Journal of Microbiology (2013) Vol. 51, No. 5, pp. 644–650
DOI 10.1007/s12275-013-3040-x

Involvement of Phosphatidylinositol 3-Kinase/Akt Signaling Pathway in β1 Integrin-Mediated Internalization of *Staphylococcus aureus* by Alveolar Epithelial Cells

Jia-He Wang[1†*], Ke Zhang[2†], Nan Wang[1], Xiao-Min Qiu[1], Yi-Bing Wang[3], and Ping He[1]

[1]*Department of Geriatrics, Shengjing Hospital of China Medical University, Shenyang, 110004, P. R. China*
[2]*Department of Developmental Cell Biology, Key Laboratory of Cell Biology, Ministry of Public Health, Key Laboratory of Medical Cell Biology, Ministry of Education, China Medical University, Shenyang, 110004, P. R. China*
[3]*Department of Urology, Shengjing Hospital of China Medical University, Shenyang, 110004, P. R. China*

(Received January 18, 2013 / Accepted April 15, 2013)

The invasion of *Staphylococcus aureus* into alveolar epithelial cells is regarded as the key step for *S. aureus* lung infection. However, the mechanism of internalization of *S. aureus* by alveolar epithelial cells is not clear, and was the aim of this investigation Human lung adenocarcinomic epithelial cells and A549 cells were used. Human β1 integrin and rat β1 integrin were detected by real-time reverse transcription (RT)-PCR. The expressions of β1 integrin, Akt and p-Akt were detected by Western blot analysis. To further investigate the role of β1 integrin in *S. aureus* internalization by alveolar epithelial cells, we next performed siRNA-mediated knockdown of β1 integrin expression. In this study, we found that *S. aureus* invades human alveolar epithelial cells and rat primary alveolar epithelial cells. The β1 integrin ligand competitive inhibitor, GRGDS-peptide, blocked the internalization of *S. aureus* by A549 cells. Knockdown of β1 integrin also inhibited the internalization of *S. aureus*. In addition, the PI3K/Akt signaling pathway in alveolar epithelial cells was activated by the infection with *S. aureus*. Furthermore, Akt phosphorylation was abolished by transient transfection with β1 integrin siRNA in A549 cells challenged with *S. aureus*. Our results suggest that the phosphatidylinositol 3-kinase/Akt signaling pathway plays an important role in β1 integrin-mediated internalization of *S. aureus* by alveolar epithelial cells.

Keywords: *Staphylococcus aureus*, phosphatidylinositol 3-kinase, β1 integrin, alveolar epithelial cells, internalization

Introduction

Staphylococcus aureus is one of the most common Gram-positive bacterial pathogens in humans (Genestier *et al*., 2005). It is an opportunistic pathogen and the major causative agent of numerous hospital- and community-acquired infections (Feng *et al*., 2008). *S. aureus* pneumonia remains an important cause of mortality and morbidity. *S. aureus* infections are increasingly difficult to treat because of the high percentage of antibiotic-resistant strains (McElroy *et al*., 2002). A better understanding of the underlying pathogenic mechanism of *S. aureus* infection might improve clinical intervention.

S. aureus has long been regarded as an extracellular pathogen, because it is rarely observed inside cells *in vivo* and because it secretes a range of toxins that are cytolytic to many host cell types (Almeida *et al*., 1996; Joh *et al*., 1999; McElroy *et al*., 1999; Mairbaurl *et al*., 2002). However, recent *in vitro* studies demonstrate that *S. aureus* is internalized and survives inside non-phagocytic cells such as alveolar epithelial cells (Almeida *et al*., 1996). *S. aureus* adhesion to and invasion of alveolar epithelial cells is regarded as the key step for *S. aureus* pneumonia (Almeida *et al*., 1996; Joh *et al*., 1999; McElroy *et al*., 1999). However, the mechanism of internalization of *S. aureus* by alveolar epithelial cells is not clear.

Integrins are a family of cell surface-expressed receptors, composed of one α subunit and one β subunit. Integrins serve as transducers capable of transmitting extracellular adhesion events into intracellular signaling cascades, which can then result in the initiation of a variety of cellular activities including cell spreading and migration (Giancotti and Ruoslahti, 1999; Fowler *et al*., 2003). Bacterial invasion of host cells also required the activation of a signaling pathway. Phosphatidylinositol 3-kinases (PI3K) are a family of related intracellular signal transducer enzymes that have been linked to a diversity of cellular functions, including cell growth, proliferation, differentiation, motility, survival and intracellular trafficking. Several studies using different bacteria or bacterial virulence factors have documented the activation of the PI3K-Akt signaling pathway (Oviedo-Boyso *et al*., 2011). In this study, we found that β1 integrin mediated the internalization of *S. aureus* into alveolar epithelial cells and that it is also required for the activation of the PI3K/Akt signaling pathway.

