ITSN-1 involvement in pathological conditions. So far, the existing data related to ITSN-1s participation in pathophysiological processes have been accumulated by studying Down syndrome and Alzheimer's disease, in which the ITSN-1 gene is upregulated.[133] A clear association between endocytic abnormalities and the pathophysiological mechanisms of Down syndrome has been established in neurons.[134] Enlarged early endosomes, marked variation in their size, and an increase of the total number were reported.[135] While in neurons, ITSN-1 overexpression induces severe impairment of endocytosis,[133,136] in fibroblasts isolated from Down syndrome patients, reports indicate an increase in internalization rate.[137] Genetic approaches to elucidate the function of mammalian ITSN-1 have yielded contradictory results, ranging from complete lethality to unaffected viability; the gene trap insertion strategy applied to ITSN-1 locus is lethal,[138] while the homologous recombination of the same locus resulted in viable and fertile mice with defects in the endocytic pathway.[139] Similar problems, perhaps compounded by the possibility of obtaining phenotypes from "null" alleles, explain the loss of function of ITSN and lethality in *Drosophila*,[140] while ablation of the ITSN gene in *Caenorhabditis elegans* results in live worms.[141]

Because ITSNs participate in the activation of different mitogenic kinases,[107,142] their involvement in cell growth, proliferation, and cancer is gaining attention. Low levels of ITSN-2 expression were associated with poor prognosis of breast cancer patients after adjuvant chemotherapy.[143] Low levels of ITSN-1 are reported in several cancers, lung cancer included (http://www.proteinatlas.org/ENSG00000205726 /summary).[144]

Overexpression of ITSN-1 induces oncogenic transformation of rodent fibroblasts.[145,146] Future work is needed to investigate the role of ITSN-1s in the context of human tumorigenesis and to delineate the mechanisms and pathways modulated by the members of ITSN family.

Our recent data suggest that ITSN-1s deficiency in mouse lungs, resulting in epithelial and endothelial

cell apoptosis and alveolar damage, causes increased paracellular permeability and patchy protein-rich interstitial edema,[105] altogether resembling common features of ALI in humans. The peak in airway space enlargement due to alveolar cells apoptosis (epithelium and endothelium) in mouse lungs, as indicated by the 40% increase in mean linear intercept value, was reached at day 10 of ITSN-1s deficiency. Activated caspase-3 in mouse lungs was detected at day 3 of KD_{ITSN} and reached the highest levels after 10 days of KD_{ITSN} by reference to control lungs.[107] Therefore, the alveolar damage may be a multifactorial process: the initial loss of the capillary bed, essential for growth and stability of alveolar septa and activation of the elastolytic activity of apoptotic cells, all potentiated by inhibition of ITSN-1s expression in the epithelial cells, contributed to alveolar damage, loss of alveolar membrane integrity, and pulmonary edema, as also described in other settings.[147] Studies from patients who died of ALI have shown pneumocytes with DNA fragmentation and activation of the pro-apoptotic Bax protein,[148,149] two mechanisms triggered by ITSN-1s deficiency in human lung microvascular endothelial cells.[150]

There is evidence that reactive oxygen species (ROSs) generated by inflammatory cells, as well as epithelial and endothelial cells, are responsible for lung damage and abnormal repair.[151] Mitochondrial dysfunction and ROS production by mitochondria can contribute to apoptotic cell death[150,152] and thus participate in cellular processes involved in the maintenance of lung integrity. KD_{ITSN} in lung endothelial cells increased ROS production significantly,[150] and therefore it is not surprising that affecting ITSN-1s expression and function is involved in the development of ALI/ARDS.

Until recently, the role of ITSNs in lung pathology has not been addressed. This unique KD_{ITSN} mouse model, without lethality, connects ITSN-1s to ALI/ARDS and offers the proper substrate to investigate the effects of a disrupted endothelial barrier with intense endothelial and epithelial cell apoptosis on mouse lung vascular and alveolar architecture. Understanding the molecular mechanisms involved in upregulation of proliferative signaling, apoptosis resistance, and stimulation of dead-cell replacement might provide potential avenues for therapeutic intervention.

