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## The role of CpG in intestinal cancer cell model

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Background: Recent reports have shown different effects of immunostimulatory sequences containing CpG motifs on various immune cells. However, the exact role of CpG oligodesoxynucleotide(ODN) in the human gut is unclear.

Aim: In the present study, we assessed potential effects of CpG ODN on non immune cell (epithelial cell line HT-29) on a dose-response and time -course basis.

Methods: epithelial cell line HT-29 was treated with CpG ODN (CpG 2006) and lipopolysaccharide (LPS) at (5, 10, 25, 50 µg/ ml) and (1, 5, 10 µg/ ml) concentrations, respectively. Following treatments, dose- response and time-course cytotoxicity (using a colorimetric method), MMP-2 activity (using gelatin zymography) and apoptosis (using annexin-v flowcytometry method) assays were performed. To verify specific CpG effects, chloroquine treatment was used for its inhibitory effect on endosomal acidification process.

Results: cytotoxicity analysis of CpG ODN showed that CpG ODN increases proliferation of cells at concentration 10-25 µg/ml. Significant differences were observed between treated and untreated groups (p< 0.05). MMP-2 activity analysis showed no significant differences between different groups of treated and untreated cells. Moreover, rate of apoptosis for

CpG ODN treated cells at 5, 10, 25  $\mu$ g/ml concentrations was 0.8 %, 6.46 % and 14.21 %, respectively, showing an increased apoptosis to necrosis ratio.

Conclusion: intestinal epithelial cell line HT-29 is potentially highly responsive to CpG therapy in vitro and exhibits deviated cancer cell characteristic phenotypes.