†These authors contributed equally to this work.
*For correspondence. E-mail: cmuwjh@yeah.net

Materials and Methods

Reagents

Monoclonal anti-GAPDH (glyceraldehyde-3-phosphate dehydrogenase) antibodies were purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, USA). β1 integrin polyclonal antibody was from Upstate (USA). Akt and phosphorylated Akt (p-Akt) polyclonal antibodies were obtained from Santa Cruz Biotechnology, Inc. F-12K and fetal bovine serum (FBS) were purchased from Gibco BIL Company (Gibco, USA). Cell isolation and tissue-culture reagents were obtained from Invitrogen life Technologies (Sweden). Todd Hewitt broth was purchased from BD Biosciences. All other reagents were obtained from Sigma-Aldrich (USA).

Bacterial culture

The *S. aureus* wild-type strain 8325-4 (Shengjing Hospital of China Medical University, China) was used in this study. The strain was grown overnight in Todd Hewitt broth. Overnight cultures were washed twice with endotoxin-free phosphate-buffered saline (PBS) before resuspension in PBS for all experiments.

Cell culture

Human lung adenocarcinomic epithelial cells, A549, were obtained from the American Type Culture Collection (CCL-185). This transformed human cell line, which was established from explanted lung carcinoma, had typical characteristics of alveolar epithelial type II (ATII) cells, namely, small, cuboidal cells that constitute approximately 60% of the pulmonary alveolar epithelium. They were cultured in F-12K medium supplemented with a final concentration of 10% FBS. Cells were incubated at 37°C in an atmosphere with 5% CO_2 and 95% relative humidity.

Alveolar epithelial cell isolation

Alveolar type II cells were isolated from rat lungs by using elastase as described previously (Mairbaurl *et al.*, 2002). Briefly, rats were anesthetized by intraperitoneal injection with 100 mg/kg pentobarbital sodium salt, and the lung was perfused with PBS while being ventilated with air. Alveolar type II cells were isolated by elastase digestion, mincing of lung tissue, filtration, and differential adhesion to IgG-coated plates as previously described. Non-adherent cells were suspended in DMEM (Sigma, D-5546) supplemented with 10% FBS, glutamine (4 mM), and gentamicin (50 μg/ml), and were plated on tissue culture-treated Nuclepore filters (0.4-μm and 12-mm Transwell; Costar, USA) at a seeding density of 1.5×10^6 cells/cm^2. Both purity and viability of Alveolar type II cells were >85%.

Real-time reverse transcription (RT)-PCR

Total RNA was extracted from cells using Trizol reagent (Invitrogen), treated with DNase I (Ambion) to remove potential genomic DNA contamination and purified using an RNeasy Mini Kit (Qiagen). Total RNA concentration was measured and the purity of the samples was estimated by the OD ratios (A_{260}/A_{280}, ranging within 1.8–2.2). cDNA was synthesized from 2 μg of DNA-free total RNA in a 25 μl reaction volume using Moloney murine leukemia virus (M-MLV) reverse transcriptase (Promega, USA). cDNA samples were diluted 10-fold for real-time PCR reactions. Gene-specific transcription levels were determined in a 20 μl reaction volume, in duplicate, using SYBR Green and an ABI 7500 real-time PCR system (Applied BioSystems). The sense primer for human β1 integrin was 5′-GCAAGTTGCAGTT TGTGGATCA-3′; and the antisense primer was 5′-TGCC ACCAAGTTTCCCATCT-3′. The sense primer for rat β1 integrin was 5′-GGAATGCCTACTTCTGCACGAT-3′; and the antisense primer was 5′-TGCTGCGATTGGTGACGT T-3′. GAPDH was chosen as the internal control. The sense primer for human GAPDH was 5′-GAAGGTGAAGGTCG GAGTC-3′; and the antisense primer was 5′-GAAGATGG TGATGGGATTTC-3′. The sense primer for rat GAPDH was 5′-CCCTTCATTGACCTCAACTACAT-3′, and the antisense primer was 5′-GCCAGTAGACTCCACGACATA-3′. Real-time PCR products were analyzed by agarose gel electrophoresis and were verified by DNA sequencing.

Western blot analysis

Briefly, cells were washed once with ice-cold PBS containing 1 mM Na_2VO_4 and extracted with lysis buffer (50 mM Tris; pH 8.0, 150 mM NaCl, 5 mM EDTA, 5% Glycerol, 1% Triton X-100, 25 mM NaF, 2 mM Na_2VO_4, 10 mg/ml of each aprotinin, leupeptin, and pepstatin). The cell lysates were frozen and thawed three times and were further centrifuged at 14,000×g for 10 min at 4°C to pellet insoluble material. The supernatant of cell extracts was analyzed for protein concentration by a DC protein assay kit based on the Lowry method (Bio-Rad, USA). Equal amounts of protein (50 mg) from each sample were separated on 10% sodium dodecyl sulfate-polyacrylamide gels and transferred to PVDF membranes (MSI, USA). Membranes were blocked in 5% nonfat dry milk in Tris-buffered saline containing 0.05% Tween-20 (TBS-T) and then incubated with rabbit polyclonal for β1 integrin, Akt and p-Akt (1:1000) overnight at 4°C. GAPDH (1:2000) was used to control for equal protein loading. The immunoblots were then washed three times with TBS-T buffer, incubated with a horseradish peroxidase-conjugated secondary antibody (goat anti-rabbit IgM, Santa Cruz), and developed using chemiluminescent substrate (Pierce, USA). To quantify and compare levels of proteins, the density of each band was measured by densitometry.