ITSN-1s REGULATION OF TRANSCELLULAR AND PARACELLULAR PERMEABILITY

An intact lung microvascular endothelium is essential for maintaining adequate pulmonary and systemic homeostasis.[52] The endothelium forms a tightly regulated barrier that not only separates the blood content from the surrounding tissues but also filters it before it reenters the systemic circulation. The endothelium is an active organ endowed with important physiological properties: it promotes an antiaggregation surface; creates and maintains the endothelial barrier; synthesizes, metabolizes, and uptakes vasoactive compounds that modulate vascular tone; and regulates ventilation–perfusion matching, hemofluidity, and interactions with blood-borne cells (Table 2). Most endothelial functions are constitutive; some of them are, however, induced upon endothelial activation as a result of exposure to inflammatory stimuli. In ALI/ARDS of different etiologies (sepsis, trauma), the endothelium is the initial site of injury in the lung, leading to increased microvascular permeability and pulmonary edema; the mechanisms responsible for opening and resealing the barrier and endothelium participation in these events have been the subject of many excellent reviews.[84,92-94] Protein and solute transport across the endothelium involves a highly selective and organized transcellular pathway carried dominantly via caveolae and, to a lesser extent, via clathrin-coated vesicles.[52,92,153,154] Caveolae density, expressed as units per square micrometer of lung endothelial cell surface, is a relatively stable endothelial parameter demonstrated to vary only in diabetes.[155] It was assumed that the presence of caveolin-1 was sufficient for the formation of caveolae,[156] but later it was shown that the proteins belonging to the cavins family were also necessary for caveolae biogenesis.[157,158] As caveolae biogenesis is an emerging field, the findings reported in the KD_{ITSN} mouse, demonstrating that the caveolae density in endothelia is reduced, most probably as a consequence of impaired dynamin-2 recruitment to the endocytic site and deficient caveolar fission, are relevant.[159] Caveolae are associated with a plethora of cel-

lular processes (i.e., endocytosis, cell proliferation, differentiation, and apoptosis, as well as cell migration) recently linked to the pathogenesis of idiopathic pulmonary fibrosis, lung cancer, inflammation, and vascular dysfunction.[160-164] Thus, our observations not only enhance the understanding of caveolar function but also link ITSN-1s to caveolae release from endothelial plasma membrane and thus to controlling their number. Moreover, the observations implicate ITSN-1 in the pathogenesis of diseases in which these prominent endothelial structures participate. It has been demonstrated that the reduced number of caveolae affects the caveolin-1/endothelial nitric oxide synthase (NOS) interaction and increases the production of NO and vascular permeability.[165,166] Clinical studies and animal models suggest that NO is involved in the pathogenesis of lung injury.[167] Acute ITSN-1s deficiency 72 hours after siRNA$_{ITSN}$ delivery caused a decrease in caveolae number and dysfunction of the endothelial barrier in mouse lungs; open interendothelial junctions, with more than 30-nm-wide intercellular gaps and heavily labeled by 18–20-nm-diameter tracer particles, were often recorded (Fig. 1).[105] As result, patchy protein-rich interstitial edema, a common feature of ALI in humans, was often detected (Fig. 2A).[105] ITSN-1s deficiency, resulting in alterations of endothelial barrier integrity and enhanced vascular permeability, may be a consequence of increased NO production; the participation of NO in this process has not been explored yet, and thus the

precise steps, the molecules involved, and how ITSN-1s regulates pertinent signaling pathways remain to be established. Moreover, the interactions of ITSN-1s with CdGAP, downstream cdc42 activation, and stress fiber/actin bundle formation[168,169] may be part of the molecular mechanisms engaged by ITSN-1s deficiency to alter the interendothelial junctions and to enhance vascular permeability.

