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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Xuejing Men

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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, thereforeIL-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, Ideveloped 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 effectthrough 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 studyis 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

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	=	*Metridia* Luciferase
NFκB	=	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

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