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The Neisseria gonorrhoeae Type IV pilus promotes resistance to an iron-dependent antibiotic streptonigrin

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Background

The Neisseria gonorrhoeae Type IV pilus is a multifunctional, dynamic fiber involved in host cell attachment, DNA transformation, and twitching motility. We reported that the pilus is also required for resistance against hydrogen peroxide-, antimicrobial peptide LL-37-, and non-oxidative, neutrophil-mediated killing. We then determined that the hydrogen peroxide, LL-37, and neutrophil hypersensitivity phenotypes in non-piliated cells involved elevated intracellular labile iron. The mechanism(s) of pilus-dependent resistance through iron homeostasis is yet unknown.

Aim/Methods

We used two genetic approaches to identify genes that are involved in resistance. In the first approach, we performed Tn-Seq by exposing a transposon mutant library to the iron-dependent antibiotic streptonigrin or the vehicle control. In the second approach, we treated multiple, independent ΔpilE cultures to six rounds of streptonigrin, backcrossed the genomic DNA into a strain that uses CRISPR interference to knock-down pilE transcription, CRISPRi-pilE, and sequenced transformants that were resistant to streptonigrin even in the absence of PilE.

Results

A library of over 15,000 unique insertions was exposed to streptonigrin and 107 mutants were depleted and 55 mutants were enriched following treatment. We identified pilus assembly, antibiotic efflux, energy and electron transport, DNA damage repair, oxidative stress response, envelope biosynthesis, metabolism, amino acid biosynthesis, transcription, and posttranslational regulation genes impacted streptonigrin sensitivity. The mutant with the greatest increase in resistance to streptongrin was ngo0059 which encodes HpaC, a reductase in a two-component FAD-dependent monooxygenase. Sequencing of the CRISPRi-pilE strain backcrossed with in vitro evolved, streptonigrin-resistant ΔpilE genomic DNA also identified a hpaC(G277T) or HpaC(Gly93Cys) mutation.

Conclusions

The data suggest that HpaC and its role in cellular oxidation-reduction is involved in the pilus-dependent resistance against streptonigrin. Given that the pilus is also important for resistance to hydrogen peroxide and

LL-37, we will be testing if HpaC affects piliation and sensitivity to these oxidative and non-oxidative killing mechanisms. Further analysis of the hits from these genetic screens will determine which pathways are responsible for the pilus modulation of labile iron pools.