UNIVERSITI PUTRA MALAYSIA

CYTOKINE PRODUCTION BY A HUMAN ENDOTHELIAL CELLLINE IN RESPONSE TO CANDIDA ALBICANS

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CYTOKINE PRODUCTION BY A HUMAN ENDOTHELIAL CELL LINE IN RESPONSE TO *CANDIDA ALBICANS*

By

LIM PEI CHING

Thesis submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Master of Science

October 2005

DEDICATION

To my parents, who put up with me, and

Jin Hoong, who encourage and accompany me always.

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Master of Science

CYTOKINE PRODUCTION BY A HUMAN ENDOTHELIAL CELL LINE IN RESPONSE TO *CANDIDA ALBICANS*

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Chairman: Professor Seow Heng Fong, PhD

Faculty: Medicine and Health Sciences

Candida albicans is the most common aetiological agent that causes haematogenously disseminated candidiasis. Under conditions that compromise the host immune system, *C. albicans* disseminates from mucosal sites and results in a progressive disease associated with high rates of mortality. Cytokines are important immunomodulators in coordinating the host defense against *C. albicans* infection. Human endothelial cells are known to produce various types of cytokines in response to pathogen invasion. The present study was undertaken to identify the cytokines that are involved in the host defense against *C. albicans*, as well as, to determine the importance of direct cell-to-cell contact in triggering expression of cytokines. In addition, the involvement of Toll-like receptor (TLR)2, TLR4 and nuclear factor-κB (NF-κB) in the host defense against *C. albicans* were also examined. Expression of cytokines by endothelial cells in response to *C. albicans* was investigated by using an *in vitro* model of human umbilical vein endothelial cell line (HUVEC) co-cultured with *Candida* spp. Both conventional and real time PCR showed that among the cytokines studied, only granulocyte-macrophage colony-stimulating factor (GM-CSF) was found to be differentially expressed in

HUVEC upon stimulation with *C. albicans*. Elevated levels of GM-CSF were found in the co-culture of HUVEC with *C. albicans* but not in the other non-albicans *Candida* spp. Three additional *C. albicans* strains co-cultured with HUVEC also showed a similar pattern of increased GM-CSF expression, although at different levels from strain to strain. This provided evidence that the induction of GM-CSF was not confined to only a particular clinical strain of *C. albicans*. On the other hand, *C. dubliniensis*, which possessed a similar phenotype as *C. albicans* failed to stimulate a similar pattern of GM-CSF expression in HUVEC. The induction of GM-CSF was then found to be contact-dependent via the use of cell culture insert to physically separate *C. albicans* from adhering to the HUVEC monolayer. Pretreatment with anti-TLR2 and anti-TLR4 antibodies showed that TLR4 but not TLR2 was involved in the induction of GM-CSF expression by HUVEC. In addition, pretreatment with SN50 inhibitor also demonstrated that NF-κB may be involved in stimulating expression of GM-CSF transcript. In conclusion, we have discovered that HUVEC is involved in the innate immune response to *C. albicans* by producing GM-CSF cytokine through the activation of TLR4 and also NF-κB transcription factor in a contact-dependent manner.

PERPUSTAKAAN SULTAN ABDUL SAMAD
UNIVERSITI PUTRA MALAYSIA

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

PENGZAHIRAN SITOKIN DI DALAM JUJUKAN SEL MANUSIA ENDOTELIUM YANG DIARUH OLEH *CANDIDA ALBICANS*

Oleh

LIM PEI CHING

Oktober 2005

Pengerusi: Profesor Seow Heng Fong, PhD

Fakulti: Perubatan dan Sains Kesihatan

Candida albicans adalah punca penyakit kandidiasis yang menyebar melalui darah. Apabila sistem pertahanan badan menjadi lemah, *C. albicans* boleh menyebar melalui mukosa ke dalam organ dalaman dan menyebabkan penyakit kandidiasis menjadi semakin serius dan membawa kepada kadar kematian yang tinggi. Sitokin penting dalam mengkoordinasikan sistem pertahanan untuk melawan jangkitan *C. albicans.* Jujukan sel manusia endotelium diketahui boleh menghasilkan pelbagai sitokin untuk melawan serangan patogen. Tujuan penyelidikan ini adalah untuk mengenalpasti sitokin yang terlibat dalam sistem pertahanan yang melawan serangan *C. albicans* dan untuk mengkaji kepentingan sentuhan dalam jujukan sel manusia endotelium (HUVEC) dan *C. albicans* dalam pengzahiran sitokin. Tambahan pula, penglibatan 'Toll-like receptor' (TLR)2 dan TLR4 serta Factor nuklear-κB (NF-κB) dalam sistem pertahanan terhadap *C. albicans* juga dikaji dalam penyelidikan ini. Pengzahiran sitokin dalam HUVEC yang diaruh oleh *C. albicans* dilakukan melalui penggunaan sebuah model kultur luaran antara HUVEC dengan *C. albicans*. Kedua-dua teknik tradisional dan "real time" RT-PCR telah menunjukkan di antara sitokin-sitokin yang disiasat, hanya granulocyte-