Bacterial invasion assay

The bacterial invasion assay was performed as described previously (McElory *et al.*, 2002). Briefly, confluent A549 cells or primary rat alveolar epithelial cells were incubated for 1 h in serum-free DMEM and then washed twice in PBS. 10^6 CFU of *S. aureus* in DMEM were added to the cells (approximately 1.5×10^6 cells/well). *S. aureus* was cocultured with cells for 2 to 5 h. At the end of the coculture period, cells were washed twice with PBS and then incubated for 1 h in the presence of 100 μg/ml gentamicin. The cells were then washed three times with PBS and lysed with 0.5% Triton X-100. The cell lysate was diluted and plated out in triplicate on tryptic soy agar plates supplemented with sheep

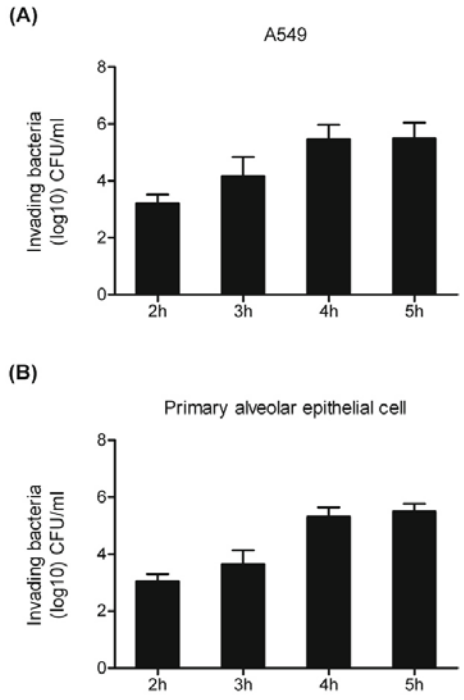

Fig. 1. *S. aureus* invades alveolar epithelial cells. (A) Human alveolar epithelial cell line A549 and (B) rat primary alveolar epithelial cells were co-cultured with *S. aureus* 8325-4 for 2 to 5 h. After treatment of the culture with 100 μg/ml gentamicin, cells were collected and CFU for surviving bacteria was determined.

blood (BD Biosciences). The plates were incubated overnight at 37°C, and the number of CFU was measured to determine the number of intracellular *S. aureus* cells. For the inhibitor study, A549 cells was incubated with GRGDS or SDGRG for 30 min before the addition of bacteria. A549 cells was also incubated with DMSO as a control.

RNAi

The small interfering RNA (siRNA) target sequences that effectively mediate silencing of β1 integrin were reported previously (Maribaurl *et al.*, 1997): 5′-AATGTAACCAAC CGTAGCA-3′ for β1.1 and 5′-GCGCATATCTGGAAATT TG-3′ for β1.2. A non-silencing siRNA sequence, 5′-TTCTC CGAACGTGTCACGT-3′, was used as a control. A549 cells were transfected with the 21-nucleotide synthetic siRNA duplex. The expression of β1 integrin was examined by Western blot analysis.

Statistical analysis

Each experiment was carried out in duplicate or triplicate and three or four independent experiments were performed. Results were expressed as means±standard deviation (SD) and analyzed with SPSS 11.5 software. Results were compared using analysis of variance (ANOVA). When ANOVA showed a statistically significant difference, a group-by-group comparison was performed using a t-test with Tukey's correction for multiple comparisons. Statistical significance was set at $P<0.05$.

