Suppression of Antimicrobial Resistance in MRSA Using CRISPRs, Part II

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Clustered Regularly Interspaced Short Palindromic Repeats (CRISPRs) are genetic elements that function with CRISPR-Associated (Cas) proteins to cleave genes. The CRISPR-dCas9 system is modified to suppress gene transcription. Methicillin-Resistant Staphylococcus aureus (MRSA) is a dangerous human pathogen that is resistant to many antibiotics. This is due to the mecA methicillin resistance gene coding for penicillin binding protein 2A (PBP2a), which inhibits the activity of beta-lactam antibiotics. Previously, two CRISPR-dCas9 systems were designed to target the promoter region of the mecA gene in MRSA to make it susceptible to beta-lactam antibiotics. Disk diffusion tests showed that the target on the coding strand significantly reduced antibiotic resistance in MRSA, whereas the target on the noncoding strand did not. This year, previous susceptibility results were confirmed using microbroth serial dilutions, which showed a reduction in resistance but not clinical susceptibility. The CRISPR system targeting the coding strand was the only one to reduce antibiotic resistance and thus was chosen for continued testing. mecA gene expression levels were analyzed using quantitative Real-Time Polymerase Chain Reaction (RT-qPCR). These results showed there was a 2.6-fold decrease in mecA gene expression in the CRISPR-treated sample when compared to a mock-treated control. A latex agglutination test was conducted to examine differences in protein expression between the control and CRISPR-containing bacteria. Results showed a 16-fold difference in PBP2a production compared to the control. In conclusion, a 2.6-fold decrease in gene expression and 16-fold decrease in protein production was not enough to make MRSA susceptible to beta-lactam antibiotics by clinical standards.

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