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Report

Essential Regulation of Lung Surfactant Homeostasis by the Orphan G Protein-Coupled Receptor GPR116

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SUMMARY

GPR116 is an orphan seven-pass transmembrane receptor whose function has been unclear. Global disruption of the *Gpr116* gene in mice revealed an unexpected, critical role for this receptor in lung surfactant homeostasis, resulting in progressive accumulation of surfactant lipids and proteins in the alveolar space, labored breathing, and a reduced lifespan. GPR116 expression analysis, bone marrow transplantation studies, and characterization of conditional knockout mice revealed that GPR116 expression in ATII cells is required for maintaining normal surfactant levels. Aberrant packaging of surfactant proteins with lipids in the *Gpr116* mutant mice resulted in compromised surfactant structure, function, uptake, and processing. Thus, GPR116 plays an indispensable role in lung surfactant homeostasis with important ramifications for the understanding and treatment of lung surfactant disorders.

INTRODUCTION

The vital process of mammalian breathing requires alveoli to expand and contract without collapsing, a remarkable feat that depends upon the surface tension-reducing properties of the lipid-rich surfactant film that lines the alveolus (Trapnell et al., 2003). Pulmonary surfactant, which also functions in innate immunity, is composed of 90% lipids (primarily phosphatidylcholine [PC]) and 10% protein (surfactant proteins A [SP-A], SP-B, SP-C, and SP-D). In premature infants, insufficient production of surfactant leads to neonatal respiratory distress syndrome (RDS). Surfactant deficiency also contributes to the pathogenesis of acute lung injury (ALI) and acute RDS (ARDS), disorders that can afflict patients of all ages and carry a mortality rate greater than 25% (Lewis and Veldhuizen, 2006; Raghavendran et al., 2011). Conversely, accumulation of surfactant in the alveolar space leads to pulmonary alveolar proteinosis (PAP) (Carey and Trapnell, 2010). Although important progress has been made in understanding surfactant catabolism by macrophages, a process dependent upon GM-CSF signaling (Carey and Trapnell, 2010; Sakagami et al., 2009), mechanisms regulating surfactant homeostasis in the alveolar space remain largely unexplained. Furthermore, whole-lung lavage, the standard of care for PAP, only helps a subset of patients, has numerous complications, and does not address the pathogenic mechanisms at work (Borie et al., 2011). Thus, new methods of regulating surfactant levels and activity are urgently needed and could have an impact on a myriad of lung diseases.

RESULTS AND DISCUSSION

Genetic Disruption of *Gpr116* Results in a Shortened Lifespan Associated with Pulmonary-Specific Abnormalities

G protein-coupled receptor (GPCR) superfamily members mediate cell signaling in response to a diverse array of extracellular stimuli and comprise over one-third of the drug targets of modern medicine (Tang et al., 2012). While searching a large panel of gene expression libraries for endothelial GPCRs (Seaman et al., 2007; St Croix et al., 2000), we identified *Gpr116*, also called Ig-Hepta in rats (Abe et al., 1999), as a pan-endothelial-expressed gene of unknown function (Figures S1A–S1H; see also Wallgard et al., 2008). *Gpr116* was broadly expressed in normal unfractionated tissues with highest expression in lung

