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Epidermal fatty acid-binding protein is increased in rat lungs following in vivo treatment with keratinocyte growth factor

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Abstract

Exogenous application of keratinocyte growth factor protects the lung against a variety of injurious stimuli. KGF-treatment leads to pronounced hyperplasia of alveolar epithelial type II cells and to stabilization of surfactant homeostasis after lung injury. Epidermal fatty acid-binding protein is involved in the synthesis of surfactant phospholipids and acts as an antioxidant scavenging reactive lipids. We treated adult rats with recombinant human keratinocyte growth factor (Palifermin) via intratracheal instillation and analyzed the expression of epidermal fatty acid-binding protein mRNA and protein by quantitative RT-PCR, immunoblotting as well as immunohistochemistry. Keratinocyte growth factor-treatment in vivo leads to an increased expression of epidermal fatty acid-binding protein mRNA and protein in the total lung. Epidermal fatty acid-binding protein mRNA expression per alveolar epithelial type II cell remains constant as shown in isolated type II cells. Epidermal fatty acid-binding protein immunoreactivity is seen in most if not all hyperplastic alveolar epithelial type II cells, and is mainly localized to the cytoplasm. The increase in epidermal fatty acid-binding protein gene expression associated with type II cell hyperplasia might contribute to the molecular mechanisms mediating lung protection by keratinocyte growth factor.

Keywords: Epidermal fatty acid-binding protein; Keratinocyte growth factor; Lung; Alveolar epithelial cell type II

1. Introduction

Keratinocyte growth factor (KGF, or fibroblast growth factor 7) plays important roles in lung development, inflammation and repair (for review: Ware & Matthay, 2002). This growth factor is mainly produced by fibroblasts and smooth muscle cells. Epithelial cells expressing the high affinity receptor FGFR2-IIIb are the targets of KGF. Exogenous application of KGF protects

Abbreviations: AEII, alveolar epithelial type II cell; E-FABP, epidermal fatty acid-binding protein; GAPDH, glyceraldehydephosphate dehydrogenase; KGF, keratinocyte growth factor; rHuKGF, recombinant human KGF; SP, surfactant protein

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the lung in various models of acute lung injury including hyperoxia, bleomycin, acid aspiration, mechanical injury, radiation, infections and even graft-versus-host disease (Ware & Matthay, 2002).

The mechanisms by which KGF protects the lung are not fully understood. However, the stimulation of alveolar epithelial type II cells (AEIIs) and the stabilization of the surfactant homeostasis are likely to play a pivotal role. Application of exogenous KGF induces proliferation of AEIIs, which in vivo results in AEII hyperplasia with a peak at 2–3 days after treatment (Ulich et al., 1994; Fehrenbach et al., 1999; Yano et al., 2000). KGF induced AEII hyperplasia resembles reactive hyperplasia that is seen in human alveolar epithelia during recovery from injury (Bachofen & Weibel, 1982; Bardales, Xie, Schaefer, & Hsu, 1996).

AEIIs produce and secrete surfactant, a phospholipid protein complex consisting of about 90% lipids and 10% proteins. Pulmonary surfactant reduces the surface tension, stabilizes the alveoli and prevents alveolar flooding. The impairment of the surfactant system, which is observed in acute lung injury/respiratory distress syndrome, can be beneficially affected by exogenous surfactant application (for review: Lewis & Brackenbury, 2003). The expression of surfactant proteins (SP) is upregulated at the mRNA level for SP-A, SP-B and SP-D in total rat lungs treated with KGF, and the amount of SP-A and SP-D is increased in the bronchoalveolar lavage fluid (Yano et al., 2000). In an experimental model of graft-versus-host disease, SP-A was reported to be an essential mediator of the KGF induced protection of the lung (Haddad et al., 2003). The regulation of the phospholipid components of surfactant by KGF is not fully understood. In vitro, KGF stimulates surfactant phospholipid production by AEIIs from premature rabbits, fetal and adult rat lungs (Ikegami, Jobe, & Havill, 1997; Chelly, Mouhieddine-Gueddiche, Barlier-Mur, Chailley-Heu, & Bourbon, 1999; Mason et al., 2003). In contrast, after KGF-treatment in vivo, Fehrenbach et al. (2003) demonstrated that during hyperplasia number and volume of lamellar bodies, the intracellular storage form of the phospholipid component of surfactant, was reduced per cell whereas the total volume of lamellar bodies per lung remained constant. Likewise, KGF increases the mRNA levels for the SPs per lung because of AEII hyperplasia, but the mRNA levels per cell are slightly diminished (Yano et al., 2000).

When isolated AEIIs are cultured in vitro, they rapidly differentiate towards a phenotype resembling type I cells and downregulate the expression of SPs (McElroy & Kasper, 2004). In contrast, in the presence of KGF the synthesis of SPs is maintained (Mason et al., 2002). Under these conditions, mRNA levels of epidermal fatty acid-binding protein (E-FABP) are increased in comparison to AEIIs cultured in the absence of KGF (Mason et al., 2003). E-FABP is a small 15 kDa protein which belongs to the family of cytoplasmatic fatty acid binding proteins. In the lung, E-FABP is involved in the synthesis of dipalmitoyl phosphatidylcholine, the most abundant phospholipid component of surfactant (Guthmann et al., 2004). Therefore, E-FABP is an essential factor needed for the synthesis of functional surfactant. In addition, E-FABP scavenges toxic lipid peroxidation products, which menace the lung during acute injury (Chow, Herrera Abreu, Suzuki, & Downey 2003; Bennaars-Eiden et al., 2002). Given the potential importance of E-FABP for surfactant homeostasis and lung protection, we investigated the expression of E-FABP in vivo in rats, which were treated with human recombinant KGF (rHuKGF) by means of immunohistochemistry, quantitative RT-PCR and immunoblotting.

2. Materials and methods

2.1. Treatment of the experimental animals

Bioactive, purified, endotoxin free rHuKGF (Palifermin) produced in *Echerichia coli* was provided by Amgen (Thousand Oaks, CA, USA). Male LEW ($RT1^{l}$) and DA ($RT1^{av1}$) rats were raised under pathogen free conditions according to the FELASA standards and provided by Harlan Winkelmann (Borchen, Germany). On day 1, animals weighing 200–270 g were anaesthetized by short time inhalation of isofluran (Forene, Abbot, Wiesbaden, Germany), intubated orally with a 16 G intravascular catheter (Insyte, Beckton Dickinson, Sandy, USA), and instilled via the trachea with 5 mg rHuKGF/kg body weight or an equivalent volume of PBS (200–270 μl). The treatment was repeated on day 2. On day 4 the rats were anaesthetized with 90 mg/kg body weight Ketamin hydrochloride (Ketavet, Pharmacia, Erlangen, Germany) and 0.1 mg/kg Medetomidine hydrochloride (Dormitor, Pfizer, Karlsruhe, Germany). Additionally they received an intravenous injection of 1000 international Units of Heparin (Ratiopharm, Ulm, Germany) per kilogram body weight and an intramuscular injection of 0.25 mg/kg Atropin sulfate (Braun Melsungen, Melsungen, Germany). The lungs were excised and the right lung was either cut in small pieces and snap frozen in liquid nitrogen for RT-PCR and Western blot experiments or fixed for immunohistochemistry. We only used the right lungs because the left lungs were used in transplantation studies, which will be described else-