macrophage colony-stimulating factor (GM-CSF) sahaja yang diaruhkan secara berbeza oleh *C. albicans*. Peningkatan pengzahiran GM-CSF hanya berlaku di dalam kultur model HUVEC dengan *C. albicans* dan bukannya dengan spesies *Candida* yang lain. Tiga tambahan *C. albicans* yang lain juga dikulturkan dengan HUVEC dan menunjukkan cara pengzahiran GM-CSF yang sama dan mengesahkan pengzahiran GM-CSF adalah bukan disebabkan oleh *C. albicans* yang tertentu sahaja. Di samping itu, *C. dubliniensis* yang mempunyai sifat yang sama dengan *C. albicans* telah gagal mengaruh pengzahiran GM-CSF di dalam HUVEC. Pengaruhan GM-CSF didapati bergantung kepada sentuhan antara HUVEC dengan *C. albicans* apabila sebuah pengisi kultur sel digunakan untuk memisah secara fizikal *C. albicans* daripada melekat pada HUVEC. Penggunaan anti-TLR2 antibodi dan anti-TLR4 antibodi menunjukkan bahawa TLR4 dan bukan TLR2 yang terlibat dalam pengzahiran GM-CSF yang diaruhkan oleh *C. albicans*. Sementara itu, SN50 menunjukkan pengzahiran GM-CSF juga bergantung kepada faktor transkripsi NF-κB yang bertanggungjawab dalam memulakan transkripsi GM-CSF sitokin. Kesimpulannya, HUVEC adalah terlibat dalam sistem pertahanan badan yang tidak terancang apabila dirangsangkan oleh *C. albicans* dengan pengzahiran GM-CSF melalui pengaktifan TLR4 serta factor transkripsi NF-κB yang bergantung kepada kewujudannya sentuhan antara HUVEC dengan *C. albicans*.

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TABLE OF CONTENTS

	Page
DEDICATION	ii
ABSTRACT	iii
ABSTRAK	v
ACKNOWLEDGEMENTS	vii
APPROVAL	ix
DECLARATION	xi
LIST OF TABLES	xvi
LIST OF FIGURES	xvii
LIST OF ABBREVIATIONS	xxiii

CHAPTER

1	INTRODUCTION	1
2	LITERATURE REVIEW	4
	2.1 *Candida spp.*	4
	2.1.1 *Candida albicans*	5
	2.1.2 *Candida dubliniensis*	6
	2.1.3 *Candida glabrata*	6
	2.1.4 *Candida tropicalis*	7
	2.1.5 *Candida parapsilosis*	7
	2.1.6 *Candida rugosa*	8
	2.2 Pathogenicity	8
	2.2.1 Adherence	8
	2.2.2 Dimorphism	9
	2.2.3 Hydrolytic enzymes	11
	2.2.3.1 Secreted aspartyl proteinase (SAP)	11
	2.2.3.2 Phospholipases (PL)	12
	2.3 Candidiasis	13
	2.3.1 Mucocutaneous candidiasis	13
	2.3.1.1 Oral thrush	13
	2.3.1.2 Vaginal thrush (Vulvovaginal candidiasis)	14
	2.3.2 Systemic candidiasis	15
	2.3.2.1 Haematogenously disseminated candidiasis	15
	2.3.2.2 Epidemiology of candidemia	15
	2.3.2.3 Mechanism of invasion through endothelial Cells	18
	2.3.3 Neonatal candidiasis	20
	2.4 Innate immune response towards candidiasis	21
	2.4.1 Toll-like receptors (TLRs)	21
	2.4.2 Nuclear factor-κB (NF-κB)	25
	2.4.3 Cytokines	26
	2.4.4 Prostaglandins E_2 (PGE_2)	29

