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**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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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.

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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 <i>Metridia</i> 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 <i>Metridia</i> 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

CD	=	Crohn's Disease
CD14	=	Cluster of Differentiation 14
CDAI	=	Crohn's Disease Activity Index
DMEM	=	Dulbecco's Modified Eagle Medium
DMSO	=	Dimethylsulfoxide
ER	=	Endoplasmic Reticulum
FBS	=	Foetal Bovine Serum
GWAS	=	Genome-wide Association Studies
HEK293	=	Human Embryonic Kidney cell line 293
JAK1	=	Janus Kinases 1
LPS	=	Lipopolysaccharide
IBD	=	Inflammatory Bowel Disease
IEC	=	Intestinal Epithelial Cell
IFN- γ	=	Interferon- γ
MD-2	=	Myeloid Differentiation factor 2
MAPK	=	Mitogen Activated Protein Kinase
MetLuc	=	<i>Metridia</i> Luciferase
NF κ B	=	Nuclear Factor kappa B
NOD	=	Nucleotide-binding Oligomerisation Domain
PRR	=	Pattern Recognition Receptors
ROS	=	Reactive Oxygen Species
SEAP	=	Secreted Alkaline Phosphatase
SNP	=	Single Nucleotide Polymorphism

STAT	=	Signal Transducer and Activator of Transcription
Th2	=	Type 2 T-helper
TLR	=	Toll-like Receptor
Tr1	=	Type 1 T-regulatory
Tyk	=	Tyrosine Kinases
TNF- α	=	Tumour Necrosis Factor- α
TGF	=	Transforming Growth Factor
UC	=	Ulcerative Colitis
WST-1	=	Water Soluble Tetrazolium-1

List of Tables

Table 1. IL-10 haplotypes of the three promoter SNPs of interest in IBD pathogenesis.	3
Table 2. IL-10haplotypes 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 24hours after transfection	67
Figure 4.2 Luciferase activity at different amounts of plasmid DNA and lipid:DNA ratios in pMetLuc2-control vector 48hours after transfection	68
Figure 4.3 SEAP activity at different amounts of plasmid DNA and lipid:DNA ratios in pMetLuc2-control vector 24hours after transfection	69

Figure 4.4 SEAP activity at different amounts of plasmid DNA and lipid:DNA ratios in pMetLuc2-control vector 48hours 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 μ M 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 1IL-10 promoter sequence

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

Appendix 3IL-10 pathway

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

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

Appendix 6Turmeric sample preparation

Appendix 7Structure of natural curcuminoids

Appendix 8Absorption and metabolism of curcumin

Appendix 9Research Output