Results

S. aureus invades human lung epithelial cells and rat primary alveolar epithelial cells

Bacterial invasion into alveolar epithelial cells is a prerequisite

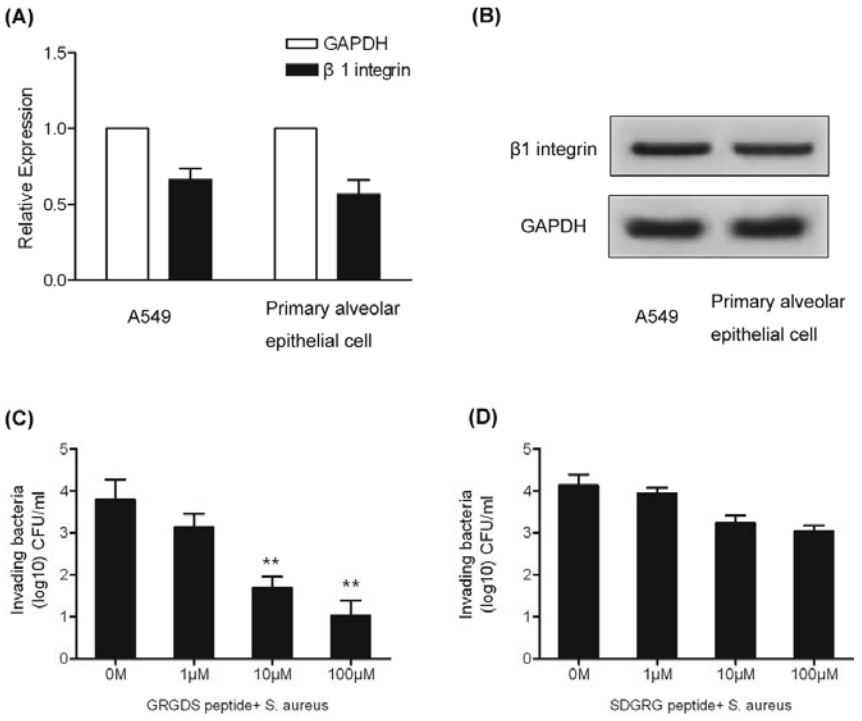

Fig. 2. β1 integrin inhibitor prevents the internalization of *S. aureus* into A549 cells. (A) Real-time PCR and Western-Blot (B) were used to detect the expression of β1 integrin. (C) GRGDS-peptide, which functions as a competitive inhibitor of β1 integrin ligands, inhibited the internalization of *S. aureus* by A549 cells in a dose dependent manner. (D) The control peptide, SDGRG, had no effect on prevention of the internalization. The results represent three independent experiments. Data are the Mean±SD. $^{**}P<0.01$.

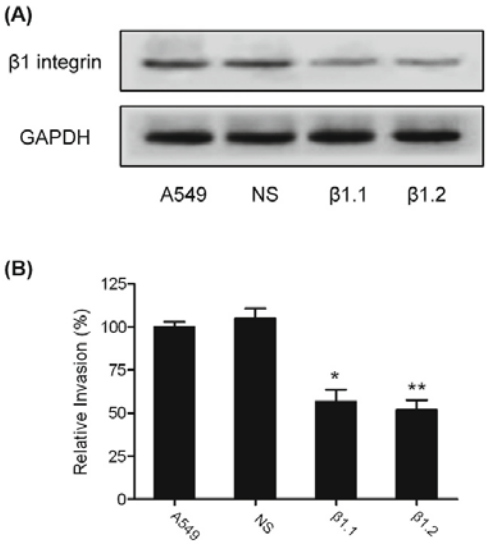

Fig. 3. Knockdown of β1 integrin blocks the internalization of *S. aureus* into alveolar epithelial cells. (A) A549 cells was transiently transfected with β1 integrin siRNA or non-silencing siRNA, and the expression of β1 integrin in the cells was detected by Western blotting. The transfected A549 cells were then tested for bacterial internalization (B). The results represent three independent experiments. Data are the Mean±SD. *P< 0.05, **P<0.01.

for the invasion of deeper tissue and dissemination of the bacteria. To assess the ability of *S. aureus* to invade lung epithelial cells, invasion assays were performed as described in the 'Material and Methods'. As shown in Fig. 1, *S. aureus* strain 8325-4 invaded the A549 human alveolar epithelial cells (Fig. 1A) and rat primary alveolar epithelial cells (Fig. 1B) in a time-dependent manner. For both mammalian alveolar epithelial cells, the maximum number of internalized bacteria was detected at 4 h after inoculation. A non-pathogenic bacterium, *E. coli* DH5α, was not detected either in A549 cells or in the rat primary alveolar epithelial cells (data not shown). These results showed that *S. aureus* has the ability to invade alveolar lung epithelial cells.

β1 integrin is required for the internalization of *S. aureus* by alveolar epithelial cells

We examined β1 integrin mRNA expression in the A549 cells and rat primary alveolar epithelial cells by real-time RT-PCR. The results showed that the transcripts of β1 integrin are present in both cell lines (Fig. 2A). Furthermore, Western blot results showed the presence of β1 integrin in the mammalian alveolar epithelial cells (Fig. 2B). In an attempt to analyze whether β1 integrin was involved the internalization of *S. aureus* by alveolar epithelial cells, GRGDS-peptide, which

