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Transcriptional Analysis Indicates Mode of Action of Novel Antibiotic MGB-BP-3 in *Staphylococcus aureus*

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ABSTRACT

Background: MGB-BP-3 (MGB) is a novel antibiotic candidate derived by chemical-synthesis and SAR from Distamycin - a natural product antibiotic that acts by binding to the minor groove of DNA. MGB has a high bactericidal activity against a broad range of Gram-positive bacteria. Its oral formulation, developed for the treatment of *Clostridium difficile* infections, is currently progressing through a clinical Phase I trial. Intravenous formulation of MGB, under development for the treatment of nosocomial systemic Gram-positive infections, is in the late preclinical stage of development. This study investigates the mode of action of this novel antibiotic.

Methods: To allow better understanding of MGB's mode of action (MoA), RNA-Seq analysis was undertaken on *S. aureus* following challenge with 0.5 x MIC (0.1 µg/mL) MGB-BP-3. Triplicate samples of RNA were extracted at 10 min after challenge. Approximately 5 - 7.5 million sequencing reads were obtained per sample (average length between 100 - 170 bp) using an Ion Torrent PGM. The data were analysed using CLC Genomics Workbench 7.5.1 software (Qiagen). The 'Empirical Analysis of DGE' (Differential Gene Expression) Tool was used, with Bonferroni-adjusted p-value.

Results: RNA-Seq analysis identified 698 transcripts showing significant changes in expression profile, which were confirmed by quantitative RT-PCR. Amongst these, 62 essential genes showed transcriptional arrest. Key enzymes of glycolysis were enhanced whereas the pentose phosphate pathway was reduced; flux through the TCA cycle was likely reduced significantly as citrate synthase and isocitrate dehydrogenase were reduced. These changes are associated with energy depletion. In addition, biosynthesis of nucleotides and certain amino acids were altered but it is not yet clear if the changes are directly due to MGB action or an indirect effect.

Conclusion: We propose that the bactericidal mode of action of MGB-BP-3 is at the transcriptional level of essential genes.

INTRODUCTION

MGB-BP-3 (Fig 1) is a novel antibiotic candidate derived by chemical-synthesis and SAR from Distamycin - a natural product antibiotic that acts by binding to the minor groove of DNA. It that has very strong antibacterial activity against all susceptible and multi-resistant Gram-positive pathogens tested, including methicillin-resistant and susceptible *Staphylococcus* species, pathogenic *Streptococcus* species, Vancomycin-Resistant and susceptible *Enterococcus* and *Clostridium difficile.*

The oral formulation of MGB-BP-3 is in a clinical Phase I study for the treatment of *C. difficile* infections and its intravenous formulation is in the final stages of preclinical development for the treatment of hospital acquired Gram-positive infections.

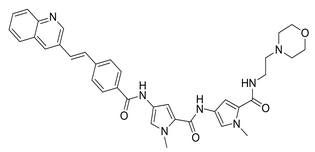


Figure 1. Molecular structure of MGB-BP-3

This study investigates the mode of action of this novel antibiotic and applies RNA-sequencing transcriptomics to determine the effect of drug on global gene expression of *Staphylococcus aur*eus.

HYPOTHESIS AND OVERALL METHODOLOGY

It was hypothesised that MGB-BP-3 acts to interfere with transcription by blocking the promoters of specific essential genes. To identify the specific target genes for MGB-BP-3 and to monitor the bacterial cell stress response to the drug, RNA sequencing technology was applied.

RNA-Seq METHOD WORKFLOW AND QC VALIDATION

Culture *Staphylococcus aureus* with and without MGB
(3 biological replicates) → QC check

Isolate high quality total RNA:

RNA protect bacterial reagent
Mechanical cell lyses with zirconia beads (0.1 mm)
RNA isolation with phenol/chloroform extraction
Silica filter purification of total RNA
DNase I treatment
Removal of DNase I and divalent cations → QC check

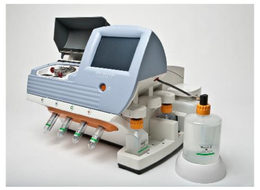
total RNA concentration per sample standardized to 4.5 µg in 26 µl

Depletion of ribosomal RNA (ribozero kit)

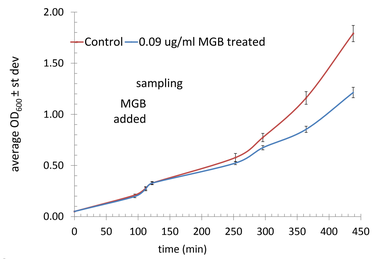
RNA-seq. library preparation → QC check

Barcoded libraries pooled at equal conc. (ctrl vrs. treated)
ctrl and MGB treated RNA-seq. samples diluted to 20 pM → QC check

Sequencing with Ion Torrent PGM

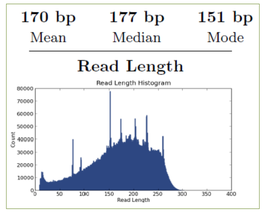


RESULTS – data generation

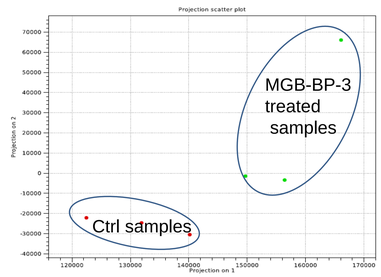


RNA-Seq analysis was undertaken on *S. aureus* following challenge with 0.5 x MIC (0.1 µg/mL) MGB-BP-3. Triplicate samples of RNA were extracted at 10 min after challenge.