3 MATERIALS AND METHODS 30
3.1 Study design 30
3.2 Cell culture 33
3.2.1 Human umbilical vein endothelial cell (HUVEC) line 33
3.2.2 *Candida* spp., media and growth conditions 34
3.3 Randomly Amplified Polymorphism DNA (RAPDs) 35
3.3.1 *Candida* spp., media and growth conditions 35
3.3.2 DNA isolation 35
3.3.3 RAPD-PCR 36
3.3.4 Analysis of RAPD profile and statistical test 37
3.4 Infection assays 38
3.4.1 Growing of *Candida* spp. 38
3.4.2 Infection of HUVEC with *Candida* spp. 38
3.4.3 Infection assay with cell culture insert (adherence blockage assay) 40
3.4.4 Infection assay with inhibitors (inhibition assay) 41
3.5 RNA analysis 41
3.5.1 RNA isolation using Tri reagent 41
3.5.2 Reverse transcription 43
3.5.3 Amplification of housekeeping gene and cytokine genes 44
3.5.3.1 PCR reagents 44
3.5.3.2 Conventional PCR 45
3.5.3.3 Real time PCR 45
3.5.3.4 Comparative C_T method 46
3.6 Protein analysis 47
3.6.1 Enzyme-Linked Immunosorbent Assay (ELISA) 47
3.6.1.1 GM-CSF ELISA 47
3.6.1.2 PGE_2 ELISA 48
3.7 Statistical analysis 49

4 RESULTS 50
4.1 HUVEC co-cultured with *C. albicans* and non-albicans *Candida* spp. 50
4.1.1 Extracted RNA 50
4.1.2 Amplification of cytokines by conventional two step RT-PCR 52
4.1.3 Quantification of GM-CSF protein by ELISA 59
4.1.3 Quantification of GM-CSF mRNA transcript by real time PCR 61
4.1.5 Quantification of PGE_2 protein by ELISA 66
4.2 HUVEC co-cultured with four strains of *C. albicans* 68
4.2.1 Genotyping of *C. albicans* by RAPD-PCR 68
4.2.2 Extracted RNA 71
4.2.3 Amplification of GM-CSF by conventional PCR 73
4.2.4 Quantification of GM-CSF protein by ELISA 76
4.2.5 Quantification of GM-CSF mRNA transcript by real time PCR 78

4.2.6 Quantification of PGE_2 protein by ELISA 83
4.3 HUVEC co-cultured with *C. albicans* and *C. dubliniensis* 85
4.3.1 Extracted RNA 85
4.3.2 Amplification of GM-CSF cytokine by conventional two step RT-PCR 87
4.3.3 Quantification of GM-CSF protein by ELISA 90
4.3.4 Quantification of GM-CSF mRNA transcript by real time PCR 92
4.3.5 Quantification of PGE_2 protein by ELISA 97
4.4 HUVEC co-cultured with *C. albicans* in cell culture insert (adherence blocking assay) 99
4.4.1 Extracted RNA 99
4.4.2 Amplification of GM-CSF by conventional two step RT-PCR 101
4.4.3 Quantification of GM-CSF mRNA transcript by real time PCR 104
4.5 Inhibition assay 109
4.5.1 Extracted RNA 109
4.5.2 Quantification of GM-CSF transcript by real time PCR 110

5 DISCUSSION 116
5.1 Co-culture model of *Candida* spp. with HUVEC 116
5.2 Release of cytokines in response to *C. albicans* and non-albicans *Candida* spp. 118
5.3 Differential expression of GM-CSF induced by *C. albicans* and non-albicans *Candida* spp. 119
5.4 Expression of GM-CSF induced by genotypically different *C. albicans* strains 122
5.5 Comparison of GM-CSF expression induced by *C. albicans* versus *C. dubliniensis* 123
5.6 Production of PGE_2 in HUVEC stimulated with *Candida* spp. 124
5.7 Adherence mediates GM-CSF expression in HUVEC in response to *C. albicans* 125
5.8 Inhibition assay 126
5.8.1 The role of TLR and GM-CSF production in HUVEC stimulated by *C. albicans* 126
5.8.2 The role of NF-κB transcription factor and GM-CSF production in HUVEC stimulated with *C. albicans* 129

6 CONCLUSIONS AND FUTURE RECOMMENDATIONS 131
6.1 Conclusions 131
6.1.1 Cytokines production in HUVEC treated with *Candida* spp. 131
6.2 Future recommendations 134

REFERENCES 135

APPENDICES 153

BIODATA OF THE AUTHOR 174

LIST OF TABLES

Table		Page
2.1	The frequency of *Candida* species isolated from bloodstream infection in limited Asia countries: Malaysia, Singapore, Thailand, Taiwan and also Japan.	17
2.2	Summary of cytokines relevant to *C. albicans* infection and their major functions.	28
3.1	*Candida* spp., source and site of isolation.	34
3.2	Target genes, primers and nucleotide sequences.	44
4.1	Relative expression of GM-CSF mRNA transcripts in HUVEC co-cultured with *C. albicans* and non-albicans *Candida* spp.	65
4.2	Genetic similarities of four *C. albicans* strains were determined by the calculated similarity coefficient (S_{AB}) value.	70
4.3	Relative expression of GM-CSF mRNA transcripts in HUVEC co-cultured with four different strains of *C. albicans*.	82
4.4	Relative expression of GM-CSF mRNA transcript in HUVEC co-cultured with *C. albicans* and *C. dubliniensis*, respectively.	96
4.5	Relative expression of GM-CSF mRNA transcript in HUVEC co-cultured with *C. albicans* ATCC 14053 in the absence or presence of cell culture insert, respectively.	108
4.6	Relative expression of GM-CSF mRNA transcript in HUVEC co-cultured with *C. albicans* 2714 in the absence or presence of respective inhibitors.	115

