Establishing the role of glycans and lipids in the mechanisms of Tpp1/Tpp2 (Bin) toxin in target insect and cancer cells.

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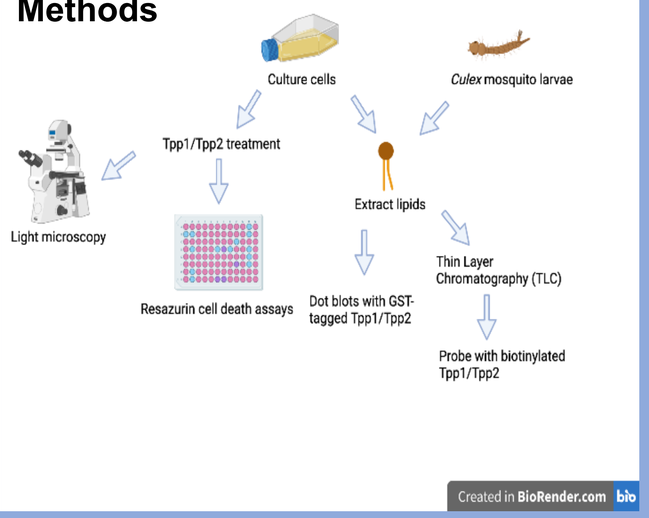
Introduction

- Tpp1/Tpp2 two-part toxin (toxin_10 family) from *Lysinibacillus sphaericus,* targets larvae of *Culex* and *Anopheles* mosquitoes [1,2,3].
- Mechanisms of toxicity not fully understood – Evidence for receptor binding/ pore formation/ toxin internalization and apoptosis[4,5,6,7].
- Toxicity reported in a range of mammalian cancer cell lines including HepG2, in the absence of putative receptor (Cqm1)[8].
- Evidence for apoptosis in cancer lines[8,9]. Mechanisms of specificity for cancer cells currently unknown.
- Studies on other invertebrate-active pore forming toxins (Cry5B and Cry14A) have shown glycans mediate toxin/receptor binding[10,11].
- Tpp1/Tpp2 shown to interact with L-fucose, L-arabinose, and glycoproteins[12].

Aims

- Evaluate Tpp1/Tpp2 toxicity in two *Culex* mosquito cell lines and HepG2 cancer cells.
- Investigate interactions between Tpp1 and Tpp2 with sugars and lipids extracted from *Culex* larvae and cells and cancer cell lines, including HepG2 cells.

Methods



Results

1. Tpp1/Tpp2 toxicity demonstrated in *Culex* mosquito cells and HepG2 cells (Figure 1).

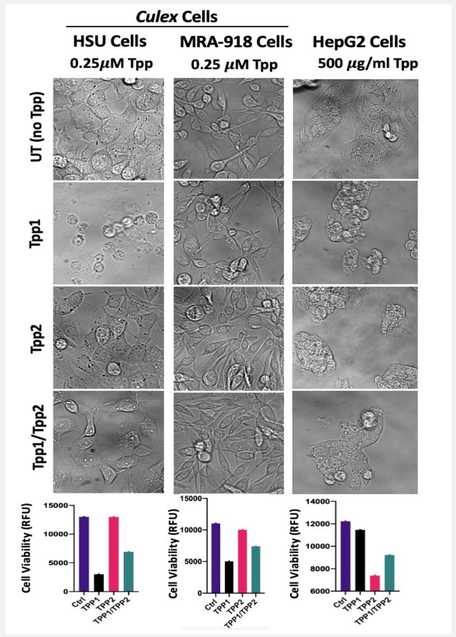


Figure 1. Tpp1/2 toxicity in HSU, MRA-918 and HepG2 cells. Cells imaged 24h post toxin treatment. Resazurin cell death assay which measures mitochondrial function, 24h post toxin treatment (Insect) 48h post toxin treatment (HepG2), the bars on the graphs represent Relative Fluorescence Units (RFU).

2. Thin Layer Chromatography (TLC) shows differences in extracted lipid profiles of *Culex* mosquito and human cancer cell lines (Figure 2).