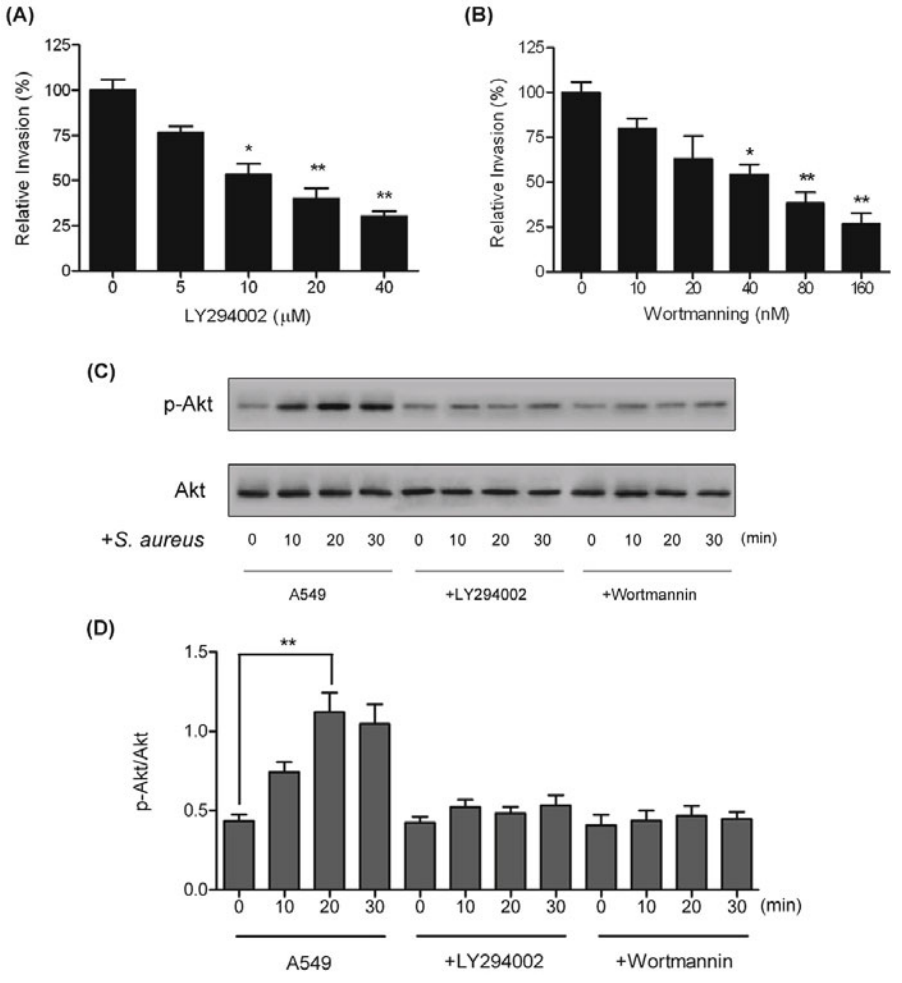

Fig. 4. PI3K/Akt activation in A549 cells infected with *S. aureus*. Confluent A549 cells monolayers were treated with LY294002 (A) or Wortmannin (B) for 30 min before the addition of *S. aureus*, and then the bacterial invasion assays were conducted. Results are presented as percentage of the control, which was defined as 100%. Data are Means±SD of three independent experiments done in triplicate. *P<0.05, **P<0.01. (C) Confluent monolayers of A549 cells were incubated with *S. aureus* for indicated periods of time. When indicated, the A549 monolayers were treated with LY294002 or Wortmannin for 30 min prior to *S. aureus* infection, and the level of Akt phosphorylation in A549 cells was analyzed by Western blot. (D) The relative abundance of each band to its own Akt was quantified. The adjusted Means±SD (n=3) of each band are shown above each blot. *P<0.05, **P<0.01.

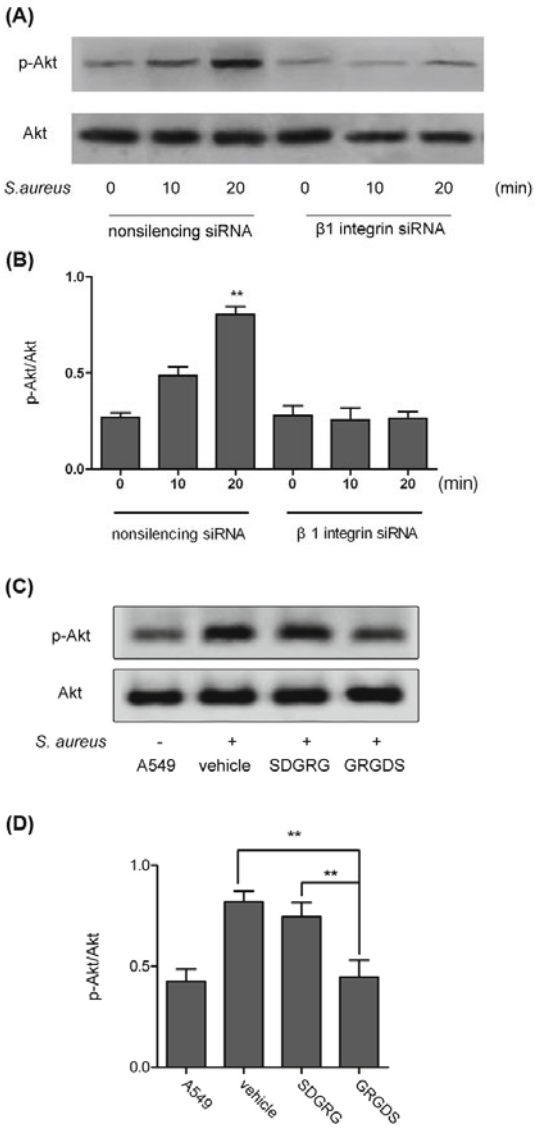

Fig. 5. β1 integrin is required for *S. aureus*-induced Akt phosphorylation. (A) A549 cells transiently transfected with β1 integrin siRNA or non-silencing siRNA were incubated with *S. aureus* for the indicated periods of time; then the phosphorylated Akt (p-Akt) and Akt were detected by Western blot analyses. (B) The relative abundance of p-Akt and Akt quantified from the results of (A). Each graph shows the adjusted mean with standard deviation (n=3). $^{**}P<0.01$. (C) Confluent A549 cells were treated with GRGDS or SDGRG and then incubated with *S. aureus*. The level of Akt phosphorylation in A549 cells was examined by Western blot analyses. (D) The relative abundance of p-Akt and Akt quantified from the results of (C). Each graph shows the adjusted mean with standard deviation (n=3). $^{**}P<0.01$.

functions as a competitive inhibitor of β1 integrin ligands, was used in the *S. aureus* invasion assay. As shown in Fig. 2C, GRGDS blocked *S. aureus* uptake in a dose-dependent manner, whereas a reverse-order control peptide, SDGRG, had no significant effect (Fig. 2D), although there was a slight decrease at 10 μM and 100 μM SDGRG. These results suggested that β1 integrin is likely to be involved in internalization of *S. aureus* by alveolar epithelial cells.

