Oligonucleotide Transcription Factor Decoys as tools to control bacterial transcription

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Background

At Procarta Biosystems Ltd. we are developing Transcription Factor Decoy (TFD) technologies to combat Antimicrobial Resistance. TFDs are short synthetic oligonucleotides that contain a binding site for specific bacterial transcription factors. TFDs inhibit genetic pathways crucial for growth, thereby killing the pathogen.

• Translocation of sufficient numbers of TFD’s into the bacterial cytoplasm is the key challenge. We have achieved this previously using a nanoparticle delivery system comprised of a lipidic delivery agent known as CM2[1].
• CM2 nanoparticles successfully deliver TFD’s to Gram-negative and Gram-positive bacteria by interacting with bacterial membrane components such as Cardiolipin and LPS [2].
• Reducing the ratio of CM2 to TFD would allow for delivery of more TFD’s, improving the effective dose and reducing cytotoxicity.

Using a click chemistry approach, we have conjugated a modified CM2 delivery molecule to a TFD oligonucleotide targeting the Fumarate Nitrate Reductase (FNR) transcription factor, an essential regulator of anaerobic respiration.

Aims

• To synthesise a conjugated molecule based on our precursor TFD delivery technology.
• To demonstrate antimicrobial efficacy in vitro and in Galleria mellonella larvae models.
• To elucidate mechanism of uptake and activity of the CM2-TFD Conjugate

Methods

Confocal Microscopy was used to assess delivery of the precursor technology to a range of bacteria, primarily Escherichia coli, Pseudomonas aeruginosa and Acinetobacter baumannii.
• The bacterial membrane is stained with WGA TMR SSS.
• CM2 nanoparticles are autofluorescent, allowing us to examine how they interact with the membrane.
• Conjugating TFDs with Rhodamine Green allows imaging of internalisation and acts as a transfection agent without CM2 present.

Conjugate Synthesis was achieved using a click chemistry approach. Click chemistry indicates a simple, high yielding reaction; in this case a copper-catalysed azide-alkyne cycloaddition (CuAAC).

• Successful synthesis was confirmed using HPLC and native PAGE.

Minimum Inhibitory Concentration experiments were conducted against a panel of E. coli isolates, cultured both aerobically and anaerobically.
• Under anaerobic conditions the FNR regulon is essential for growth, inhibiting FNR using a TFD should kill bacteria.
• An FNR knockout E. coli mutant was used as a reporter for FNR-essential conditions.

Galleria mellonella larvae were used as an in vivo model of bacterial infection.
• Larvae were infected with 1x10⁶ CFU per larva of E. coli ATCC 25922 and treated with either TFD conjugates, Levofloxacin (positive control) or PBS (negative control).
• Survival was monitored over a period of 96 hours.

Results

Conjugate Synthesis and Characterisation

• HPLC confirmed successful synthesis, exhibiting a single peak on a C18 column, with no peaks present to indicate free CM2 head groups or TFD’s.
• Native PAGE gels showed a single clear band, indicating successful synthesis and a monodispersed size population.

Killing Resistant E. coli in vitro

<table>
<thead>
<tr>
<th>Compound</th>
<th>E. coli ATCC 25922</th>
<th>E. coli ATCC BAA 2469</th>
<th>E. coli NCTC 13846</th>
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<tbody>
<tr>
<td></td>
<td>Aerobic</td>
<td>Anaerobic</td>
<td>Aerobic</td>
</tr>
<tr>
<td>Levofloxacin</td>
<td>0.008</td>
<td>0.015</td>
<td>0.08</td>
</tr>
<tr>
<td>CM2-TFD Conjugate</td>
<td>32</td>
<td>0.125</td>
<td>16</td>
</tr>
</tbody>
</table>

MIC results in μg/mL

• The CM2-TFD conjugate exhibited very good antimicrobial activity against E. coli under anaerobic conditions, including those strains resistant to Carbapenem and Colistin, unlike Levofloxacin.

Antimicrobial Activity in Galleria mellonella

• Preliminary in vivo data showed that TFD conjugates can protect G. mellonella against E. coli infection. Fine tuning of dosing regimen will be required to increase efficacy.

Understanding Mechanism of Delivery

• Confocal microscopy illustrated bacterial uptake of the precursor CM2 nanoparticle. CM2-TFD conjugates currently lack the necessary fluorescence for confocal imaging.
• Rhodamine-TFD conjugates are not antimicrobial but are structurally analogous to CM2-TFD conjugates and fluoresce brightly, making them a useful tool in understanding TFD conjugate translocation.

(a) - Internalisation of the CM2 nanoparticles in P. aeruginosa and interaction with Cardiolipin in the inner membrane.
(b) - Transfection of a Rhodamine-TFD conjugate into E. coli.

Conclusion

CM2-TFD conjugates showed early promise as effective antimicrobials in E. coli, including strains highly resistant to traditional antibiotics. Preliminary in vivo testing supported this and imaging of the precursor technologies indicated a mechanism of internalisation in a range of species. Conjugates were synthesised successfully and efficiently, meaning they can now be designed to target essential transcription factors in other high risk bacterial pathogens. Mechanism and quantification of uptake will also be further investigated.