Stp1 and Stk1: The Yin and Yang of Vancomycin Sensitivity and Virulence in Vancomycin-Intermediate Staphylococcus aureus Strains

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(See the article by Cameron et al, on pages 1677–87.)

Reversible phosphorylation and dephosphorylation of proteins play an important role in a variety of cellular processes in both eukaryotes and bacteria [1, 2]. Typically, the phosphorylation of proteins, activated by extracellular signals, is mediated specifically by protein kinases and is coupled to dephosphorylation reactions catalyzed by phosphatases. Originally described in eukaryotes, it has been recently shown in several studies that eukaryotic-type serine/threonine kinases (STKs) and phosphatases are also expressed in prokaryotes [1–3]. Ser/Thr phosphorylation has now emerged as a critical regulatory pathway in bacteria to control various cellular functions including stress response, biofilm formation, cell wall biosynthesis, and metabolic and developmental processes [1, 2, 4]. Despite these studies, only a small number of phosphorylation substrates have been identified in bacteria. In addition, the biological impact of phosphorylation and dephosphorylation on target protein function has been described in only a few cases.

A single Stk (Stk1 or PknB–Stk1, which is preferable to conform to the standard terminology), comprising an N-terminal intracellular catalytic domain, a single transmembrane segment, and C-terminal extracellular PASTA (penicillin-binding protein and serine/threonine associated) domains, is conserved in all sequenced Staphylococcus aureus genomes. Interestingly, some of the methicillin-resistant S. aureus (MRSA) strains encode a second kinase (Stk2, SA0077 in N315), but Stk2 does not contain the extracellular PASTA domain. Mass spectrometric analysis has identified 6 phosphoserine and 3 phosphothreonine residues in Stk2, with a phosphorylated serine residue located in the activation loop that is crucial for its activity (unpublished data).

Genomic and transcriptional analysis indicate the presence of a phosphatase gene designated stp1, upstream of stk1, that is cotranscribed with stk1 in S. aureus [5]. The phosphatase Stp1 (SA1062 in N315) is a preserved 247 a.a. cytoplasmic protein possessing phosphatase 2C-specific motifs found in all sequenced S. aureus genomes [2]. In one study, Betalor minus et al [5] reported that mutants lacking Stp1 displayed thickened cell walls and increased resistance to the glycyglycine endopeptidase lysostaphin compared with the wild-type N315. Stk1 is also one of the targets of dephosphorylation for Stp1 [6]. However, the effect of an stk1 mutation on increased sensitivity to cell wall–active antibiotics is more variable, in part, due to background resistance, with methicillin-resistant S. aureus (MSSA) strain 8325-4 showing no change in sensitivity [2] and MRSA strains such as N315 [5], USA300, and COL [7] showing varying degrees of increased sensitivity to β-lactam antibiotics but not to vancomycin, aminoglycosides, or fluoroquinolones.