Knockdown of β1 integrin inhibits the internalization of *S. aureus* by alveolar epithelial cells.

To further investigate the role of β1 integrin in *S. aureus* internalization by alveolar epithelial cells, we next performed siRNA-mediated knockdown of β1 integrin expression. Two different siRNAs (β1.1, β1.2) were transiently transfected into A549 cells, and the silencing effect was examined by Western blot analysis. The siRNA mediated β1 integrin silencing, based on a comparison with the non-silencing control (Fig. 3A). A549 cells transiently transfected with the siRNA were subjected to bacterial internalization assays. Transfection with β1 integrin siRNA significantly reduced *S. aureus* invasion (Fig. 3B), whereas the control siRNA did not. These results indicated that knockdown of β1 integrin in A549 cells reduces *S. aureus* internalization by A549.

Activation of PI3K/Akt is involved in internalization of *S. aureus* by alveolar epithelial cells

Previous studies have demonstrated that the PI3K/Akt signaling pathway is involved in bacterial invasion of host cells. To investigate the signaling pathway involved in internalization of *S. aureus* by alveolar epithelial cells, the effects of specific kinase inhibitors on *S. aureus* invasion were examined. A549 cells and rat primary alveolar epithelial cells were pretreated with PI3K inhibitor LY294002 or Wortmannin for 30 min before the *S. aureus* internalization assay. Treatment with either LY294002 or Wortmannin reduced *S. aureus* internalization of A549 cells in a dose-dependent manner (Figs. 4A and 4B). Akt, also called PKB, a serine/threonine kinase, is a downstream effector of PI3K in bacteria-induced intracellular signaling in eukaryotic cells. Next, to assess the effect of the inhibitors on the activation of the PI3K/Akt signaling pathway, we analyzed the level of phosphorylated Akt (p- Akt). The phosphorylation of Akt in A549 cells was increased in response to *S. aureus* infection. The peak phosphorylation of Akt appeared at 20 min (Figs. 4C and 4D); however, PI3K inhibitor LY294002 or Wortmannin blocked the increase of p-Akt. These results indicated that the presence of *S. aureus* can lead to the activation of the PI3K/Akt signaling pathway, which was required for *S. aureus* internalization into alveolar epithelial cells.

β1 integrin is critical for activation of the PI3K signaling pathway during staphylococcal infection

To investigate the relationship between the β1 integrin and PI3K activation in the process of *S. aureus* internalization, A549 cells were transfected either with β1 integrin siRNA or with a control siRNA. Then, the cells were challenged with *S. aureus* and the level of Akt phosphorylation was measured. The increase in Akt phosphorylation was abolished by transient transfection with β1 integrin siRNA in A549 cells challenged with *S. aureus* (Figs. 5A and 5B). Furthermore, treatment with β1 integrin inhibitor GRGDS prevented the increase of p-Akt in the A549 cells challenged with *S. aureus*, whereas treatment with the reverse-peptide SDGRG did not (Figs. 5C and 5D). These results indicated that β1 integrin is involved in *S. aureus* internalization, and is required for PI3K/Akt activation in alveolar epithelial cells.

Discussion

Staphylococcus aureus is a major cause of human infections resulting in a variety of diseases including endocarditis, osteomyelitis, pneumonia, toxic shock syndrome and septicemia (Lowy, 1998). Traditionally considered an extracellular pathogen, *S. aureus* can, *in vitro*, enter a variety of non-specialized phagocytic cells such as endothelial cells (Hamill *et al*., 1986; Beekhuizen *et al*., 1997), epithelial cells (Almeida *et al*., 1996; Bayles *et al*., 1998), and osteoblasts (Hudson *et al*., 1995). In the present study, we found that *S. aureus* can invade cultured human lung epithelial A549 cells and rat primary alveolar epithelial cells.

Different bacterial pathogens have developed different mechanisms to modulate cell responses during pathogen–host interactions. Different *S. aureus* isolates associated with disease have a common prominent characteristic: expression of a series of MSCRAMM adhesins (microbial surface components recognizing adhesive matrix molecules) (Patti *et al*., 1994; Foster and Hook, 1998). MSCRAMMs can facilitate the organism's adherence to and/or invasion of non-phagocytic cells by interacting with extracellular matrix (ECM) components of the host, such as collagen, fibrinogen and fibronectin (Fn) (Foster and Hook, 1998). It is known that internalization of *S. aureus* into cultured eukaryotic cells involves a 'sandwich' mechanism. The Fn-binding MSCRAMMs, FnbpA or FnbpB, first bind Fn to the bacteria. This Fn coat is then recognized by Fn-binding integrins on mammalian cells, leading to internalization of the bacteria (Dziewanowska *et al*., 1999; Peacock *et al*., 1999; Sinha *et al*., 1999; Fowler *et al*., 2000, 2003; Liang and Ji, 2006).

