Effects of Gastrokine-1 in Bacterial Biofilm Formation

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Background and hypothesis:

Gastrokine-1 (GKN1) is produced and secreted by the epithelial cells of the gastric mucosa. It is highly expressed in normal gastric mucosa, and it plays a role in gastric mucosal protection, anti-inflammatory processes and epithelial cell proliferation. While GKN-1 is typically absent in the colon, emerging evidence suggests it may have a protective effects in intestinal inflammatory conditions, such as colitis. Adherent-Invasive *Escherichia coli* (AIEC) is a gut pathobiont associated with Crohn's disease that invades intestinal epithelial cells and can form biofilms. Citrobacter rodentium, a murine pathogen, serves as a model for enteropathogenic *E. coli*-induced colitis. We hypothesize that GKN1 treatment will inhibit or reduce bacterial biofilm formation on intestinal epithelial cells.

Experimental design:

Human colorectal carcinoma cell line HCT-116 was cultured on circular glass coverslips (1.8 cm diameter). Cells were pre-treated with recombinant GKN1 before incubation with bacterial suspensions of either AIEC or C. rodentium. The co-culture was maintained overnight (24 hours) under static conditions. A parallel set of samples was prepared without GKN1 treatment as a control. Post-incubation, cells were stained for biofilm and bacterial visualization, mounted, and imaged using fluorescence and confocal microscopy. The experiment was extended to 48 hours in an attempt to amplify observable differences between treated and untreated conditions.

Results:

To date, microscopy analysis has not revealed a discernible difference in fluorescence signal or bacterial presence between GKN1-treated and untreated HCT-116 cells. This was consistent across both 24-hour and 48-hour incubation periods, using both fluorescence and confocal imaging.

Conclusion and potential impact:

Although our results did not support the original hypothesis, this experiment highlights the challenge of translating known gastric effects of GKN1 to colonic cell models. GKN1 is well-documented to maintain gastric epithelial integrity and modulate mucin production, yet its role in intestinal epithelial–bacterial interactions remains poorly defined.

The lack of observed biofilm inhibition suggests several possible explanations, including cell line limitations, protein activity loss, or ineffective localization of GKN1 in this in vitro setup. These findings warrant further investigation, such as testing different cell lines, using in vivo colitis models, or exploring dose and timing variations of GKN1 application.

Despite the inconclusive outcome, this study provides a valuable starting point for exploring GKN1 as a therapeutic or modulatory agent in the context of intestinal infections and inflammation.