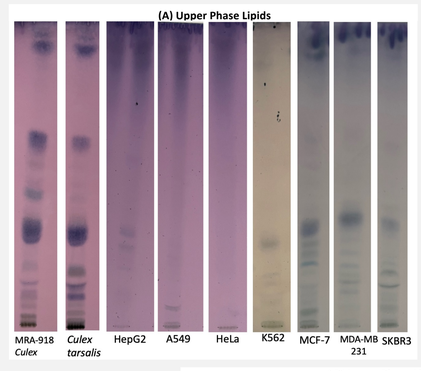


Figure 2. TLC comparison of the extracted upper and lower phase lipid profiles from *Culex* cells and human cancer cell lines. Upper phase contains polar lipids such as gangliosides. Lower phase contains most lipids including non polar and simple lipids.

3. Tpp1 and Tpp2 bind to lipids from *Culex* larvae/ cells and some human cancer cells (Figure 3).

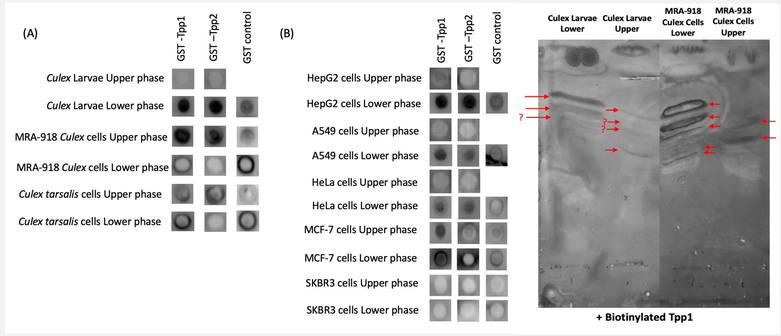


Figure 3. Tpp1/2 binding to lipids extracted from *Culex* larvae and *Culex cells* and cancer cell lines. GST-Tpp1 and Tpp2 binding to whole lipid extracts from (A) Culex larvae and cell lines (B) human cancer cell lines, on dot blots. (C) TLC on lipids extracted from Culex larvae and cells with biotinylated Tpp1 probe showing multiple bands for Tpp1 binding in lower and upper phase lipids.

4. No Tpp1/2 interactions with sugars were detected on dot blots (Figure 4).

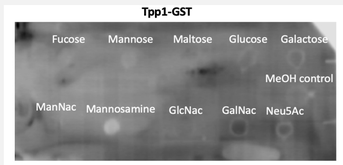


Figure 4. Tpp1 and Tpp2 sugar dot blots. Sugars were blotted directly onto the membrane and incubated with GST-Tpp1 or GST-Tpp2. No sugar binding was observed for either protein.

Discussion

- Tpp1/Tpp2 displayed toxicity in two *Culex* cell lines and HepG2 cells in the same patterns as previously reported.
- Tpp1 and Tpp2 bind lipids from both *Culex* larvae and cells. Additionally, we showed for the first time Tpp1 and Tpp2 binding lipids from cancer cells, particularly HepG2 cells, which could be important for toxin binding or toxicity.
- Extracted lipid profiles of *Culex* cells and human cancer cells are different. Some cancer lines e.g. HepG2 have lipid profiles more similar to *Culex* cells, which may relate to the ability of Tpp1 and Tpp2 to bind those lipids.
- No evidence of Tpp1 and Tpp2 interactions with any sugars tested. May suggest the proteins do not interact with glycolipids or the glyco- motifs of lipids or that they bind to a currently untested sugar.

Future Work

- Identify which *Culex* and cancer cell lipids Tpp1 and Tpp2 bind on TLCs and investigate their role in mediating toxicity.
- Investigate the necessity of glycosylation for Tpp1 and Tpp2 binding to *Culex* and cancer cell lipids utilising Miglustat (inhibits glycosphingolipid biosynthesis) and Kifunensine (inhibits N-glycosylation)
- Investigate role of lipids and glycans in mediating toxicity in other insecticidal proteins.

References

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Methods figure created in BioRender.com.

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