In an article published in this issue of the Journal by Cameron et al [8], the authors report the effect of an stp1 mutation on increased vancomycin resistance in 1 laboratory-derived series and 4 clinical sets of vancomycin-intermediate S. aureus (VISA) isolates generated from patients with invasive S. aureus syndromes. Using whole genome sequencing and comparative genomics, they identified previously observed mutations in vraG (single-nucleotide polymorphism [SNP]), agrA (nonsense), dltA (SNP), and yqf (SNP), as well as in stp1 (deletion), in the VISA strains compared with the isogenic parent strains. Based on this and 2 other studies, a pattern has emerged in the generation of VISA strains [8–10]. First, the number of mutations is limited, with 5–13 mutations in Cameron et al’s study [8] and 31 and 4 SNPs in others [9, 10]. This finding is surprising and hints at the utility...
of comparative genomics of clinical isolates as a valid means of tracking mutations linked to the emergence of other antibiotic resistances (eg, imipenem). Second, unlike those reported by Mwangi et al [9], these mutations were not cumulative but occurred independently during passage in vivo and in vitro, suggesting selection of a resistant subpopulation as a common occurrence rather than stepwise accumulation of mutations upon prolonged vancomycin exposure. The fact that distinct sets of mutations can occur in 5 sets of strains argues for the heterogeneity of genetic changes associated with the VISA phenotype. Third, the categories of mutations associated with the VISA phenotype are broad, encompassing genes involved in cell wall metabolism, regulation, transcription, protein synthesis, and an ATP-binding cassette transporter, thus failing to ascribe a single category of mutations (eg, cell wall metabolism) to the emergence of VISA isolates. Despite the absence of a consistent category of gene mutations to account for the VISA phenotype, several of these mutations may shed light on plausible mechanisms. For instance, vraG, encoding an efflux pump, and dltA, enabling decoration of surface teichoic acids with δ-alanine, can confer surface positive charge to S. aureus [11]. SNP mutations of vraG and dltA, as found in Cameron et al’s study [8], may promote gene function, thus netting an increase in surface positive charge and hence decreased binding of positively charged antibiotics such as vancomycin. yvqF (SA1702), lying upstream of vraS, is part of the vraSR operon [12]. Mutation of yvqF, in association with intermediate vancomycin resistance, has been described in the study by Cameron et al [8] and several other studies [9, 13, 14]. In a recent study, Gardete et al [15] showed that introduction of native yvqF on a multicopy plasmid into the VISA strain of USA300 caused an increase in vancomycin susceptibility, whereas introduction of wild-type vraSR into the revertant strain reestablished VISA phenotype. The yvqF/vraSR operon seems to function as an on-off switch, with mutations in yvqF turning on the vraSR expression to increase vancomycin resistance, whereas mutations in vraS are accompanied by loss of resistance [15]. This conjecture was supported by the finding that mutation of yvqF has not been found in association with vraS mutation in clinical isolates in 2 studies [9, 13]. A notable absence is a mutation in walK (previously yycG) in clinical VISA isolates in Cameron et al’s study and other studies, whereas strains passed in vitro in vancomycin commonly contained walK mutations [8, 16]. Mutations in YycH and/or YycI, both negative regulators of WalKR [17], are also found in Cameron et al’s study and another study [9], indicating that an intact WalK is likely integral to development of this resistance in clinical strains. Whether WalK contributes to increased cell wall thickness described in VISA strains remains to be determined.

Based on data from by Cameron et al [8], we can now add to this list Stp1, the mutation of which has led to a VISA phenotype (4 μg/mL) in a clinical isolate. A separate stp1 mutant was then recreated via allelic exchange in the parental but vancomycin-susceptible strain A5937, resulting in an increase in vancomycin minimum inhibitory concentration (from 1.5 to 3 μg/mL but <4–8 μg/mL established for VISA). This finding is consistent with another study in regard to mutations contributing to the VISA phenotype in clinical isolates. Notably, Renzoni et al found that an stp1 mutant in ISP794 (8325 background) also exhibited borderline intermediate vancomycin susceptibility (2–4 μg/mL) compared to the parent strain [10]. One mechanism by which an stp1 mutant may contribute to the VISA phenotype [18] is to alter cell wall biosynthesis via enhanced phospho-signaling to increase cell wall thickness, as has been observed in Cameron et al’s study and 2 other studies [5, 8, 10]. Microarray studies here [8] were also consistent with the metabonomic studies of stp1 mutants [19], showing enhanced cell wall synthesis (increased expression of uppS encoding an undecaprenyl pyrophosphatase synthase) accompanied by higher levels of UDP-MurNAc substrate compared with the wild type. Nevertheless, the specific phosphorylation substrates that are dephosphorylated by Stp1 to increase vancomycin susceptibility remain undefined.

Peleg et al observed that VISA isolates are less virulent than their vancomycin-susceptible counterparts in the invertebrate Galleria mellonella model [20]. A VISA derivative of MRSA clone USA300 has also been found to display down-regulation of major virulence determinants in microarray analysis [15]. In support of these findings, the study of Cameron et al showed that the stp1 mutant of a clinical isolate, which exhibited reduced susceptibility to vancomycin, expressed a lower level of δ-hemolysin, consistent with reduced agr function, and attenuation in virulence in a murine bacteremia model compared with the parental strain. Similarly, an stp1 mutant of MSSA strain Newman was also found to be less virulent than the isogenic parent using a murine sepsis model [21]. The above findings are concordant with the recent observation that an stk1 mutant of MRSA USA300 exhibited an elevated level of agr-related transcripts and enhanced virulence in a murine subcutaneous mode of infection [7]. The reason for the attenuation of virulence in an stp1 mutant with reduced susceptibility to vancomycin is not yet clear. However, we envision that resistance may impose a fitness cost in terms of growth of bacteria within the host environment; this cost can be mediated by divergent phospho-signaling pathways of regulatory proteins that control cell wall metabolism and bacterial virulence. An alternative explanation may be that downregulation of virulence genes may provide a strategy for the VISA strains (ie, stp1 derivatives) to avoid detection by the host immune system.

Notes

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References


