Using Anthrax toxin (Atx) for drug delivery

When people think of toxins in relation to medicine, they usually think of the use of the toxins' warhead. This warhead has been used to kill things like cancer cells when directed by a cell-targeting antibody. However, until now little attention has been given to the protein architecture that has evolved (and is part of the toxin) to deliver the toxin warhead to its target intracellular compartment.

The warhead of a protein toxin is a macromolecule that, in order to function, requires access to a specific intracellular compartment. It is not unreasonable to wonder of the architecture responsible for warhead delivery could be used to deliver other macromolecule, perhaps therapeutic macromolecular drugs?

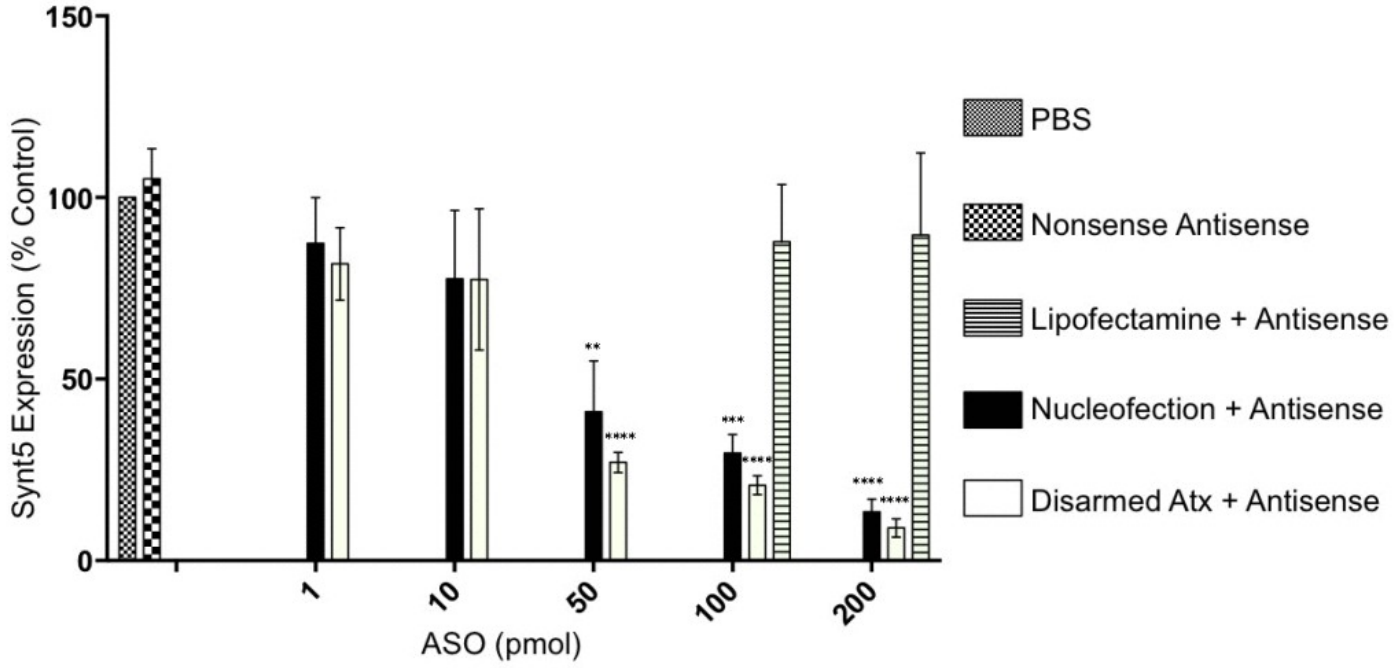

Fig. 1. Synt5 Modulation in HeLa Cells in Response to Varying ASO Dose (n=3 ± SEM at 24h) PBS and the missense antisense treatments were used as negative controls and Nucleofection served as a positive control. Here the disarmed Atx was shown to deliver an antisense drug with the same efficiency as nucleofection.

Here we have done just that. We have taken a class of drug (*i.e.* antisense and small interfering RNAs aka siRNA) that on their own are not very good at getting to their target compartment. We have attached them to the delivery architecture of a protein toxin that has historically been very successful at getting to that very same intracellular target compartment required for antisense or

siRNA function.

Antisense and siRNA drugs are particularly interesting as they have the ability to turn off a specific gene, leaving none-target genes alone. This is useful if the over-expression of the gene you are targeting causes a disease, or the target gene belongs to a virus, as there is the capacity to make people better.

Naturally we were keen to ensure that the recombinant technology we had employed to remove the toxins warhead was robust, so we also monitored not only antisense and siRNA delivery efficiency, but also any resulting toxicity relative to other commonly used chemically synthesised antisense delivery vehicles.

IC_{50} (µg/mL; n=6 ±SEM)	HeLa cells	Vero cells
25 KDa branched PEI	2.9 ± 0.6	7.3 ± 0.1
0.8 KDa branched PEI	2.4 ± 0.2	7.4 ± 0.3
20 KDa linear PEI	3.0 ± 0.1	6.9 ± 0.5
ASO	100+	100+
PA	100+	100+
LFn-GAL4	100+	100+
LFn-PKR	100+	100+
PA:LFn-GAL4:ASO	100+	100+
PA:LFn-PKR:siRNA	100+	100+

Table 1. In vitro toxicity after 72h

Here we have used disarmed anthrax toxin (Atx) to deliver both antisense and siRNA drugs.

As can be seen from figure 1a, the down-regulation of the target gene (Synt5) is profound and comparable to very high efficiency nucleofection (our positive control). Further, the delivery of siRNA is shown (Fig. 1b.), and again activity comparable to nucleofection was recorded.

The toxicity profile of this delivery system is sown in Table 1 and relative to poly(ethylamine) a well known DNA delivery agent, little toxicity was observed.

All of these experiments were conducted *in vitro* (*i.e.* using cells grown in a laboratory), which begs the question, will this system work in a whole body? This remains to be seen, though it is without a doubt the next step towards seeing if this technology can translate into a medicine.

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Publication

Disarmed anthrax toxin delivers antisense oligonucleotides and siRNA with high efficiency and low toxicity.

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J Control Release. 2015 Nov 9