Årsrapport

Nutrigenomic study of mechanisms of colon cancer protective effects of a probiotic bacteria – identification of genes accessible to dietary manipulation

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Background

Colorectal cancer is the second most common cause of cancer related mortality in Europe, with environmental factors especially diet, identified as having a crucial role in its etiology in part through the modulation of the gut microflora. Probiotics have extensive experimental support for their potential to reduce risk for colon cancer. However, little data exist to explain the mechanisms for these anticancer effects.

The objectives set out in the application were as follows:

i) To evaluate, using genomic, proteomic and bioassay methodologies, the mechanisms underlying the established *in vivo* anticarcinogenic properties of a model probiotic bacteria, *Bifidobacterium longum*.

ii) To utilize i) as a model system to attempt to identify host genes, relevant to disease prevention, which are accessible to modulation by dietary components. Interest will focus on genes involved in cell cycle control, cell adhesion, angiogenesis, signaling and immunity.

We also pointed out in the application that this project will, for the first time, examine the effect of probiotic bacteria on global gene expression in colonic cells and identify their targets (including pathways of oncogenesis). Because of the unbiased nature of the functional genomics approach, the results will also be of the utmost value to understanding how probiotics protect against other diseases, such as inflammatory bowel disease and allergy. Finally, the results of this project will hopefully identify colonic genes, of relevance for disease development, which are accessible to manipulation by dietary agents. The manipulation of these genes will supply us with powerful novel molecular tools to develop/select even more effective probiotic strains for anticancer and other protective effects in the gut. They can also be exploited, by the 'food industry' for the development of other novel foods (functional foods) aimed at protecting against colon cancer and other gut-related diseases.

Activities during year 1 of project and initial results

Initially, it was planned to use *Bifidobacterium longum* as the model probiotic bacteria for this project largely based on the support from animal models for anticarcinogenic effects of this bacteria. However, just prior to the initiation of the project, a large EU-funded dietary intervention study was completed, which showed for the first time that the probiotic,

Lactobacillus rhamnosus GG exerted colon cancer protective effects in humans (1). For this reason, it was decided to use *Lactobacillus rhamnosus* GG as model probiotic in our studies. Also, in our application (which spanned 3 years), it was stated that we would start by examining the effect of probiotic bacteria on global gene expression in colonic cells *in culture* and subsequently confirm important findings *in vivo* (in germfree & conventional specific pathogen free mice colonized with the probiotic bacteria). However, in view of the funding level and funding period, the decision was taken to initiate the project with the *in vivo* experiments.

Thus, we have initiated the identification of biomarkers involved in the host microbial interplay, locally in whole colon and systemically in the liver and spleen by a global genome expression analyses approach on germfree (GF) and conventional specific pathogen free (SPF) mice, which are unchallenged or challenged with the probiotic, *Lactobacillus rhamnosus* GG (LGG).

Experimental setup*:* The organs described above were collected from groups (4 animals/group) of GF and SPF raised adult NMRI mice and GF mice colonized for 3 weeks with the probiotic strain *Lactobacillus rhamnosus* GG (LGG).

Whole genome expression analyses*:* Each of 21,000 mouse genes are represented by one 60-mer oligonucleotide (Compugen) probe spotted on polylycin coated slides. Three ?g total RNA of each sample was amplified and indirectly labelled with a fluorophor (Cy3 or Cy5) as well as a control RNA (opposite dye) and hybridized to a slide in MAUI hybridization chamber. The slides were scanned with Axon GenePix 4000B scanner and scrutinized with GenePix version 6.1.

Bioinformatics*:* Lowess normalization of each microarray slide was performed using the R package. Global mean normalization between slides was then applied. To compare different samples or groups, significance analyses of microarray (SAM) analyses were performed, a statistical analyses method based on a modified t-test.

Results: Results to date of the global gene expression analyses are presented in Table 1.

Table 1. Number of differentially expressed genes (SAM) in whole colon, liver & spleen comparing conditions.

Comparison	Colon	Liver	Spleen
GF/SPF (high; low SPF vs GF)	**162** (46;116)	**429** (229;200)	**380** (364;16)
GF/LGG (high; low LGG vs GF)	**367** (0;367)	**9** (1;8)	**50** (47;3)
LGG/SPF (high; low SPF vs LGG)	**520** (520;0)	**9** (8;1)	**835** (569;266)

It is clear from Table 1 that the presence of a gut flora plays a significant role in the regulation of gene expression in the host, with 162 genes differentially expressed in the colon, 429 in the liver and 380 in the spleen, when one compares GF and SPF conditions. Of considerable interest for the goals of the present project was the observation that the probiotic, *Lactobacillus rhamnosus* GG (LGG) influenced expression of 367 genes in the colon, 9 in the

liver and 50 in the spleen. Interestingly, when grouping them according to biological function, genes involved in fat metabolism and immunity were overrepresented.

Activities planned for year 2

Verification of genes/markers found in the microarray by real time PCR followed by immuno-histochemistry and in situ hybridizations, in mice. Human orthologues will be identified. The identified human orthologues will be matched against known data bases of cancer and other major health problems like allergy, type II diabetes, obesity, IBD, rheumatoid arthritis and psoriasis. We will also begin to use the markers found when comparing GF, LGG colonized and SPF mice to monitor/screen the effects of new probiotic strains in the mouse models.

References

1. **Rafter J**, Bennett M, Caderni G, Clune Y, Dunne C, Hughes R, Karlsson PC, KlinderA, Meaney K, O'Riordan M,. O' Sullivan GC, Pool-Zobel B, Rechkemmer G, Roller M, Rowland I, Salvadori M, Van Loo J, Watzl B, Collins JK.
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Gastroenterology, submitted.