The extent to which the endothelial vesicular trafficking is affected by KD$_{ITSN}$ is severe,[105] and hence, compensatory mechanisms were developed to keep the cells alive and to maintain lung homeostasis; this was an unforeseen effect of KD$_{ITSN}$. Under these circumstances, upregulation of alternative endocytic pathways and their morphological intermediates (enlarged endocytic structures, membraneous rings, and tubules open to the cell surface or apparently with no communication with the extracellular milieu, all heavily labeled by tracer particles) compensate for deficient caveolae internalization and transport across the lung endothelium (Fig. 2A, 2a1, 2B [arrows and outlined area], 2D).[105] Interestingly, the functionality of this alternative transport increased at 24 days of KD$_{ITSN}$, leading to partial resealing of interendothelial junctions and reduced interstitial edema. At this time point, mice still displayed increased microvascular permeability, a sequela of ALI, but neither the gold albumin (18–20-nm diameter) nor the DNP-BSA (dinitrophenol–bovine serum albumin; 6–10-nm diameter) tracers were able to penetrate the inter-
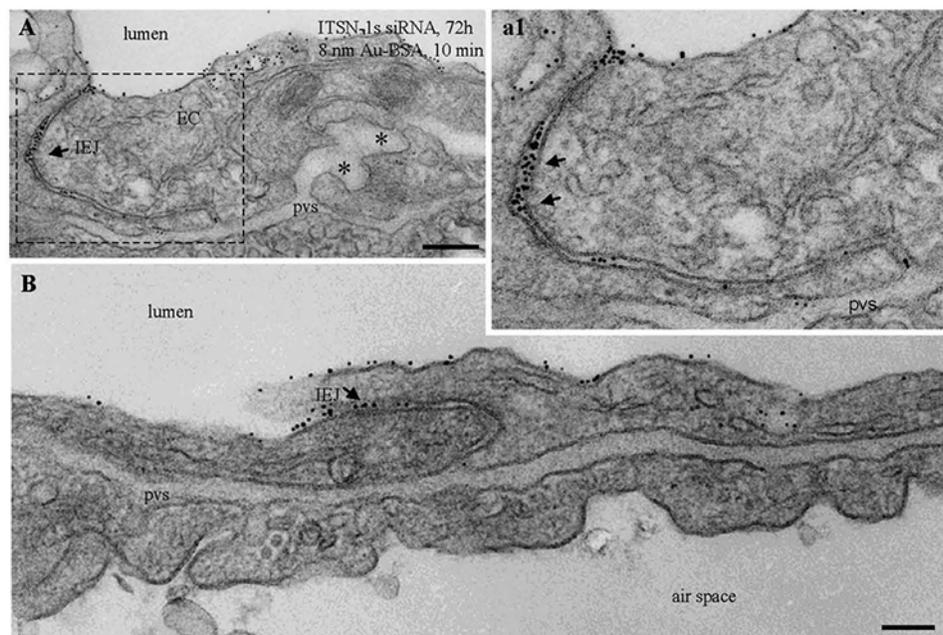

Figure 1. Increased endothelial permeability and impaired interendothelial junctional integrity in mouse lung endothelium acutely depleted of intersectin-1s (ITSN-1s); representative electron micrographs showing open interendothelial junctions (IEJs) labeled throughout their length by 8-nm gold albumin (Au-BSA) particles. Arrows in A (magnified in a1) point to three or four Au-BSA particles located close to each other in the same plan, indicative of the wide opening of the IEJ. Gold particles are also associated with the abluminal exit of IEJs. Note also the limited number of caveolae and the dilation of the pericapillary space (asterisks). pvs: perivascular space; siRNA: small interfering RNA. Scale bars: 200 nm (A), 100 nm (B).
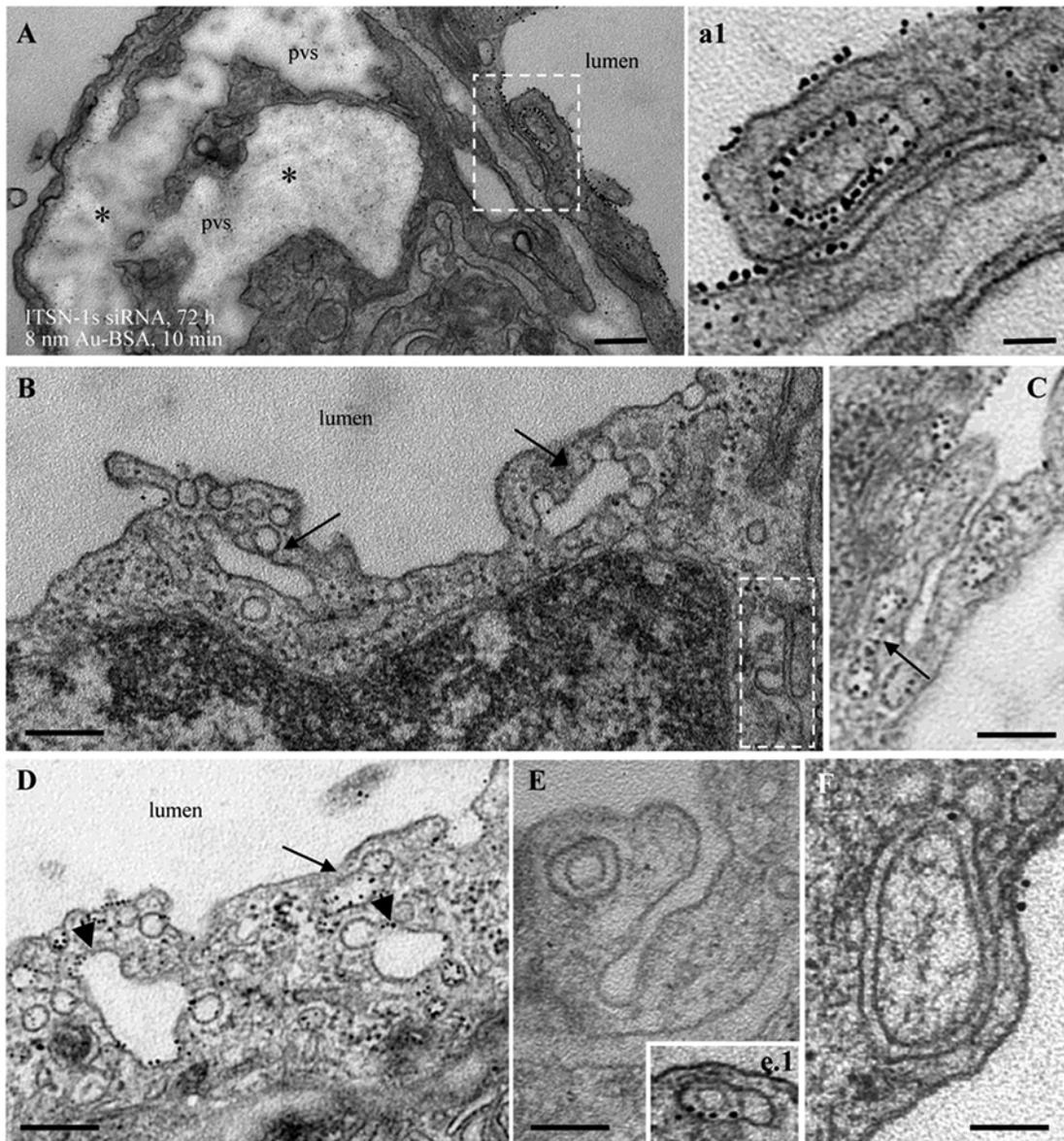
Figure 2. Acute perturbation of intersectin-1s (ITSN-1s) expression induces pleomorphic endocytic/transcytotic intermediates; representative electron microscopic (EM) images of membranous rings (*A, a1* [area outlined in *A*], *E, e.1, F*), tubular elements (*B–D*, arrows), and enlarged endosomes (*D*, arrowheads), loaded with 8-nm gold albumin (Au-BSA) and associated with caveola-like morphology. Two tubular structures open to the lumen are shown in *C* and *E*. The EM image shown in *E* was selected from a mouse lung specimen not perfused with 8-nm Au-BSA. Note also the severe dilation of the perivascular space (pvs) and the proteinaceous edema (*A*). Asterisks have the same meaning as in Figure 1. siRNA: small interfering RNA. Scale bars: 250 nm (*A, B*); 200 nm (*C, D*); 100 nm (*a1, E*); 150 nm (*F*).