β1 integrin also plays an important role in bacterial internalization and invasion. Gustavsson *et al*. (2002) transfected a β1-integrin-deficient cell line with wild type β1A, β1B, or mutants of the β1A subunit and found that, although both β1A and β1B bound to Yersinia, only β1A was able to mediate internalization of the bacterium. Agerer *et al*. (2003) have shown that β1 integrin mediated the invasion of *S. aureus* into 293T cells. In the present study, we found that a competitive inhibitor of β1 integrin ligands, GRGDS-peptide, could block *S. aureus* internalization in a dose-dependent manner, whereas a reverse-order, control peptide, SDGRG, had minimal effect. Moreover, knockdown of β1 integrin expression in the A549 cell line by a specific siRNA also attenuated the internalization of *S. aureus*. Therefore, based on our results, we concluded that β1 integrin plays an important role in the internalization of *S. aureus* into alveolar epithelial cells.

β1-integrin-mediated phagocytosis of invasin-expressing bacteria requires the activation of cellular signaling pathways. Enteropathogenic *Yersinia* species promote uptake into host cells by binding β1 integrins via Rac1-mediated signals, whereas Cdc42 signaling is not essential for *Yersinia* uptake (Wong and Isberg, 2005). Fowler *et al*. (2003) demonstrated that Src family kinase-specific inhibitors effectively block *S. aureus* internalization into HeLa cells mediated by β1 integrins. The host-cell actin cytoskeleton and Src family protein-tyrosine kinases are essential for *S. aureus*, but not *S. carnosus*, invasion into 293T cells mediated by β1 integrins (Agerer *et al*., 2003). In our study, we found that the PI3K inhibitors LY294002 and wortmannin blocked the internalization of *S. aureus*. In addition, *S. aureus* infection activated Akt phosphorylation, an indicator of PI3K activation. Next, we applied β1 integrin knock-down procedures before measuring Akt phosphorylation in A549 cells challenged with *S. aureus*. The results showed that the activation of Akt phosphorylation was abolished by β1 integrin siRNA and inhibitor GRGDS. Our findings suggest that the PI3K/Akt signaling pathway is involved in *S. aureus* internalization, which is required for β1 integrin-mediated internalization by alveolar epithelial cells.

In summary, our findings indicated the involvement of the PI3K/Akt signaling pathway in β1 integrin-mediated *S. aureus* internalization by alveolar epithelial cells. The study will not only provide new insights into *S. aureus* infection, but may also suggest novel therapeutic approaches to control and eradicate bacterial infections. Further investigations are needed to determine the dynamic interaction between the pathogen and its host in the course of disease, which will be a challenging task for future studies.

Acknowledgements

This work was supported by the National Natural Science Foundation of China (81101224, 81101225), Outstanding Scientific Fund of Shengjing Hospital (201206), the National Natural Science Foundation of Liaoning province (201202270), to tackle key problems in science and technology of Liaoning province (2011225020), and to tackle key problems in science and technology of Shenyang city (F12-193-9-20).

References

Agerer, F., Michel, A., Ohlsen, K., and Hauck, C.R. 2003. Integrin-mediated invasion of *Staphylococcus aureus* into human cells requires Src family protein-tyrosine kinases. *J. Biol. Chem.* **278**, 42524–42531.

Almeida, R.A., Matthews, K.R., Cifrian, E., Guidry, A.J., and Oliver, S.P. 1996. *Staphylococcus aureus* invasion of bovine mammary epithelial cells. *J. Dairy Sci.* **79**, 1021–1026.

Bayles, K.W., Wesson, C.A., Liou, L.E., Fox, L.K., Bohach, G.A., and Trumble, W.R. 1998. Intracellular *Staphylococcus aureus* escapes the endosome and induces apoptosis in epithelial cells. *Infect. Immun.* **66**, 336–342.

Beekhuizen, H., van de Gevel, J.S., Olsson, B., van Benten, I.J., and van Furth, R. 1997. Infection of human vascular endothelial cells with *Staphylococcus aureus* induces hyperadhesiveness for human monocytes and granulocytes. *J. Immunol.* **158**, 774–782.

Dziewanowska, K., Patti, J.M., Deobald, C.F., Bayles, K.W., Trumble, W.R., and Bohach, G.A. 1999. Fibronectin binding protein and host cell tyrosine kinase are required for internalization of *Staphylococcus aureus* by epithelial cells. *Infect. Immun.* **67**, 4673–4678.

Feng, Y., Chen, C.J., Su, L.H., Hu, S., Yu, J., and Chiu, C.H. 2008. Evolution and pathogenesis of *Staphylococcus aureus*: Lessons learned from genotyping and comparative genomics. *FEMS Microbiol. Rev.* **32**, 23–37.

Foster, T.J. and Hook, M. 1998. Surface protein adhesins of *Staphylococcus aureus*. *Trends Microbiol.* **6**, 484–488.

