

**Probiotic-Derived Factors as Potential Chemopreventive  
Agents for Intestinal Mucositis and Colon Cancer**

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## ABSTRACT

**Background:** Colorectal cancer is one of the most deadly forms of cancer in humans. Current chemotherapeutic agents have limited efficacies for colon cancer treatment, and concurrently increase the risk of developing intestinal mucositis. Mucositis is the term for painful inflammation and ulcerating lesions of the mucous membranes lining the digestive tract. Intestinal mucositis is a common side-effect of cancer chemotherapy. To date, there are no satisfactory clinical treatments available to combat mucositis and colorectal cancer.

Probiotics are live bacteria, some of which have been demonstrated to secrete bioactive factors capable of exerting beneficial effects; especially in the digestive system. The potential for probiotic-derived factors to combat intestinal mucositis and colon cancer has yet to be thoroughly investigated. In particular, the effects of factors derived from the same probiotic strain and cultured in different growth media have not been investigated in either setting.

**Objective:** The project described in this thesis aimed to identify therapeutically effective bioactive formulations (probiotic-derived factors) capable of protecting the digestive tract from the devastating effects of mucositis and their potential for colon cancer management. The capacity for supernatants (SNs) from *Escherichia coli* Nissle 1917 (EcN) and *Faecalibacterium prausnitzii* (Fp) cultured under different growth conditions in the settings of mucositis and colorectal cancer models *in vitro* and *in vivo* were evaluated.

**Materials and Methods:** EcN was grown in Luria-Bertani (LB) broth, tryptone soya broth (TSB), de Man Rogosa Sharpe (MRS) broth, and M17 broth supplemented with 10% (v/v) lactose solution (M17). Fp was grown anaerobically in M2 medium supplemented with 30% rumen fluid. Rat epithelial IEC-6, human colonic adenocarcinoma Caco-2 and T-84 cells were treated with DMEM (control), growth media alone (without factors) or SNs, in the presence and absence of 5-FU. Cell viability (MTT), reactive oxygen species (ROS), cell apoptosis and cell monolayer permeability were determined.

Optimal SNs from the *in vitro* studies were further investigated in female dark agouti rats (101-146g). Animals received a daily oral gavage of EcN or Fp SNs (1mL) respectively from day 0 to 8. At day 5, rats were injected with either saline or 5-Fluorouracil (5-FU; 150 mg/kg). At kill, small intestinal tissues were collected for determination of myeloperoxidase (MPO) activity and histological assessment.

**Results and Conclusions:** This thesis comprises the following 5 chapters:

The potential mechanism of new nutraceuticals including probiotics, prebiotics and synbiotics, which may be suited to the treatment of intestinal mucositis and colon cancer, is extensively reviewed in **Chapter 1**.

**Chapter 2** evaluated the capacity for SNs derived from EcN, cultured under different growth conditions, to prevent 5-Fluorouracil (5-FU)-induced intestinal epithelial cell damage. EcN SNs were able to reduce cell death, late apoptosis and enhance cell barrier function against 5-FU induced damage in IEC-6 cells, which indicated a potential role for EcN SN in the treatment of mucositis.

**Chapter 3** evaluated the effects of EcN SNs grown in different growth media on the viability of Caco-2 colon cancer cells in the presence and absence of 5-FU. EcN derived factors, especially grown in LB medium, were most effective at decreasing the viability of Caco-2 cells. This could indicate a potential role for this EcN SN in chemoprevention for colon cancer.

**Chapter 4** evaluated the effects of SNs derived from Fp on 5-FU treated intestinal cells (IEC-6, Caco-2 and T-84). The capacity of factors derived from Fp and EcN to prevent 5-FU induced damage in a rat model of mucositis was also investigated. Fp SN exerted toxic effects to colon cancer Caco-2 cells. However, in both Caco-2 and T84 cells, Fp SN partially prevented the decrease in cell barrier integrity caused by 5-FU administration. Furthermore, 5-FU-injected rats administered Fp SN or EcN SN partially prevented body weight loss and normalized water intake and faecal output compared with 5-FU controls. These results suggested a growth inhibitory mechanism of Fp SN action on transformed epithelial cells that could be mediated by effects on tight junctions. Factors derived from Fp SN and EcN SN could therefore have a role in reducing the severity of intestinal mucositis.

**Chapter 5** summarises and highlights the key findings from this thesis and indicates potential future directions. In particular, identification, isolation and characterization of the components of SNs require further investigation. The determination of the underlying mechanisms associated with the beneficial effects of specific probiotic derived factors would provide valuable information for the treatment of mucositis and colon cancer.