endothelial junctions, meaning that resealing mechanisms were returning the endothelial barrier to its perm-selective status.[105] Patchy areas of mild septal edema were still present, implying that the resealing did not return the structure of the junctional complexes to their initial status, because of continuous ITSN-1s deficiency; additional studies are needed to fully define this intermediary state of interendothelial junction opening (>3 nm but <6 nm). KD_{ITSN} in mouse lungs had a dual outcome: (1) acute (3 days)

KD_{ITSN}-induced impairment in caveolae transport function, significant disruption of junctional integrity, and acute pulmonary edema and (2) chronic (24 days): KD_{ITSN} upregulated alternative transport pathways to compensate for deficient caveolae transport function and partially restored the endothelial barrier integrity, maintaining, however, a mild edema.[105] Thus, ITSN-1s is positively one of the factors needed for barrier integrity, and prolonged ITSN-1s deficiency and upregulation of compensatory endocytic

pathways may be part of a protective mechanism against lung injury associated with severe disruption of endothelial barrier function; this may apply in the case of acute inflammatory reaction, when the ITSN-1s protein is cleaved by the cytotoxic protease granzyme B (GrB),[170] inducing a long-term ITSN-1s deficiency. Evidence indicates that the bronchoalveolar fluid of ARDS patients contains highly upregulated messenger RNA levels for GrB and perforin.[171] Moreover, Bem and colleagues[172] demonstrated that combined GrA/GrB deficiency participates in progression of ALI/ARDS in pneumovirus-infected mice. The absence of GrA and GrB results in delayed neutrophil recruitment, diminished activation of caspase-3, and reduced lung permeability. More work is needed to identify the targets of these cytotoxic proteases with proinflammatory and pro-apoptotic activity and the physiological significance of their cytotoxic activity. While the extent of pulmonary edema depends on several factors, it is attractive to speculate that ITSN-1s cleavage by GrB, resulting in decreased ITSN-1s expression, may be part of the pathophysiology of increased permeability and pulmonary edema in ALI/ARDS. ITSN-1s, because of its ability to regulate caveolae release from the endothelial plasma membrane during transendothelial transport as well as actin cytoskeleton remodeling and stress fiber formation associated with interendothelial junctions, mediates a unique cross talk between transcellular and paracellular transport pathways, with a critical role for barrier integrity and lung homeostasis.

