Synthetic aptamers as potential novel efflux pump inhibitors of the TolC channel in E. coli strains.

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Introduction and goals

Antibiotic resistance is more than ever one of the most contemporary challenges threatening the health system worldwide. According to World Health Organization, previous cases of bacterial infections—once treatable with antibiotics—can now be lethal due to the uncontrolled misuse of these agents. One of the main triggers of antibiotic resistance is the over-expression of multi-drug resistant (MDR) efflux pumps. These pumps allow the bacterium to pump antibiotics out of the cell and therefore desensitize the cells to the antibacterial inhibitory effect. Most of the efflux pumps present in Gram negative bacteria, including E. coli, are composed of three main components: AcrB (inner membrane transporter), AcrA (periplasmic adapter protein bridging the three proteins together), and TolC (channel in the outer membrane). In this project, we propose to design small chains of nucleic acids called aptamers to bind to and block the outer membrane channel of the efflux pump, which is a protein called TolC, to impede antibiotic resistance bacteria from effluxing antibiotics.

Methods

How do we generate aptamers?

- Selection of ligand sequences that bind to a target
- Partitioning of aptamers from non-aptamers via affinity methods
- Amplification of bound aptamers

Results of Efflux Assay

- No accumulation of ethidium bromide was noticed in rounds 5 and 9 indicating that the aptamers resulting from these selections were unable to bind to the TolC channel and thus could not inhibit the efflux.
- The tenth round of selection recorded an intense accumulation of ethidium bromide implying that the aptamers selected had higher binding affinity to the outer membrane channel of the TolC pump blocked the efflux of the ethidium bromide to the outside of the cell.
- Surprisingly, when testing round 11, no accumulation of ethidium bromide was noted. This is likely because the efflux inhibitory activity appeared at round 10 and disappeared at round 11 implying some amplification of non-specific aptamers after round 11.
- We postulate that excessive cycles of selection might amplify non-specific products whereas fewer cycles would produce insufficient yields.

Summary and Future Work

- We generated 108 base DNA aptamers using a whole-cell SELEX methodology after eleven rounds of selection and we tested 9, 10, and 11 ability to block the efflux by conducting an in-vitro efflux assay.
- Of the rounds tested, only R10 showed an increased accumulation of fluorescent ethidium bromide within the E. coli cells. This activity disappeared at R11 indicating that the tenth round of selection was optimal and contained the most enriched pool of aptamers that can recognize and interact with the outer membrane channel TolC in E. coli serving as a molecular plug. We postulate that these aptamers might be a significant efflux pump inhibitor.
- Future work will tackle the cloning of the aptamer of interest into E. coli plasmid followed by sequencing steps to identify the base sequence of the aptamer generated.

Synthetic DNA Aptamers As Potential Novel Efflux Pump Inhibitors Of The TolC Channel In E. coli

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How do we measure efflux in vivo?

E. coli cells are grown in a nutrient-rich liquid medium then transformed with phosphate-buffered saline.

A fluorescent dye called ethidium bromide is added to the cells along with a small-molecule compound whose efflux-inhibiting activity we work to bind.

Ethidium bromide accumulates in efflux-depleted cells, causing the cells to fluoresce.

Ethidium bromide is pumped out of efflux-repleted cells so the cells do not fluoresce.