Copyright is owned by the Author of the thesis. Permission is given for a copy to be downloaded by an individual for the purpose of research and private study only. The thesis may not be reproduced elsewhere without the permission of the Author.
The development of an assay for evaluating the expression of human interleukin-10 promoter region gene linked to inflammatory bowel disease and its application in turmeric assessment

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in

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New Zealand.

Xuejing Men

2013
Abstract

Inflammatory bowel disease (IBD) appears in two forms, Crohn's disease (CD) and ulcerative colitis (UC), which are debilitating diseases with less than satisfactory treatments. Despite years of study, the aetiology of this chronic inflammation remains unclear. Evidence from epidemiological and clinical studies supports that it is a complex interaction among environmental, genetic and immune-regulatory factors. Therefore, gene-nutrition based approaches are suggested to be an appropriate candidate in the future prevention and treatment of IBD.

Different geographic and racial prevalence of IBD are observed in many epidemiological studies, with highest rates found in developed countries and in Caucasian populations. However, the prevalence has increased dramatically in traditional low-incidence areas during the last two decades, and the racial gap is also closing, indicating that both environmental factors such as diet and genetic predispositions contribute to the IBD susceptibility.

The imbalance between pro- and anti-inflammatory cytokines is known to be the key contributor of IBD pathogenesis. Interleukin-10 (IL-10), an anti-inflammatory cytokine, is expressed in many different cells of the adaptive and innate immune system including T regulatory cells, activated macrophages, B regulatory lymphocytes and many other cell types. It plays important part in the regulation of immune response, as was demonstrated in spontaneous colitis in IL-10 deficient mice models, therefore IL-10 is crucial in the IBD pathogenesis.

Three single nucleotide polymorphisms (SNPs) in the promoter region of IL-10 gene, -1082 G/A, -819 C/T and -592 C/A, have been identified to related to IL-10 production and IBD susceptibility, with -1082 G/A as the most relevant SNP. In this research study, I developed a
cell-based luciferase reporter assay in which the reporter expression is investigated under the control of promoter containing the variants of interest.

Turmeric has a long historical use in Asian medicine for treatment of various diseases. It was shown to exert strong anti-inflammatory effect through multiple molecular targets and mechanisms of action. In the second part of my research study, I tested turmeric samples for its ability to alter IL-10 production in the risk polymorphic variant, using the developed assay. The results suggest that curcumin, the bioactive component of turmeric, has the ability to increase IL-10 transcription in the low-producer (ACC) haplotype.

The in vitro model of IL-10 promoter assay established in this study is a novel and valuable tool in assessing IL-10 production at transcriptional level. Furthermore, it provides the possibility of high-throughput screening of food to overcome the functional change of SNPs that are important in human IBD.
Acknowledgements

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Thanks to AgResearch and Nutrigenomics New Zealand for their supply of the food samples and experimental equipment that made the assay development and testing possible.

I would like to extend my appreciation and respect to my chief supervisor, Dr Mark McCann, for his insights, knowledge, patience and understanding. Thank you for helping me and encouraging me to my first step into science. I would also like to thank Kelly Armstrong for going beyond the call of duty to help me, not only as a colleague, but also as a friend.

Many thanks go to all the staff at AgResearch for their assistance in many different ways. It has truly been a wonderful experience.

Last but not least, I would like to thank my partner, my best friend, Jiatao Yu, for the love and support throughout. It is such a blessing to have you in my life. I am extremely grateful for my family, who have always been my inspiration and strength.
Table of content

Abstract .................................................................................................................................................. ii
Acknowledgement ................................................................................................................................. iv
Abbreviations ......................................................................................................................................... viii
List of Tables ........................................................................................................................................... x
List of Figures .......................................................................................................................................... xi
List of Appendices .................................................................................................................................... xiii

1 Introduction .......................................................................................................................................... 1
  1.1 Background: .................................................................................................................................... 1
  1.2 Significance of the study ...................................................................................................................... 3
  1.3 Aim and objectives: ............................................................................................................................. 4
      1.3.1 Aim: ........................................................................................................................................ 4
      1.3.2 Objectives: ................................................................................................................................. 5
  1.4 Overview of the study: ......................................................................................................................... 5