RESOLUTION OF ALI: ITSN-1s INVOLVEMENT IN ALVEOLAR AND ENDOTHELIAL REPAIR

Although cellular turnover is relatively slow in normal developed lungs, post-ALI rapid repair of the denuded and injured alveolar surface is crucial to survival. This implies that quiescent endothelial and alveolar type II cells regain the capacity to proliferate and differentiate. The signaling pathways for lung remodeling, repair, and regeneration are not well defined but possibly recapitulate the ontogeny.[173] Lung development requires coordinated interactions among epithelial, endothelial, and mesenchymal cells, and it is likely that alveolar repair does so as well. For example, VEGF, a major angiogenic and permogenic factor, is partly produced by lung mesenchymal and alveolar

type II cells, showing the interdependence of cells with regard to vascular and alveolar growth and maintenance. Inadequately regulated healing response after ALI/ARDS causes pulmonary fibrosis. Patients surviving the initial phase of ALI can progress to restoration of the normal pulmonary architecture or to accumulation of fibrotic tissue and lung dysfunction. The outcomes of persistent and progressive lung injury include multiple organ failure, fibrosing alveolitis, and pulmonary vascular obliteration with pulmonary hypertension and death.[132,174] The genetic, cellular, and molecular factors that contribute to each of these outcomes remain largely unknown.[112] It was postulated that the preferential growth of fibroblasts toward fibrosis may be a direct result of cell necrosis, particularly of the epithelium.[123,175] Also, disrupted or delayed repair of alveoli, interstitium, or the alveolocapillary membrane is sufficient to promote excessive proliferation and collagen deposition.[176] Some mechanistic insights about the lung fibrotic process were obtained from the bleomycin-induced ALI/ARDS rodent model.[23] Although extensively used, the bleomycin has been seen as "overwhelming stimulus" with little relevance to human post-ALI/ARDS pulmonary fibrosis.[12] Other animal models of ALI, such as high-dose LPS, cecal ligation and puncture, and oleic acid, have a high mortality, without allowing the study of lung-repairing process.

Long-term KD_{ITSN} in mouse lung allowed us a detailed analysis of the time course of lung injury and repair and provided mechanistic insights into the signaling pathways responsible. Mouse lung endothelial cell death, as caused by KD_{ITSN}, was a prerequisite for the subsequent endothelial cell proliferation, repair, and remodeling process. Within days, these normally quiescent cells with a low turnover rate began to hyperproliferate, indicating that the Erk1/2 MAPK survival signaling, lost because of KD_{ITSN}, had been reestablished. Expression of TGFβ, a cytokine that regulates diverse and often contradictory functions in a milieu- and cell type–dependent manner,[128] was increased. Endothelial cell death induced an increased expression and activation of TGFβ in the early stages of lung injury, peaking at day 3, when Erk1/2 MAPK signaling was significantly decreased because of KD_{ITSN}. It is well documented that TGFβ is a multifunctional cytokine involved in endothelial cell proliferation, survival, and maintenance of vascular in-

tegrity[128] and that Ras/Erk1/2 MAPK is a major signaling pathway downstream of Alk5, the broadly expressed TGFβ-R type 1.[106] Apparently, upregulation of BMP-2/4 proteins by chronic KD$_{ITSN}$ worked synergistically with TGFβ and VEGF to induce endothelial cell proliferation via activation of Erk1/2 MAPK pathway.[107] Paracrine and autocrine growth factors released by apoptotic endothelial cells caused increased survival, proliferation, and alteration of their phenotype. In order to reestablish lung tissue homeostasis, KD$_{ITSN}$ switched the TGFβ/Alk5 signaling from the typical Smad2/3 activation toward the less common Ras/Erk1/2 MAPK pathway, with protective effects on endothelial cells and lung vasculature causing increased microvessel density (Fig. 3), endothelial phenotypical changes, and alveolar repair.[107] Stimulation, via growth factors, of the Erk1/2 MAPK pathway also inactivated the pro-apoptotic Bad protein by phosphorylation of Ser112 and Ser155 residues, conferring on endothelial cells hyperproliferative and apoptosis-

resistance properties. Prolonged inhibition of ITSN-1s led to increased microvessel density, critical for repair and remodeling of the alveolar capillary membrane.[107]