Fowler, T., Wann, E.R., Joh, D., Johansson, S., Foster, T.J., and Hook, M. 2000. Cellular invasion by *Staphylococcus aureus* in-

volves a fibronectin bridge between the bacterial fibronectin-binding MSCRAMMs and host cell beta1 integrins. *Eur. J. Cell Biol.* **79**, 672–679.

Fowler, T.J., Wary, K.K., and Höök, M. 2003. Src kinase has a central role in *in vitro* cellular internalization of *Staphylococcus aureus*. *Cell. Microbiol.* **5**, 417–426.

Genestier, A.L., Michallet, M.C., Prevost, G., Bellot, G., Chalabreysse, L., Peyrol, S., Thivolet, F., Etienne, J., Lina, G., Vallette, F.M., and *et al.* 2005. *Staphylococcus aureus* Panton-Valentine leukocidin directly targets mitochondria and induces Bax-independent apoptosis of human neutrophils. *J. Clin. Invest.* **115**, 3117–3127.

Giancotti, F.G. and Ruoslahti, E. 1999. Integrin signaling. *Science* **285**, 1028–1032.

Gustavsson, A., Armulik, A., Brakebusch, C., Fassler, R., Johansson, S., and Fallman, M. 2002. Role of the ß1-integrin cytoplasmic tail in mediating invasin-promoted internalization of *Yersinia*. *J. Cell. Sci.* **115**, 2669–2678.

Hamill, R.J., Vann, J.M., and Proctor, R.A. 1986. Phagocytosis of *Staphylococcus aureus* by cultured bovine aortic endothelial cells: Model for post adherence events in endovascular infections. *Infect. Immun.* **54**, 833–836.

Hudson, M.C., Ramp, W.K., Nicholson, N.C., Williams, A.S., and Nousiainen, M.T. 1995. Internalization of *Staphylococcus aureus* by cultured osteoblasts. *Microb. Pathog.* **19**, 409–419.

Joh, D., Wann, E.R., Kreikemeyer, B., Speziale, P., and Hook, M. 1999. Role of fibronectin-binding MSCRAMMs in bacterial adherence and entry into mammalian cells. *Matrix Biol.* **18**, 211–223.

Liang, X. and Ji, Y. 2006. Alpha-toxin interferes with integrin-mediated adhesion and internalization of *Staphylococcus aureus* by epithelial cells. *Cell. Microbiol.* **8**, 1656–1668.

Lowy, F.D. 1998. *Staphylococcus aureus* infections. *N. Engl. J. Med.* **339**, 520–532.

Mairbaurl, H., Mayer, K., Kim, K.J., Borok, Z., Bartsch, P., and Crandall, E.D. 2002. Hypoxia decreases active Na transport across primary rat alveolar epithelial cell monolayers. *Am. J. Physiol. Lung Cell Mol. Physiol.* **282**, 659–665.

Mairbaurl, H., Wodopia, R., Eckes, S., Schulz, S., and Bartsch, P. 1997. Impairment of cation transport in A549 cells and rat alveolar epithelial cells by hypoxia. *Am. J. Physiol.* **273**, 797–806.

McElroy, M.C., Cain, D.J., Tyrrell, C., Foster, T.J., and Haslett, C. 2002. Increased virulence of a fibronectin-binding protein mutant of *Staphylococcus aureus* in a rat model of pneumonia. *Infect. Immun.* **70**, 3865–3873.

McElroy, M.C., Harty, H.R., Hosford, G.E., Boylan, G.M., Pittet, J.F., and Foster, T.J. 1999. Alpha-toxin damages the air-blood barrier of the lung in a rat model of *Staphylococcus aureus*-induced pneumonia. *Infect. Immun.* **67**, 5541–5544.

Oviedo-Boyso, J., Cortés-Vieyra, R., Huante-Mendoza, A., Yu, H.B., Valdez-Alarcón, J.J., Bravo-Patiño, A., Cajero-Juárez, M., Finlay, B.B., and Baizabal-Aguirre, V.M. 2011. The hosphoinositide-3-kinase-Akt signaling pathway is important for *Staphylococcus aureus* internalization by endothelial cells. *Infect. Immun.* **79**, 4569–4577.

Patti, J.M., Allen, B.L., McGavin, M.J., and Hook, M. 1994. MSCRAMM-mediated adherence of microorganisms to host tissues. *Annu. Rev. Microbiol.* **48**, 585–617.

Peacock, S.J., Foster, T.J., Cameron, B.J., and Berendt, A.R. 1999. Bacterial fibronectin-binding proteins and endothelial cell surface fibronectin mediate adherence of *Staphylococcus aureus* to resting human endothelial cells. *Microbiology* **145**, 3477–3486.

Sinha, B., François, P.P., Nüsse, O., Foti, M., Hartford, O.M., Vaudaux, P., Foster, T.J., Lew, D.P., Herrmann, M., and Krause, K.H. 1999. Fibronectin-binding protein acts as *Staphylococcus aureus* invasin via fibronectin bridging to integrin $\alpha_5ß_1$. *Cell. Microbiol.* **1**, 101–117.

Wong, K.W. and Isberg, R.R. 2005. Emerging views on integrin signaling via Rac1 during invasin-promoted bacterial uptake. *Curr. Opin. Microbiol.* **8**, 4–9.