Methyl donor nutrient depletion modifies multiple immune associated gene networks in C-4 II cervical cancer cells: A bioinformatics analysis. By M. Ismail, N. Poomipark, A. Panagoulia, H. J. Powers and P. S. Grabowski, Department of Oncology & Metabolism, The Medical School, The University of Sheffield, Beech Hill Rd, S10 2RX Sheffield, United Kingdom

Cervical cancer arises when high-risk strains of human papilloma virus (HR-HPV) become integrated into the host genome in cervical epithelial cells and loss of episomal expression of the viral transcriptional regulator gene E2 occurs\(^1\). Not all women who acquire HPV infection develop cervical cancer\(^2\); about 60% of HPV infections are transient and resolve on their own due to innate and adaptive immunity\(^3\). Among other factors known to influence risk of cervical cancer are high parity and use of oral contraceptives, while dietary factors influencing the methyl donor cycle may also be important in determining cervical cancer risk\(^4\). Dietary methyl donor nutrients are required for maintenance of DNA methylation, modification of which is associated with cancer progression through dysregulation of gene expression\(^5\). The aim of the present study was to identify immune-system associated gene networks and pathways that are affected both by methyl donor nutrient depletion and by chromosomal integration of HPV in cervical cells, using a bioinformatics approach.

A gene expression microarray analysis was performed in C4-II cervical cancer cells that were grown for 8 days either in Weymouth’s medium or in methionine and folate depleted Weymouth’s medium. Differentially expressed genes (DEGs) were identified and compared with those from the GSE4289 dataset in the Gene Expression Omnibus\(^6\), which identifies host gene expression changes associated with episome loss in W12 cervical keratinocyte cells undergoing selection for integrated HPV16\(^1\). Genes were considered as differentially expressed when P<0.05, based on a false detection rate P<0.05. DEGs common to both datasets and with a fold change of ≥1.5 were analysed for gene ontology enrichment using DAVID Bioinformatics Resources 6.8 and ClueGo and interpreted using the MCODE plugin in Cytoscape. Immune-associated gene clusters susceptible to modulation both by methyl donor depletion and by HPV integration were thus identified.

A total of 1229 DEGs were common to both datasets. Significantly, seven clusters of genes involved in cellular immune and antimicrobial/antiviral defence responses were also identified.

<table>
<thead>
<tr>
<th>Enriched Gene Ontology Clusters</th>
<th>Enrichment Score</th>
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<tbody>
<tr>
<td>Response to microorganisms</td>
<td>6.55</td>
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<tr>
<td>Immune system development</td>
<td>3.45</td>
</tr>
<tr>
<td>Response to biotic stimulus</td>
<td>3.32</td>
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<tr>
<td>Type 1 interferon signalling pathway</td>
<td>2.29</td>
</tr>
<tr>
<td>Regulation of interferon production</td>
<td>1.55</td>
</tr>
<tr>
<td>Interleukin-6-mediated signalling pathway</td>
<td>1.48</td>
</tr>
<tr>
<td>Cellular response to lipopolysaccharide/bacteria</td>
<td>1.47</td>
</tr>
</tbody>
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The bioinformatic analysis highlights the potential importance of methyl donor nutrients not only in the regulation of cancer associated gene networks, but also in regulating host immune defence mechanisms that might influence the development and progression of cervical cancer through modulation of infectivity and persistence of HPV.

Evaluation of “Ogi” – a fermented cereal gruel made in Nigeria, as a probiotic food for the treatment, prevention and management of diarrhoea Adetokunbo Osho RNAUrt MSc (PhD Student); Prof G.A Bonwick & Dr C. Birch (Supervisors)

Introduction: Food fermentation is generally carried out by microorganisms which are classed as lactic acid bacteria (LAB), yeast and moulds. It is proposed that some of these organisms may have potential of being probiotic, if they are live and viable at the point the product is being ingested. Consequentially, these fermented foods and beverages are sometimes described as functional foods since they are suggested to have the potential to provide health benefits other than nutrients. This claim has been investigated in various studies but results have been inconsistent. Where significant beneficial effects have been recorded, authors have been unable to provide information about what component of the fermented food matrix may have provided the effects - the activities of the fermentation organisms, metabolites or exopolysaccharides formed during fermentation. Understanding activities in the GIT when fermented foods are ingested may provide insights to which food matrix may give the therapeutic or disease prevention effect, if any.

Aim of study: To investigate the effect of orally ingested ‘Ogi’, a fermented maize product on the digestive tract using a simulated in-vitro human digestive system. To investigate the potentials of ‘ogi’ in the management of diarrhoea caused by selected bacterial pathogens.

Research questions: 1) Do the organisms involved in the production of ‘Ogi’ have probiotic potential? 2) Will ingestion of ‘Ogi’ cause a modulation in the gut microbiota, by increasing the population of Lactobacillus and Bifidobacteria species? 3) Will the fermented maize product cause an elimination of some diarrhoea causing organisms from the colon?

Method of data collection: 1) Dominant fermentation organisms will be isolated from spontaneously fermented maize and will be inoculated in sterile maize for controlled fermentation. Organisms will be identified using both culture dependent and culture independent mechanisms. Fermented product will analysed for nutrient and metabolite (such as SCFAs and peptide compounds) composition using HPLC and GC. 2) In-vitro digestion of the fermented product will be carried out in an artificial system with components of the upper GIT, using standard methods approved by INFOGEST, in order to investigate the survival of the fermentation organisms in the harsh digestion conditions. 3) Output of upper GIT digestion will be inoculated in an artificial 4 chambered colon representing the ascending colon, transverse colon, descending colon and rectum investigate the modulation the microbiota in the colon, increased production of SCFAs, lowered pH, production of antibacterial peptides in the colon and the effect on some diarrhoea causing pathogens. 4) All experiments will be done in triplicate and statistical analyses will be carried out in SPSS (version 22) using ANOVA.

Expected outcome: Organisms involved in the fermentation of maize will survive the harsh environments in the stomach and the small intestine; Intake of ‘ogi’ will cause a beneficial shift of the gut microbiota; Intake of ‘ogi’ will cause an elimination from the large intestine, of some pathogenic organisms by increasing population of Lactobacillus species, lowering colon pH with increased SCFAs, increased production of bacteriocin or improved immune response

References