KD$_{ITSN}$ mouse is an emerging model of an adaptor protein causing lung injury and endothelial cell phenotypical changes toward proliferation and apoptosis resistance, leading to repair of the injured lung. Extensive endothelial cell apoptosis, widely open inter-endothelial junctions, the rich proteinaceous interstitial edema, loss of pulmonary microvessels, and the histological images showing loss of the alveolar septa provide strong evidence that lung injury occurred. Moreover, absence of intra-alveolar neutrophil infiltrate and minimal inflammatory response characterize this emerging mouse model of ALI/ARDS. Furthermore, increased expression of growth factors, compensatory endothelial and epithelial cell proliferation resulting in increased microvascular density, and lung remodeling without fibrosis provide evi-
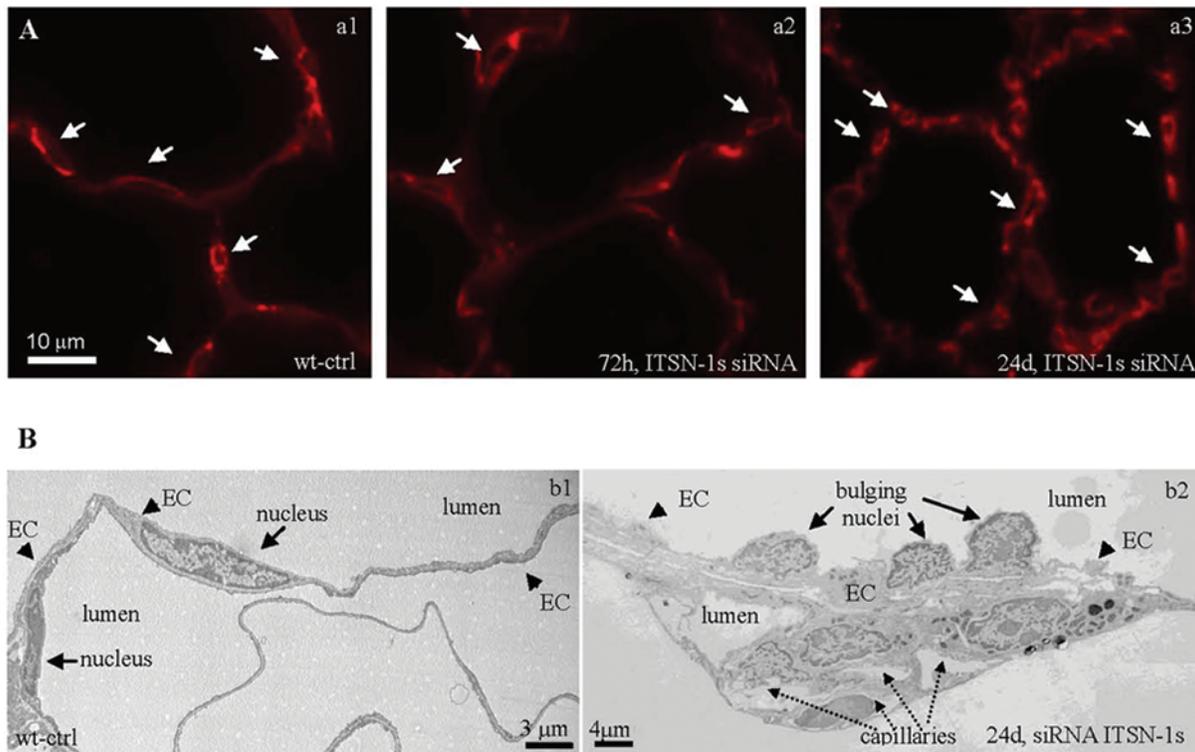

Figure 3. Chronic intersectin-1s knockdown (KD$_{ITSN-1s}$) in mouse lungs induces microvascular remodeling. A, Micrographs of GS-1 lectin staining of paraffin-embedded sections show microvessel profiles (arrows) within the alveolar walls in wild-type control (wt-ctrl) mice (a1), mice treated with small interfering RNA (siRNA$_{ITSN}$) for 3 days (a2), and mice treated with siRNA$_{ITSN}$ for 24 days (a3). Scale bar: 10 μm. B, Ultrastructural features of microvascular remodeling in KD$_{ITSN}$ mouse lungs (24 days). b1, Two vessel profiles in wt-ctrl mouse lungs display elongated endothelial cell (EC) nuclei. Note the relatively uniform thickness of the ECs throughout the vessel perimeter. b2, Segment of a midsized vessel in KD$_{ITSN}$ mouse lung shows a distorted endothelium and several nuclei protruding into the lumen (arrows). New pulmonary microvessels (dashed arrows) with narrow openings are abundant and located in very close proximity to each other.