Approximately 5 - 7.5 million sequencing reads were obtained per sample (average length between 100 - 170 bp) using an Ion Torrent PGM.



RESULTS – data analysis

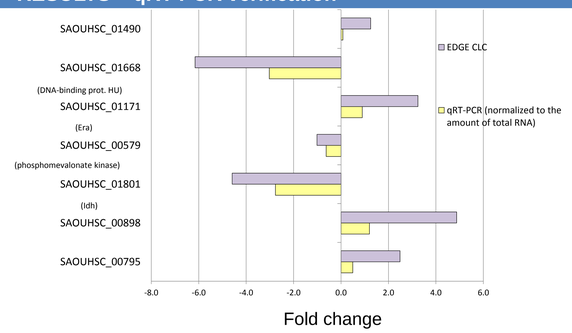


The data were analysed using CLC Genomics Workbench 7.5.1 software (Qiagen). The 'Empirical Analysis of DGE' (Differential Gene Expression) Tool was used, with Bonferroni-adjusted p-value, to trimmed reads.

Biological replicates group together in a PCA plot

RESULTS – qRT-PCR verification

When the 'fold changes' in RNA-Seq data (purple) were compared with the qRT-PCR data (yellow), the results were in agreement.



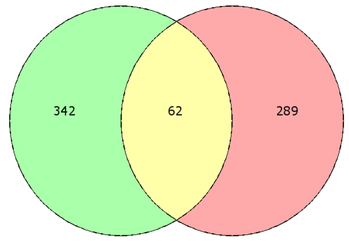
RESULTS – overview

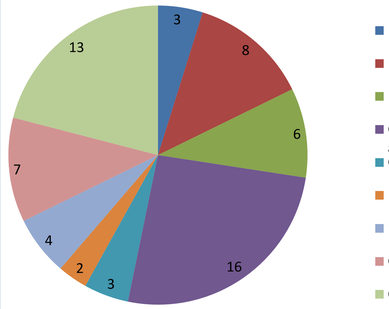
RNA-Seq analysis identified 698 transcripts showing significant changes in expression profile. Key enzymes of glycolysis were enhanced whereas the pentose phosphate pathway was reduced; flux through the TCA cycle was likely reduced significantly as citrate synthase and isocitrate dehydrogenase were reduced. These changes are associated with energy depletion. In addition, biosynthesis of nucleotides and certain amino acids were altered but it is not yet clear if the changes are directly due to MGB action or an indirect effect.

RESULTS – classification of genes with altered expression

402 genes (342 + 62) were down regulated significantly in the RNA-Seq experiment.
There are 351 essential genes (289 + 62) in *S. aureus.*

62 of the down regulated genes were essential (yellow)

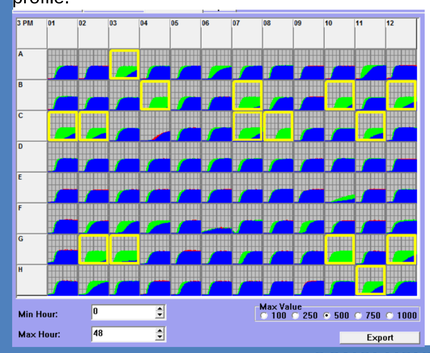




Distribution of the 62 essential genes that were down-regulated

RESULTS - Comparison of RNA-Seq with phenotypic analysis

Phenotype microarrays (Biolog) were used to further assess the effect of MGB-BP-3 on *S. aureus* metabolism at sub-inhibitory concentrations. The results were compared to the data from RNA-seq experiment. Significant phenotypes are highlighted in yellow. Changed in amino acid utilization due to the antibiotic were reflected in the RNA-Seq profile.



Biolog plate PM3B:

A3 nitrite
B4 L-isoleucine
B7 L-methionine
B10 L-serine
B12 L-tryptophan
C1 L-tyrosine
C2 L-valine
C7 D-lysine
C8 D-serine
C11 L-homoserine
G2 xanthosine
G3 uric acid
G10 D,L-a-amino-caprylic acid
G12 a-amino-N-valeric acid
H11 Gly-Met

RNA-seq data:

SAOUHSC_02729
amino acid ABC transporter-like protein
Fold change of -3.5

SAOUHSC_00151
branched-chain amino acid transport
system II carrier protein

Fold change of -2.4

SAOUHSC_02102
methionine aminopeptidase
Fold change of -2.6
Essential

CONCLUSION

RNA sequencing technology was applied and showed significant changes in the MGB-BP-3 treated *S.aureus* transcriptome where genes were both over and underexpressed. Phenotype microarrays further confirmed the effect of MGB-BP-3 to *S. aureus* metabolism. Based on the RNA-seq data analysis, we propose that the bactericidal mode of action of MGB-BP-3 is at the transcriptional level of essential genes. We will further identify links between the affected transcripts in order to identify the sequence specificity of the MGB-BP-3 compound. We aim to use this knowledge to facilitate the rational design of other antibiotic MGBs using synthetic chemistry.

REFERENCES

Chaudhuri *et al.* Comprehensive identification of essential *Staphylococcus aureus* genes using transposon-mediated differential hybridisation (TMDH). 2009. BMC Genomics 10:291.