2 Literature Review ................................................................................................................................. 7
  2.1 What is inflammatory bowel disease? ................................................................................................ 7
  2.2 Inflammatory bowel disease epidemiology ......................................................................................... 8
  2.3 Nature and nurture: modifying inflammatory bowel disease risk ............................................... 13
      2.3.1 The role of genetic predisposition ....................................................................................... 13
      2.3.2 The role of environmental factors ....................................................................................... 17
  2.4 Intestinal homeostasis and immunobiology: mediating the inflammatory bowel disease process ................................................................................................................................. 21
      2.4.1 The role of intestinal barrier ................................................................................................. 22
      2.4.2 Intestinal microbial agents and host immune regulations ..................................................... 25
      2.4.3 The role of IL-10 ................................................................................................................... 30
  2.5 Current treatment of IBD .................................................................................................................. 34
2.6 The role of personalised nutrition in IBD .........................................................36
2.7 Turmeric/Curcumin and IBD ...........................................................................37
  2.7.1 Characteristics of turmeric/curcumin .........................................................38
  2.7.2 Biological activities of turmeric/curcumin .................................................38
3 Materials and Methods ......................................................................................42
  3.1 Cell culture of 293-hTLR4A-MD2-CD14 cells ...............................................42
    3.1.1 Recovery of cells from cryostorage .........................................................43
    3.1.2 Cell maintenance and subculture ............................................................44
    3.1.3 Cryostorage of cells .............................................................................45
    3.1.4 Cell counting .........................................................................................46
  3.2 Establishment of IL-10 promoter assay .........................................................48
    3.2.1 The Metridia luciferase reporter system in the assay .........................48
    3.2.2 pMetLuc2-control and pSEAP2-control vector transfection optimisation ..51
    3.2.3 pMetLuc2-control and pSEAP2-control vector co-transfection optimisation ..........................................................55
    3.2.4 IL-10 promoter assay ...........................................................................57
  3.3 Establishment of positive control for IL-10 transcription .............................59
  3.4 Testing of turmeric samples using the IL-10 promoter assay .....................61
  3.5 Data handling ..............................................................................................64
4 Results .................................................................................................................65
  4.1 pMetLuc2-control and pSEAP-control vector transfection optimisation .......65
  4.2 pMetLuc2-control and pSEAP-control vector co-transfection optimisation ....71
  4.3 Establishment of positive control for IL-10 transcription ............................72
  4.4 Turmeric sample tests using the IL-10 promoter assay ................................74
5 Discussion and conclusion .................................................................................79
  5.1 Introduction ..................................................................................................79
5.2 The establishment of IL-10 promoter assay..........................................................79
  5.2.1 The use of 293-hTLR4A-MD2-CD14 cell line ..............................................79
  5.2.2 The use of Metridia luciferase reporter system in the assay.....................80
  5.2.3 The optimisation of experimental conditions ..............................................81
5.3 The test of turmeric Ssamples..........................................................84
5.4 Limitations of the study and future thoughts .............................................86
  5.4.1 Transient transfection to stable transfection ..............................................86
  5.4.2 IL-10 promoter gene variants to test...........................................................87
  5.4.3 Future application of IL-10 promoter assay..............................................88
  5.4.4 Theoretical considerations .........................................................................89
5.5 Conclusions..........................................................90
6 References........................................................................................................93
Appendices........................................................................................................104
## Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD</td>
<td>Crohn’s Disease</td>
</tr>
<tr>
<td>CD14</td>
<td>Cluster of Differentiation 14</td>
</tr>
<tr>
<td>CDAI</td>
<td>Crohn's Disease Activity Index</td>
</tr>
<tr>
<td>DMEM</td>
<td>Dulbecco’s Modified Eagle Medium</td>
</tr>
<tr>
<td>DMSO</td>
<td>Dimethylsulfoxide</td>
</tr>
<tr>
<td>ER</td>
<td>Endoplasmic Reticulum</td>
</tr>
<tr>
<td>FBS</td>
<td>Foetal Bovine Serum</td>
</tr>
<tr>
<td>GWAS</td>
<td>Genome-wide Association Studies</td>
</tr>
<tr>
<td>HEK293</td>
<td>Human Embryonic Kidney cell line 293</td>
</tr>
<tr>
<td>JAK1</td>
<td>Janus Kinases 1</td>
</tr>
<tr>
<td>LPS</td>
<td>Lipopolysaccharide</td>
</tr>
<tr>
<td>IBD</td>
<td>Inflammatory Bowel Disease</td>
</tr>
<tr>
<td>IEC</td>
<td>Intestinal Epithelial Cell</td>
</tr>
<tr>
<td>IFN-γ</td>
<td>Interferon-γ</td>
</tr>
<tr>
<td>MD-2</td>
<td>Myeloid Differentiation factor 2</td>
</tr>
<tr>
<td>MAPK</td>
<td>Mitogen Activated Protein Kinase</td>
</tr>
<tr>
<td>MetLuc</td>
<td>Metridia Luciferase</td>
</tr>
<tr>
<td>NFκB</td>
<td>Nuclear Factor kappa B</td>
</tr>
<tr>
<td>NOD</td>
<td>Nucleotide-binding Oligomerisation Domain</td>
</tr>
<tr>
<td>PRR</td>
<td>Pattern Recognition Receptors</td>
</tr>
<tr>
<td>ROS</td>
<td>Reactive Oxygen Species</td>
</tr>
<tr>
<td>SEAP</td>
<td>Secreted Alkaline Phosphatase</td>
</tr>
<tr>
<td>SNP</td>
<td>Single Nucleotide Polymorphism</td>
</tr>
<tr>
<td>Acronym</td>
<td>Full Form</td>
</tr>
<tr>
<td>---------</td>
<td>-----------</td>
</tr>
<tr>
<td>STAT</td>
<td>Signal Transducer and Activator of Transcription</td>
</tr>
<tr>
<td>Th2</td>
<td>Type 2 T-helper</td>
</tr>
<tr>
<td>TLR</td>
<td>Toll-like Receptor</td>
</tr>
<tr>
<td>Tr1</td>
<td>Type 1 T-regulatory</td>
</tr>
<tr>
<td>Tyk</td>
<td>Tyrosine Kinases</td>
</tr>
<tr>
<td>TNF-α</td>
<td>Tumour Necrosis Factor-α</td>
</tr>
<tr>
<td>TGF</td>
<td>Transforming Growth Factor</td>
</tr>
<tr>
<td>UC</td>
<td>Ulcerative Colitis</td>
</tr>
<tr>
<td>WST-1</td>
<td>Water Soluble Tetrazolium-1</td>
</tr>
</tbody>
</table>
## List of Tables