dence for lung tissue repair/recovery following injury. However, despite reproducing several clinically relevant phenomena, the KD_{ITSN} mouse, like any animal model of human disease, has several specific limitations. ITSN-1s deficiency induced by retro-orbital delivery of liposome/$siRNA_{ITSN}$ complexes targets mainly the vascular endothelium, while the primary target tissue in human disease is not known. Even if ITSN-1s deficiency may be relevant in clinical settings, given ITSN-1s's susceptibility to GrB cleavage, the human disease is more complex, with many other risk factors as well as genetic determinants. Despite limitations, this mouse mimics many salient features of ALI/ARDS and can be used to better understand the molecular mechanisms underlying these severe syndromes and to develop better intervention strategies.

THE GENETIC DIMENSION OF ALI/ARDS

The major challenge of ALI/ARDS studies in human patients is the fact that the syndromes are the result of a plethora of molecular scenarios taking place in different cell types and physiological backgrounds dictated by age, sex, and, most of the time, coincident pathological processes.[42] Besides this intrinsic level of complexity, a particular characteristic of ALI/ARDS pathogenesis is the extremely high proportion of iatrogenic induced illness; half the instances of ALI occur in patients subjected to mechanical ventilation for the following major causes: (1) transfusion of blood and/or blood products, (2) massive fluid resuscitation, (3) hospital pneumonia, and (4) normalization of blood gas parameters.[177-180] When critically ill patients, most of them undergoing invasive procedures, are considered, the need for individualized therapies becomes obvious. On this pathogenic background, it was thought that phenotypic variance of ill patients might be related to variations in individual genomes, an assumption that has generated a plethora of gene expression studies aimed at identifying disease susceptibility loci. Initial research using genomic and genetic strategies to find the genes implicated in ALI pathogenesis and to generate lists of putative genes involved in the susceptibility and severity of the disease was considered sufficient to generate data aimed at an individualized treatment.

These initial genetic-association studies (more than 80 performed up to 2008), evaluated in two significant articles,[181,182] identified 15 or 16 genes positively associated with the susceptibility and outcomes of all-cause ALI or ARDS. Candidate genes significantly associated with ALI and/or ARDS were genes controlling several major biological processes—inflammatory response, endothelial permeability, nitric oxide biosynthesis, phospholipid metabolism, apoptosis, cell motility, transcription, etc. The complexity of phenotypic (tissular, individual, or populational) heterogeneity caused significant difficulties in identifying the most likely candidate genes related to susceptibility and severity of ALI. Inconsistencies, and sometimes conflicting results, were attributed to either inappropriate study design or the modalities used for data implementation and/or interpretation.[183,184] These discrepancies motivated the formulation of standards to improve quality and to help perform meta-analysis of the data,[185] while leading to the consensus that such studies should be considered tools to investigate ALI/ARDS and not definitive answers for disease pathogenesis and therapeutic options. Thus, despite adding a genetic predisposition to ALI, these studies rendered the promise of an individualized treatment very challenging.

Since the genetic-linkage analysis does not have the power to detect signals of common genetic variation with modest effects that are the causes of susceptibility to common complex diseases,[186,187] genome-wide association (GWA) studies were considered more powerful and better suited for identifying putative risk variants for ALI. However, GWA studies are biased toward detecting the loci with the largest effects and not all the loci involved in the pathophysiology of a disease; also, GWA studies assume that the susceptibility loci identified represent a small subset of all the loci involved and that they represent the true players in the disease development. Thus, it became clear that a plethora of factors, such as phenotypic variance of ill patients, diversity of causes eliciting lung injury, possibility of locus heterogeneity, the sporadic nature of the disease, and low or incomplete gene penetrance, should be considered and included in the design of this type of study. Despite these limitations, the use of GWA studies for different

diseases led to important achievements, as in the cases of type 1 and type 2 diabetes[188,189] and prostate and breast cancer,[190,191] while for other diseases, such as asthma,[192] coronary heart diseases,[193] and ALI,[194] progress remains limited.

In the past 4 years or so, more than 20 GWA studies in pulmonary medicine have been published that evaluated genes associated with pulmonary function measures, the genetic basis of asthma, and susceptibility loci in chronic obstructive pulmonary disease, interstitial pulmonary fibrosis, and sarcoidosis;[195] most of the data are widely accessible and can be used as basis for further research to evaluate the genetic basis for pulmonary conditions less subjected to the GWA approach so far, such as ALI/ARDS. Therefore, we have begun building a catalog of the genes associated with the disease, with the potential to advance our understanding of lung injury; in addition, it may lead to the development of more-personalized therapies and, most importantly, may help in planning the necessary changes in medical education curricula to prepare future clinicians to handle and manage genetic, genomic, and pharmacological data. At present, the most significant and conceptually agreed-on candidate genes involved in ALI/ARDS are listed in Table 3.