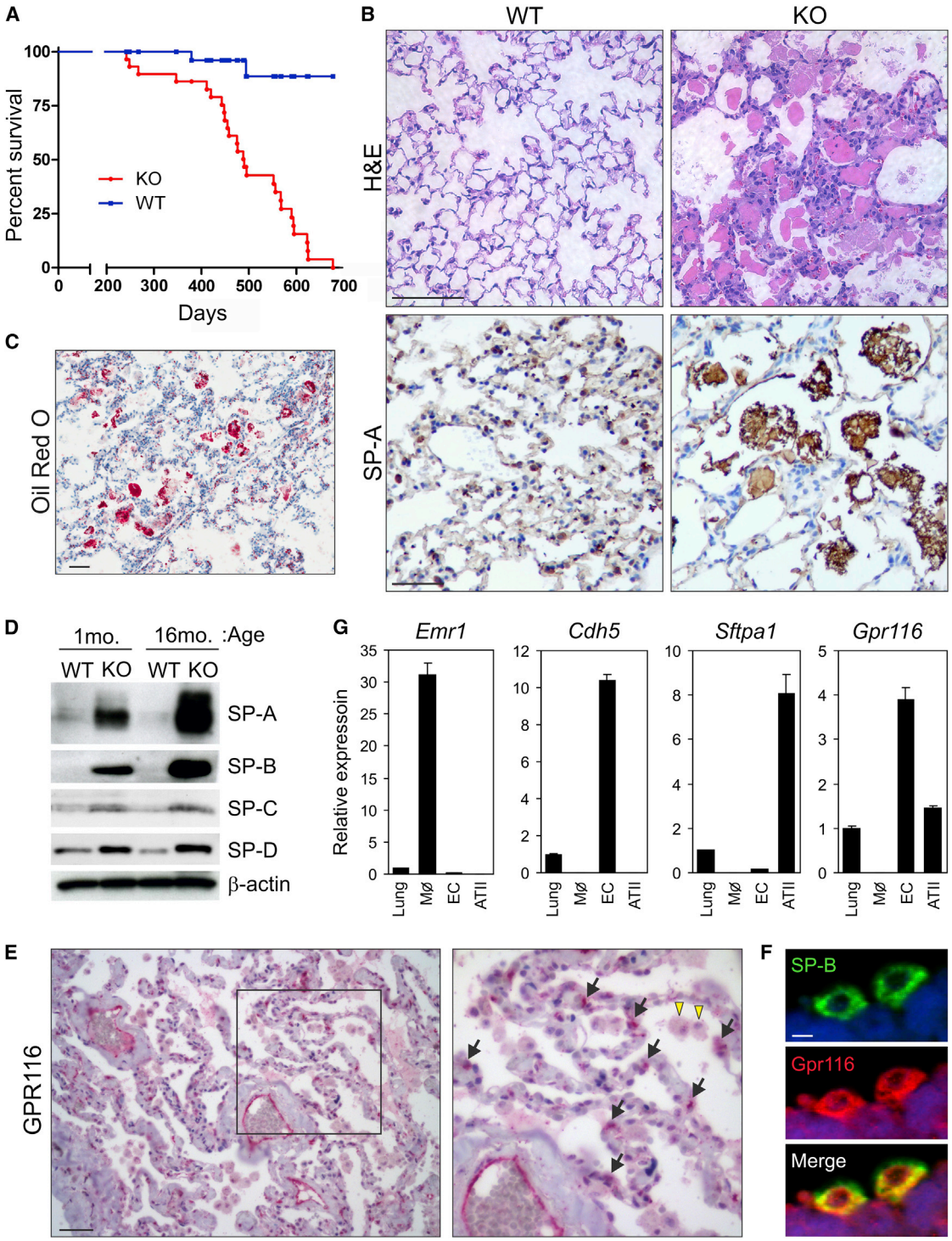

Figure 1. *Gpr116* Is Expressed in Lung ECs and ATII Cells and Is Important for Maintaining Lung Surfactant Homeostasis

(A) Survival analysis of *Gpr116* WT and KO mice.

(B) An accumulation of eosinophilic, SP-A+ material was observed in the alveolar space of 6-month-old lungs of KO mice by H&E and SP-A staining. Scale bar, 50 μm.

(C) The accumulated lung material stained positive with oil red O dye indicative of a buildup of neutral lipids in the alveolar space. Scale bar, 50 μm.

(D) Immunoblotting for SP-A, SP-B, SP-C, and SP-D in KO versus WT whole-lung lysates.

(E) Immunohistochemical staining of normal human lung. GPR116 protein (red) can be found in both ECs and ATII cells (arrows in inset), whereas alveolar macrophages were negative (yellow arrowheads). Scale bar, 100 μm.

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