Investigation of *Clostridium difficile* toxin receptor specificity and its effect on pathogenesis

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Introduction

Clostridium difficile: a toxin-producing bacteria causing intestinal infection

- *Clostridium difficile (C. difficile)* is a Gram-positive bacteria naturally present in the environment as well as in the intestinal tract of healthy humans and animals.
- When microbiome composition in the human or animal intestine is unbalanced, *C. difficile* can dominate and colonize the gut, subsequently causing *Clostridium difficile infection* (CDI). CDI symptoms range from mild-degree diarrhea to life-threatening colitis.

C. difficile toxins enter host cells mediated by multiple host-cell receptors

- Toxin B (TcdB) produced by *C. difficile* is one of the toxins involved in the development of disease. TcdB has a *glucosyltransferase* activity able to induce cell death[1,2]
- TcdB enters colonic epithelial cells via receptor-mediated endocytosis upon interaction with host-cell receptors. There are three host-cell surface receptors identified for TcdB: NECTIN3, CSPG4, and FZD1/2/7 protein, with the latter two being the predominant receptors for TcdB.

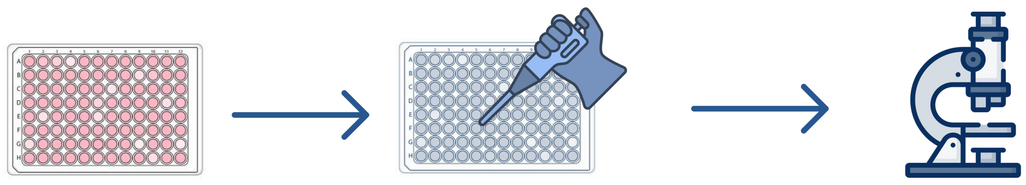
Having multiple receptors could be beneficial for TcdB pathogenesis. In this research experiment, we aimed to identify the role of different TcdB receptors in pathogenesis and host-cell morphological changes.

Method

To observe the pathological effect of TcdB in presence or absence of interaction with different host cell receptors, we utilized two different types of cell line (HeLa wild type and HeLa CSPG4$^{-/-}$) and five different types of TcdB toxins.

Table 1. Description of different types of Clostridium difficile Toxin B used

TcdB type	Description
Wild type (wt)	Wild type toxin, able to bind to FZD1/2/7 and CSPG4
F1597S	Lacks ability to bind to FZD1/2/7 receptor
ΔCROP (1-1875)	Lacks the ability to bind to CSPG4 receptor
ΔCROP F1597S	Lacks the ability to bind to both FZD1/2/7 and CSPG4 receptors
D286/288N	Lacks glucosyltransferase activity
R20291	Hypervirulent TcdB toxin lacking the ability to bind to FZD1/2/7



Seeding of HeLa cell lines (wild type and CSPG4$^{-/-}$) in a microtiter plate

Application of TcdB toxins detailed in Table 1. Toxins were applied in 1:5 dilution with 5000ng/ml and 0.05 ng/ml as the highest and lowest concentration, respectively

Observation of **cytopathic** and **cytotoxic effect** using inverted microscope

Cytopathic effect and cytotoxic effect were decided based on microscopic observation. Cytopathic effect was described as observed cell rounding, while cytotoxic effect was described as either chromatin condensation or cell blisters. Figure 1 shows the reference for normal cells (untreated cells), cytopathic effect, and cytotoxic effect.

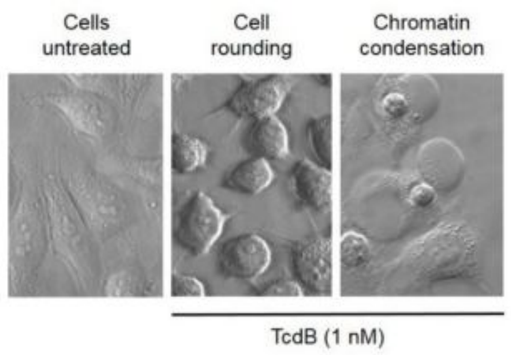


Figure 1. Morphological appearances of cells exposed to 1 nM TcdB toxins

Findings and Discussion

Table 2. Summary of observed TcdB toxin potency in HeLa wt and CSPG4 $^{-/-}$ cell line

Description	TcdB type					
	Wild type	F1597S	ΔCROP	ΔCROP F1597S	D286/ 288N	R20291
Lowest concentration inducing **cytopathic effect** in **HeLa wt cells**	0.3 ng/ml	0.3 ng/ml	0.3 ng/ml	1000 ng/ml	200 ng/ml	1.6 ng/ml
Lowest concentration inducing **cytotoxic effect** in **HeLa wt cells**	200 ng/ml	200 ng/ml	200 ng/ml	-	-	-
Lowest concentration inducing **cytopathic effect** in **HeLa CSPG4 $^{-/-}$**	1.6 ng/ml	200 ng/ml	200 ng/ml	1000 ng/ml	5000 ng/ml	200 ng/ml
Lowest concentration inducing **cytotoxic effect** in **HeLa CSPG4 $^{-/-}$**	5000 ng/ml	5000 ng/ml	-	-	-	-

•Cytopathic and cytotoxic potency of TcdB mutant devoid of FZD1/2/7 binding (**TcdB F1597S**) and TcdB mutant devoid of CSPG4 binding (**TcdB ΔCROP**) were in the **same range** as well as compared to wild type TcdB.

•This suggests that the overall **toxin potency of TcdB was not significantly affected by whether the toxin binds to multiple receptors or only one of them**. This also indicates that cellular receptor binding to TcdB happens independent of the role of other receptors.

•**Significant reduction** (3000-fold) of cytopathic and cytotoxic effect was observed in **TcdB devoid of both FZD1,2,7 and CSPG4 binding**.

•This signifies that **FZD1/2/7 and CSPG4 cellular receptors are significant factors contributing to toxin activity**. Without any of these cellular receptors, TcdB cannot enter the host cell and subsequently cause pathological effect[1]

•Interestingly, in HeLa CSPG4$^{-/-}$ cells treated with TcdB, less cytotoxic effect was observed compared to in HeLa wild type cells. This might suggest that TcdB toxin **uptake via CSPG4 receptor leads to more efficient cell destruction**.

Conclusion

TcdB uses both FZD1/2/7 and CSPG4 receptors in an independent manner
CSPG4 is more physiologically relevant for toxicity in TcdB

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