ELSEVIER

Journal of Cystic Fibrosis 14 (2015) 600 – 607

Journal of Cystic Fibrosis
www.elsevier.com/locate/jcf

Original Article

Identification of outer membrane Porin D as a vitronectin-binding factor in cystic fibrosis clinical isolates of *Pseudomonas aeruginosa*

Magnus Paulsson [a], Birendra Singh [a], Tamim Al-Jubair [a], Yu-Ching Su [a], Niels Høiby [b], Kristian Riesbeck [a,*]

[a] *Clinical Microbiology, Department of Translational Medicine, Lund University, Jan Waldenströms gata 59, SE-205 02 Malmö, Sweden*
[b] *Department of Clinical Microbiology, University Hospital, Rigshospitalet, Copenhagen, Denmark*

Received 25 December 2014; revised 16 April 2015; accepted 13 May 2015
Available online 3 June 2015

Abstract

Background: *Pseudomonas aeruginosa* is a pathogen that frequently colonizes patients with cystic fibrosis (CF) or chronic obstructive pulmonary disease (COPD). Several pathogens are known to bind vitronectin to increase their virulence. Vitronectin has been shown to enhance *P. aeruginosa* adhesion to host epithelial cells.
Methods: We screened clinical isolates from the airways of CF patients and from the bloodstream of patients with bacteremia for binding of vitronectin. Two-dimensional SDS-PAGE and a proteomic approach were used to identify vitronectin-receptors in *P. aeruginosa*.
Results: *P. aeruginosa* from the airways of CF patients ($n = 27$) bound more vitronectin than bacteremic isolates ($n = 15$, $p = 0.025$). Porin D (OprD) was identified as a vitronectin-binding protein. A *P. aeruginosa oprD* transposon insertion mutant had a decreased binding to soluble and immobilized vitronectin ($p \leq 0.001$).
Conclusions: *P. aeruginosa* isolates obtained from CF patients significantly bound vitronectin. Porin D was defined as a novel *P. aeruginosa* vitronectin-receptor, and we postulate that the Porin D-dependent interaction with vitronectin may be important for colonization.
© 2015 European Cystic Fibrosis Society. Published by Elsevier B.V. All rights reserved.

Keywords: Adhesion molecules; Extracellular matrix; Porin D; *Pseudomonas aeruginosa*; Vitronectin

1. Introduction

The Gram-negative opportunistic pathogen *Pseudomonas aeruginosa* is responsible for a substantial burden of disease and mortality, particularly for patients with other underlying diseases. *P. aeruginosa* causes various infections in humans, notably pulmonary infections, keratitis, wound infections and septicemia [1]. It has been shown to be the main pathogen causing increased morbidity and mortality in patients with cystic fibrosis (CF) [2]. *P. aeruginosa* is also associated with a high morbidity in patients with advanced stage of chronic obstructive pulmonary disease (COPD) [3], and is reported as the most common bacterial species isolated during exacerbations [4]. In parallel, *P. aeruginosa* is often found as an infectious agent in patients suffering from ventilator associated pneumonia (VAP) [5].

Though *P. aeruginosa* primarily exists in mucus plugs and sputum plaques during long term colonization of CF patients, it is known to adhere to epithelial cells, exposed basement membrane or proteins of the extracellular matrix (ECM) [6]. For both

Abbreviations: aa, amino acid; BSA, bovine serum albumin; CF, cystic fibrosis; COPD, chronic obstructive pulmonary disease; CPM, counts per minute; DBA, direct binding assay; ECM, extracellular matrix; FCS, fetal calf serum; HBD, heparin binding domain; IPTG, isopropyl-1-thio-β-D-galactoside; LB, lysogeny broth; mAb, monoclonal antibodies; NHS, normal human serum; OMP, outer membrane protein; pAb, polyclonal antibodies; PBS, phosphate buffered saline; PBST, PBS with Tween; SEM, standard error of the mean; VAP, ventilator associated pneumonia; WT, wild type bacterium.

* Corresponding author. Tel.: +46 40 338494; fax: +46 40 336234.
E-mail address: kristian.riesbeck@med.lu.se (K. Riesbeck).

http://dx.doi.org/10.1016/j.jcf.2015.05.005
1569-1993/© 2015 European Cystic Fibrosis Society. Published by Elsevier B.V. All rights reserved.

P. aeruginosa and *P. fluorescence* it has been suggested that the ECM protein vitronectin is important for bacterial adherence to respiratory epithelial [7,8].

Vitronectin is a plasma protein that exists naturally as a 75 kDa intact protein and a truncated 65 kDa form. It was first discovered for its ability to stick to glass surfaces where subsequently human primary cells could grow [9]. It has later been found to be associated with cell to cell adhesion, wound repair and regulation of the complement system. Furthermore, vitronectin promotes coating on polymer surfaces and it may aid to build up biofilms, as exemplified by coagulase-negative staphylococci on cerebrospinal shunts [10]. During wound healing, vitronectin facilitates mammalian cell adhesion and forms an ECM with other glycoproteins that is attached to mammalian cells through $\alpha_v\beta_5$ integrins, which are upregulated during inflammation [11]. Although vitronectin is mainly produced by hepatocytes, it is also released by respiratory epithelial cells and present in the healthy lung [12]. The vitronectin mRNA expression is upregulated in neutrophils derived from patients with CF and the concentration of vitronectin in the airway lumen is increased in patients suffering from sarcoidosis and interstitial lung disease, which may reflect the level of inflammation [13,14].

