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Outline of Research Result

Identification of the *Staphylococcus aureus* exotoxin transporter

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Research aims

Drug pumps contribute to the multi-drug resistance of MRSA, *Pseudomonas aeruginosa*, and *Acinetobacter baumannii*, and thus understanding their functions is important to establish clinical treatment strategies against MRSA and other antibiotic-resistant pathogenic bacteria.

S. aureus AbcA comprises six transmembrane domains and an ATP hydrolyzing domain in a single polypeptide (575 aa), which is a type III ABC transporter found in the export system of many bacteria. Overexpression of *abcA* leads to resistance against *β*-lactams, such as methicillin and cefotaxime; the phosphoglycolipid, moenomycin; the lipopeptide antibiotic, daptomycin; and dyes, such as rhodamine and ethidium bromide. These findings suggest that AbcA is a multidrug efflux system in *S. aureus*. AbcA expression is regulated by NorG, a transcription factor involved in antibiotic resistance, and other virulence regulators, including AgrBDCA, Rot, SarA, SarZ, and MgrA. Additionally, AbcA expression is induced by antibiotic-treatment and in starvation conditions or the stationary phase, wherein several *S. aureus* virulence factors are produced. These findings suggest that AbcA plays a role in *S. aureus* virulence in addition to drug efflux. However, the endogenous molecule exported by AbcA and the exact functions of AbcA in *S. aureus* virulence have not been elucidated.

S. aureus produces phenol-soluble modulins (PSM) having high lytic activity against neutrophils and erythrocytes. Additionally, deletion of genes encoding PSM decreases *S. aureus* virulence in a mouse infection model. PSM is considered to be a determinant of the high virulence of community-acquired MRSA (CA-MRSA). Moreover, PSM is involved in in *S. aureus* colony spreading.[1), 2)] Therefore, elucidating the secretory mechanism of PSM is important for understanding the molecular mechanisms underlying *S. aureus* infection.

PSM peptides do not have signal peptides recognized by Sec machinery, but its characteristics are similar to those of bacteriocins, which are antimicrobial peptides produced by various bacteria. Some *S. aureus* strains produce bacteriocins, namely Aureocin A70, with an isoelectric point of 9.9–10.0. AurT(571 aa)-secreting Aureocin A70 is a type-III ABC transporter comprising six transmembrane domains and an ATP hydrolyzing domain. We hypothesized that the transporter secreting PSM has a structure similar to that of AurT, and found that AbcA shared 58% amino acid identity with AurT.

Methods and Results

AbcA is required for PSM secretion

We searched for a protein similar to AurT from the *S. aureus* N315 genome database using protein BLAST and identified AbcA (SA0599), which had the highest homology with AurT among *S. aureus* gene products. In addition, we found 17 *S. aureus* gene products having similarity to AbcA. We constructed knockout mutants of the *S. aureus* Newman strain for these genes and measured the amount of PSM in culture supernatants. AbcA shared high identity with AurT in the transmembrane domain, Walker A and Walker B motifs that are predicted to bind ATP, the signature C motif, and the switch region, which are all conserved among bacterial ABC exporters. The amount of PSM in the culture supernatant of the *abcA* knockout mutant was less than that of the parent strain. This amount was restored by introducing the intact *abcA* gene. The growth of the *abcA*-disrupted mutant was indistinguishable from that of the parent strain. We hypothesized that PSM would accumulate in the cytosolic fraction of the *abcA* knockout mutant if PSM secretion was blocked. In agreement, the amount of PSM was higher in the intracellular fraction of the *abcA* knockout mutant than in that of the parent strain. This amount decreased with the introduction of the intact *abcA* gene in the *abcA* knockout mutant. These findings suggest that AbcA is required for PSM secretion.

The ATP binding motif of AbcA is required for PSM secretion

Because the ABC transporter utilizes energy released by ATP hydrolysis, amino acid substitutions in the Walker A or Walker B motifs, which function in ATP hydrolysis, result in the loss of the transporting activity of the ABC transporter. We constructed mutants encoding AbcA proteins harboring an amino acid substitution in the Walker A or Walker B motifs. We examined their complementation activity in the *abcA* knockout mutant. Introduction of the wild-type *abcA* gene restored the amount of PSM in the culture supernatant of the *abcA* knockout mutant to the parent level. In contrast, mutant *abcA* genes encoding K374R or D497R mutant proteins decreased the restoration activi-

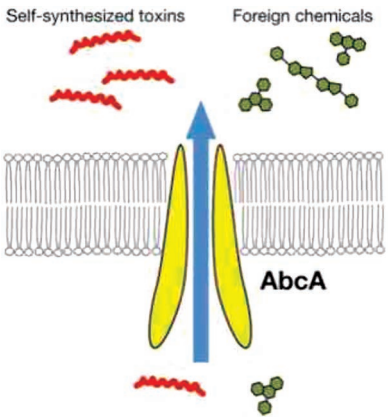

Fig. 1. AbcA has dual functions

ties compared with the wild-type *abcA* gene. The expression levels of mutant AbcA proteins were comparable with that of wild-type AbcA protein. These findings suggest that the ATP binding motif of AbcA is required for PSM secretion.

AbcA is required for *S. aureus* virulence

Based on the requirement of AbcA for PSM secretion, we investigated whether AbcA has a critical role in *S. aureus* virulence by using a murine systemic infection model. The survival rate of mice injected with the *abcA* knockout mutant was higher than that of mice injected with the parent strain. This finding indicates that AbcA has a critical role in *S. aureus* infection in mammals.

Heterologous expression of AbcA in *L. lactis* increases resistance against PSM

If AbcA directly secretes or extrudes PSM, it is assumed that heterologous expression of AbcA functions in protecting the bacteria from PSM. We thus examined whether heterologous expression of AbcA makes *L. lactis* resistant to PSM. When PSM was added to the culture, *L. lactis* strain expressing AbcA had a higher survival rate than *L. lactis* strain transformed with an empty vector. These findings suggest that heterologously expressed AbcA protects *L. lactis* from extracellularly administered PSM.

Conclusion

Our findings revealed that a multidrug resistance transporter, AbcA, is required for the secretion of the *S. aureus* cytolytic toxin PSM. Heterologous expression of AbcA in *L. lactis* protected the cells against extracellular PSM. Therefore, our results suggest that AbcA secretes self-synthesized PSM as well as extrudes extracellularly administered PSM. Furthermore, an in vivo infection experiment demonstrated that AbcA plays a role in *S. aureus* virulence. These findings provide the first evidence that a multidrug transporter has dual functions: to secrete toxins as well as to protect cells from foreign substances. Thus it possesses offensive and defensive functions, both of which are important for the bacterial infection[3] (Fig. 1).

The present study revealed that a drug resistance transporter secretes *S. aureus* cytolytic toxins, thus the link between drug resistance and virulence expression. Transporters are typically located on the cell surface, which is easily accessed by chemicals. Thus, inhibition of *S. aureus* AbcA may lead to increased sensitivity to antibiotics and decreased virulence, making AbcA a promising target for novel antibiotics against *S. aureus*.

References

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