Because this type of study focuses on the potential genetic and gene-gene interactions that may constitute the explanation for the disparities observed in the heterogeneous response of individuals to ALI/ARDS-generating factors and/or diseases, one should be aware that the interactions of gene and environment, along with the transitory or inherited alterations, may be as important as the genetic background alone. Years of genetic and genomic studies of susceptibility to ALI/ARDS led to the conclusion that gene expression is controlled not only by modification(s) of the gene but also by the interaction of the gene with the environment[213] and by epigenetic mechanisms.[214] Both ALI and ARDS are the result of a complex interplay between environmental, epigenetic, and genetic factors, along with the reality that only a fraction of individuals exposed to ALI-inducing factors (trauma, sepsis, acid aspiration) progress to develop the disease and that from this only another fraction will progress to ARDS.

Table 3. Candidate genes involved in ALI/ARDS

Symbol	Gene
ACE	Angiotensin converting enzyme[196,197]
CXCL2	Chemokine ligand 2/macrophage inflammatory protein 2[198]
GADD45A	Growth arrest and DNA-inducible alpha[199]
IL-6	Interleukin 6[200]
IL-10	Interleukin 10[201]
LBP	Lipopolysaccharide binding protein[202]
MLCK	Myosin light-chain kinase[203]
MBL2	Mannose-binding lectin[204]
NFkB1A	Nuclear factor of kappa light polypeptide gene enhancer in B-cells 1[205]
NRF2	Nuclear factor (erythroid-derived 2)-like 2[206]
PAI-1	Plasminogen activator inhibitor[207]
PBEF	Pre-B-cell colony enhancing factor[208]
SOD3	Extracellular superoxide dismutase[209]
SP-B	Surfactant protein B[210]
TGF-β	Transforming growth factor β[211]
TNF-α	Tumor necrosis factor α[212]
VEGF	Vascular endothelial growth factor[126]

The challenge for both genetic-association studies and GWA studies is the identification of the biological context in which the statistically significant putative genes act. At present, the current genotyping platforms have the power to survey only a subset of human sequence variations, as the typing of single-nucleotide polymorphisms has been limited to only 300,000–600,000, depending on the population, which after all means that they capture most of the common genetic variation in a region of interest. In other words, the reported susceptibility variants are most probably tagging the real functional variants, and thus they are not causal themselves. Consequently, all these initial discoveries must be followed by fine mapping of the regions harboring the most significant statistical signals,[215] which in the absence of prior biological information is financially prohibitive.

SUMMARY

Studies on animal and human models of ALI/ARDS are critical for a better understanding of the patho-

physiology of ALI/ARDS and for the development of novel and improved therapeutic tools for treatment of these severe syndromes. While animal models bridge cell-culture studies and human trials and provide significant help in understanding the molecular events and signaling pathways involved in ALI/ARDS, the development and progression of ALI/ARDS in animals occur in a reduced time frame and in the settings of animal pulmonary physiology, quite different from that of humans. Despite limitations, animal models of ALI/ARDS share molecular etiology with human disease and add significantly to our knowledge of how endothelial barrier dysfunction, inflammatory response, nitric oxide biosynthesis, cell motility, cell adhesion, apoptosis, and so on contribute to lung injury and repair. The KD_{ITSN} mouse model discussed in detail here seems to be relevant to the pathogenesis of human ALI/ARDS. Acute endothelial and epithelial cell apoptosis, alveolocapillary damage, loss of endothelial barrier function, and pulmonary edema, followed by compensatory proliferation and a repair process, are all caused by ITSN-1s deficiency in murine lungs. The findings are relevant, considering that ITSN-1s is a general endocytic protein, a regulator of mitochondrial apoptosis, and a GrB substrate.[171] Under inflammatory conditions and increased GrB levels, loss of full-length ITSN-1s protein may cause endocytic dysfunction, loss of prosurvival signaling, and apoptotic endothelial cell death. The findings implicate ITSN-1s, a key prosurvival protein of lung endothelium, and the biological processes regulated by it in the pathology of ALI/ARDS. Thus, the KD_{ITSN} mouse model may become a valuable tool to advance our understanding of ALI/ARDS pathophysiology and provide novel targets for treatment of these severe human syndromes.

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