The goal of this study was to investigate whether *P. aeruginosa* has vitronectin-binding surface receptors, and to evaluate the ubiquity of this interaction by screening clinical isolates. Moreover, we also aimed to identify vitronectin-binding proteins at the surface of *P. aeruginosa*, and to characterize the nature of this interaction in detail. We identified Porin D as the main vitronectin-binding receptor, which is a previously unknown function attributed to this outer membrane protein (OMP).

2. Material and methods

2.1. Bacterial strains and culture conditions

Forty-two clinical *P. aeruginosa* isolates and the reference strain PAO1 were supplied by the Clinical Microbiology Laboratory (Malmö, Sweden) and the Department of Clinical Microbiology at Rigshopitalet (Copenhagen, Denmark) [15]. All isolates were verified as *P. aeruginosa* by using MALDI-TOF. A transposon insertion mutant was obtained from the *P. aeruginosa* two-allele library (Washington university, Seattle, WA): PW2742 oprD-E12::IS*pho*A7/hah [16]. *P. aeruginosa* was grown on blood agar plates or in liquid Lysogeny broth (LB). *Escherichia coli* DH5α and *E. coli* BL21 (DE3) were also cultured in LB medium supplemented with appropriate antibiotics (Table 1).

2.2. Vitronectin direct binding assay (DBA)

Vitronectin was labeled with [^{125}I] using the Chloramine-T method [17]. One loop of *P. aeruginosa* was taken from a glycerol stock kept at −80 °C and grown overnight on blood agar plates. Bacteria were scraped from the plates and resuspended to OD_{600} = 1.0 in phosphate buffered saline (PBS) containing 1% bovine serum albumin (BSA). After washing, 15 ng of [^{125}I]-vitronectin in 100 μl PBS was added to the bacterial suspension and incubated for 1 h at 37 °C. The unbound [^{125}I]-vitronectin was removed by 2 washes with PBS. Bacterial surface-bound radioactivity was measured in a Tri-Carb liquid scintillation counter (Perkin Elmer, Waltham, MA).

Table 1
Clinical *Pseudomonas aeruginosa* isolates and laboratory strains used in the present study.

Name	Description/genotype	Reference
Clinical isolates		
PA KR794	Urine isolate	This study
PA KR796	Airway isolate from patient with CF	This study
PA KR799	Blood isolate	This study
PA KR801	Airway isolate from patient with CF	This study
Laboratory strains		
PAO1	*P. aeruginosa* reference strain	[15]
E. coli BL21(DE3)	*E. coli* laboratory strain	[19]
E. coli DH5α	*E. coli* laboratory strain	
E. coli—OprD	*E. coli* BL21(DE3) pET16b-*oprD*	This study
MPAO1	*P. aeruginosa* reference strain. Clone of PAO1 used in two allele transposon library.	[16]
PW2742 oprD-	MPAO1 mutant with transposon insert in *oprD*	
E12::IS*pho*A7/hah		[16]

2.3. Outer membrane protein identification with two-dimensional (2D) gel electrophoresis

Bacterial OMP fractions were prepared from overnight cultures based upon the method of Alteri and Moble and analyzed by 2D-SDS-PAGE [18].

2.4. Expression and purification of recombinant proteins

The full-length gene encoding for Porin D (*oprD*) were amplified from genomic DNA of *P. aeruginosa* PAO1 using primers 5′-CTGAGGATCCGGACGCATTCGTCAGCGATCAGGCC-3′ and 5′-CTGACAAGCTTCAGGATCGACAGCGGATAGTCGACGATCAG-3′. The amplified gene products were cloned into the expression vector pET26b (Novagen, Darmstadt, Germany), and used for protein expression and purification [19]. The *oprD* gene was also cloned into pET16b (Novagen) for expression of proteins at the surface of *E. coli* using primers 5′-TATACGCATATGAAAGTGATGAAGTGGAGCGCCAT-TGCA-3′ and 5′-TCAATTGGATCCTTACAGGATCGACAGCGGATAGTCGA-3′. For expression and purification, *E. coli* DE3 (Novagen) with the appropriate vector was used. Vitronectin fragments were expressed in HEK 293T cells and purified by Ni-NTA resin [18].

2.5. Antibody production

Two rabbits were immunized with 200 μg of recombinant protein emulsified in complete Freund's adjuvant (CFA; Difco and BD Biosciences, Franklin Lakes, NJ). Booster doses were injected on days 18 and 36 with the same dose of protein in incomplete FA. Blood was drawn three weeks later. Antibodies

Download English Version:

https://daneshyari.com/en/article/4208089

Download Persian Version:

https://daneshyari.com/article/4208089

Daneshyari.com