## DECLARATION

This thesis contains no material that has been accepted for the award of any other degree or diploma in any University or other tertiary institution and to the best of my knowledge and belief contains no material previously published or written by another person except where due reference has been made in the text.

I give consent for this copy of my thesis, when deposited in the University Library, being made available for loan and photocopying, subject to the provisions of the Copyright Act 1968. I also give permission for the digital version of my thesis to be made available online, via the University's digital research repository, the Library catalogue, and also through web search engines, unless permission has been granted by the University to restrict access for a period of time.

Hanru Wang

Signature:

Date: 4. 11. 15

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## **DEDICATION**

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## PUBLICATIONS DERIVED FROM THIS THESIS

1. **Hanru Wang**, Susan EP Bastian and Gordon S Howarth. Newly developed synbiotics and the chemotherapy-damaged gut? *Journal of Evidence-Based Complementary and Alternative Medicine*, 2013, 18(3), 198-208.
2. **Hanru Wang**, Caitlin L Brook, Alexandra L Whittaker, Andrew Lawrence, Roger Yazbeck and Gordon S Howarth. Effects of *Streptococcus thermophilus* TH-4 in a rat model of doxorubicin-induced mucositis. *Scandinavian Journal of Gastroenterology*, 2013, 48, 959-968.
3. Gordon S Howarth and **Hanru Wang**. Role of endogenous microbiota, probiotics and their biological products in human health. *Nutrients*, 2013, 5(1), 58-81.
4. **Hanru Wang**, Mark S Geier and Gordon S Howarth. Prebiotics: a potential treatment strategy for the chemotherapy-damaged gut? *Critical Reviews in Food Science and Nutrition*, 2014, DOI:10.1080/10408398.2012.741082.
5. **Hanru Wang**, Susan EP Bastian, Ker Y Cheah, Andrew Lawrence and Gordon S Howarth. *Escherichia coli* Nissle 1917-derived factors reduce cell death, late apoptosis and increase transepithelial electrical resistance in a model of 5-Fluorouracil-induced intestinal epithelial cell damage. *Cancer Biology and Therapy*, 2014, 15(5), 560-569.
6. **Hanru Wang**, Susan EP Bastian, Andrew Lawrence and Gordon S Howarth. Factors derived from *Escherichia coli* Nissle 1917, grown in different growth media, enhance cell death in a model of 5-Fluorouracil-induced Caco-2 intestinal epithelial cell damage. *Nutrition and Cancer*, 2015, 67(2), 316-326.
7. **Hanru Wang**, Yoga D Jatmiko, Susan EP Bastian, Suzanne Mashtoub and Gordon S Howarth. Effects of supernatants from *E.coli* Nissle 1917 and *Faecalibacterium prausnitzii* on intestinal epithelial cells and a rat model of 5-Fluorouracil-induced mucositis. (Under review)

# CONFERENCE PROCEEDINGS DERIVED FROM THIS THESIS

1. **Hanru Wang**, Susan EP Bastian, Ker Y Cheah, Andrew Lawrence, Suzanne Mashtoub and Gordon S Howarth. *Escherichia coli* Nissle 1917-derived factors reduce cell death, late apoptosis and increase transepithelial electrical resistance in a model of 5-Fluorouracil-induced intestinal epithelial cell damage. Accepted by a) Australian Society for Medical Research (SA Chapter), Adelaide, South Australia and b) Australian Gastroenterology Week, Gold Coast, Queensland, Australia (2014; Poster).
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3. **Hanru Wang**, Susan EP Bastian and Gordon S Howarth. Factors derived from *Faecalibacterium prausnitzii* further promote 5-Fluorouracil-induced cell death but increase transepithelial electrical resistance in transformed colonic epithelial cells. Accepted by a) MASCC/ISOO, annual meeting on supportive care in cancer, Copenhagen, Denmark; b) Gastro 2015 conference, Queensland, Australia and c) Australian Society for Medical Research (SA Chapter), Adelaide, South Australia (2015; Poster).
4. **Hanru Wang**, Susan EP Bastian, Suzanne Mashtoub and Gordon S Howarth. Effects of *Faecalibacterium prausnitzii* and *Escherichia coli* Nissle 1917 derived factors in a rat model of 5-Fluorouracil induced mucositis. Accepted by MASCC/ISOO, annual meeting on supportive care in cancer, Copenhagen, Denmark (2015; Poster).

## **THESIS STRUCTURE**

This thesis is presented as a ‘Thesis by Publication’ and includes a combination of published manuscripts and one paper under review. Each manuscript is formatted according to the journal’s specifications.