Cytolethal distending toxin producing *Campylobacter jejuni* promotes colorectal tumorigenesis by induction of DNA damage and cell cycle arrest

**HYPOTHESIS:**
Cytolethal distending toxin producing *C. jejuni* 81-176 promotes colorectal tumorigenesis.

**BACKGROUND/AIMS:**
Colorectal cancer (CRC) is the third most common cancer type in males and the second in females. Exploring the possible causative agents of CRC is critical for both prevention and treatment of CRC. An increased prevalence of *Escherichia* and *Campylobacter* spp has been found in colorectal cancer tumor lesions compared to normal adjacent tissues. While approximately 2-3 million campylobacteriosis cases/year occur in the United States, human infection can result in an asymptomatic carrier state. *Campylobacter jejuni*, a major cause of bacterial diarrhea in humans, produces a genotoxin named cytolethal distending toxin (CDT) which has DNAse activity and causes DNA double strand breaks. However, carcinogenic potential of CDT *in vivo* has not been demonstrated.

**METHODS:**

*In vivo experiment:* Germ-free (GF) *ApcMin/+* mice were colonized with the human clinical isolate *C. jejuni* 81-176 or 81-176 *cdtB* mutant via oral gavage (1 X 10^5 cfu), followed by 1% dextran sulfate sodium (DSS). Colitis development and tumor formation were monitored by colonoscopy. Three weeks post-DSS treatment, mice were euthanized and tumor count and size were determined. *C. jejuni* 81-176 lysate preparation: *C. jejuni* 81-176 was grown micro-aerobically on Campylobacter selective agar plates for 72h. Bacteria were harvested, suspended in PBS, pelleted at 3000rpm and washed twice in PBS. Bacteria suspensions were sonicated on ice for four 30s bursts with 30s intervals. The lysate was passed on a 0.22µm sterilized filter and protein content measured using BCA assay.

*In vitro experiment:* The small intestinal epithelial cell line IEC-6 and colon cancer cell lines HT-29 were exposed to *C. jejuni* cell lysates (WT or *cdtB* mutant; 5µg/ml) to determine DNA damage via phosphorylated histone H2AX (γH2AX) immunofluorescence, comet assay and cell cycle assay.

*Publicly available databases:* We retrieved microbiome data from publicly available databases that contained whole genome metagenome sequencing of stool from CRC patients and non-CRC patients. Through *de novo* assembled data, we identified *cdtA*, B and C protein products. The protein products were aligned to *C. jejuni* *cdtA*, B or C and those alignments showing >50% identity and >75% coverage were considered valid hits.

**RESULTS & CONCLUSIONS**

**Results:** GF *ApcMin/+* mice colonized with *C. jejuni* had significantly more (*P*=0.0253) and larger tumors (*P*=0.042) compared with the mice without *C. jejuni*. Cell lines treated with lysates of *C. jejuni* demonstrated increased γH2AX immunofluorescence and increased number of comet tails compared to control cells. At the same time, lysates of *C. jejuni* still caused G2/M arrest in cell cycle. *CdtB* mutation attenuated *C. jejuni*-induced colorectal tumorigenesis ability *in vivo*, and significantly decreased the DNA damage response in cell lines. Furthermore, lysates of *cdtB* mutant *C. jejuni* did not induce cell cycle arrest. Interestingly, the prevalence of *cdtB* was significantly higher in CRC patient than normal subjects (*P*= 0.004) suggesting a potential role of *cdtB* in carcinogenesis.

**Conclusion:** *C. jejuni* 81-176 promotes colorectal tumorigenesis through CDT-induced DNA damage and cell cycle arrest.