Table 1. IL-10 haplotypes of the three promoter SNPs of interest in IBD pathogenesis. .....3

Table 2. IL-10 haplotypes of the three promoter SNPs of interest in IBD pathogenesis and the pMetLuc2 with IL-10-variants .................................................................58

Table 3. Example of 96-well plate layout for IL-10 promoter assay in testing food samples .................................................................................................................63
List of Figures

Figure 2.1 CD prevalence worldwide ................................................................. 9
Figure 2.2 UC prevalence worldwide ............................................................... 10
Figure 2.3 Inflammatory bowel disease susceptibility loci .............................. 16
Figure 2.4 The epithelial barrier system ............................................................ 23
Figure 2.5 Simplified version of the IL-10 signalling ....................................... 31
Figure 2.6 Obtaining curcumin from turmeric .................................................. 38
Figure 2.7 Molecular targets of curcumin ......................................................... 40
Figure 3.1 HEK293 cell morphology at high and low density ........................ 43
Figure 3.2 A Standard haemocytometer with Neubauer ruling ....................... 47
Figure 3.3 Flowchart of the Ready-To-Glow™ secreted luciferase reporter assay procedure ................................................................. 49
Figure 3.4 Example of a 24-well optimisation plate ......................................... 54
Figure 3.5 Flowchart of IL-10 promoter assay in testing food components ........ 62
Figure 4.1 Luciferase activity at different amounts of plasmid DNA and lipid:DNA ratios in pMetLuc2-control vector 24 hours after transfection ......................... 67
Figure 4.2 Luciferase activity at different amounts of plasmid DNA and lipid:DNA ratios in pMetLuc2-control vector 48 hours after transfection ......................... 68
Figure 4.3 SEAP activity at different amounts of plasmid DNA and lipid:DNA ratios in pMetLuc2-control vector 24 hours after transfection ......................... 69
Figure 4.4 SEAP activity at different amounts of plasmid DNA and lipid:DNA ratios in pMetLuc2-control vector 48 hours after transfection .................................................. 70

Figure 4.5 Luciferase and SEAP activity in pMetLuc-control and pSEAP-control vectors 24 and 48 hours after co-transfection .................................................................................. 72

Figure 4.6 The effect of different concentrations of dexamethasone on 293TLR4 cell metabolic activity .................................................................................................................. 73

Figure 4.7 The effect of 1 µM dexamethasone on IL-10 promoter activity ......................... 74

Figure 4.8 Relative IL-10 transcription rate in M-ACC variant after turmeric treatment ... 76

Figure 4.9 The curcumin content of turmeric samples .................................................... 77

Figure 4.10 The effect of turmeric samples on the metabolic activity of 293TLR4 cells after 24 hours .................................................................................................................. 78
List of Appendices

Appendix 1 IL-10 promoter sequence

Appendix 2 pMetLuc2-control vector and pMetLuc2-reporter vector information

Appendix 3 IL-10 pathway

Appendix 4 The effect of 1 µg/ml LPS on IL-10 promoter variant transcription over 24 hours

Appendix 5 Preparation of ethanol extracts and reversed-phase fractions from food samples

Appendix 6 Turmeric sample preparation

Appendix 7 Structure of natural curcuminoids

Appendix 8 Absorption and metabolism of curcumin

Appendix 9 Research Output