LIST OF FIGURES

Figure		Page
2.1	Different morphotypes of *C. albicans*.	10
2.2	Oral thrush in neonate caused by *C. albicans*.	14
2.3	Haematogenously disseminated candidiasis affected (A) face, (B) hands, and (C) legs of a neonate.	20
2.4	Mammalian TLRs and respective ligands.	22
2.5	TLR signal transduction pathway.	23
3.1	Schematic flow diagram of the experimental approaches or study design undertaken in this project.	32
3.2	Diagram of the cell culture insert.	40
4.1	Total RNA extracted from HUVEC with (A) medium alone; (B) *C. albicans* 2714; (C) *C. parapsilosis* 2707; (D) *C. tropicalis* 2719; (E) *C. glabrata* 2744; (F) *C. rugosa* 2692 and (G) LPS (positive control).	51
4.2	PCR amplification of IL-6 cytokine from (A) HUVEC with medium alone, HUVEC treated with *C. albicans* 2714 and *C. parapsilosis* 2707, respectively; (B) HUVEC treated with *C. tropicalis* 2719, *C. glabrata* 2744 and *C. rugosa* 2692, respectively; (C) HUVEC treated with LPS (positive control).	53
4.3	PCR amplification of IL-8 cytokine from (A) HUVEC with medium alone, HUVEC treated with *C. albicans* 2714 and *C. parapsilosis* 2707, respectively; (B) HUVEC treated with *C. tropicalis* 2719, *C. glabrata* 2744 and *C. rugosa* 2692, respectively; (C) HUVEC treated with LPS (positive control).	54

4.4	PCR amplification of MCP-1 cytokine from (A) HUVEC with medium alone, HUVEC treated with *C. albicans* 2714 and *C. parapsilosis* 2707, respectively; (B) HUVEC treated with *C. tropicalis* 2719, *C. glabrata* 2744 and *C. rugosa* 2692, respectively; (C) HUVEC treated with LPS (positive control).	55
4.5	PCR amplification of TGF-β cytokine from (A) HUVEC with medium alone, HUVEC treated with *C. albicans* 2714 and *C. parapsilosis* 2707, respectively; (B) HUVEC treated with *C. tropicalis* 2719, *C. glabrata* 2744 and *C. rugosa* 2692, respectively; (C) HUVEC treated with LPS (positive control).	56
4.6	PCR amplification of GM-CSF cytokine from (A) HUVEC with medium alone, HUVEC treated with *C. albicans* 2714 and *C. parapsilosis* 2707 respectively; (B) HUVEC treated with *C. tropicalis* 2719, *C. glabrata* 2744 and *C. rugosa* 2692, respectively; (C) HUVEC treated with LPS (positive control).	57
4.7	PCR amplification of β2M mRNA transcripts from (A) HUVEC with medium alone, HUVEC treated with *C. albicans* 2714 and *C. parapsilosis* 2707 respectively; (B) HUVEC treated with *C. tropicalis* 2719, *C. glabrata* 2744 and *C. rugosa* 2692, respectively; (C) HUVEC treated with LPS (positive control).	58
4.8	GM-CSF protein concentration in cell culture supernatants collected from HUVEC co-cultured with *C. albicans* and non-albicans *Candida* spp.	60
4.9(A)	Representative real time PCR cycling profiles of GM-CSF and β2M from the co-cultures of HUVEC with *C. albicans* and non-albicans *Candida* spp. after 8 hours of incubation.	62
4.9(B)	Representative melting curves for GM-CSF and β2M mRNA amplification products from the co-cultures of HUVEC with *C. albicans* and non-albicans *Candida* spp. after 8 hours of incubation.	63

4.10	Relative expression of GM-CSF mRNA transcript in HUVEC co-cultured with *C. albicans* and non-albicans *Candida* spp. after 8 hours of incubation.	64
4.11	The level of PGE_2 protein in the cell culture supernatants collected from HUVEC co-cultured with *C. albicans* and non-albicans *Candida* spp.	67
4.12	RAPD profiles of four *C. albicans* strains generated by using two different random primers, (A) PA03 and (B) RP02.	69
4.13	Dendrogram of four *C. albicans* strains generated from the RAPD-PCR profiles obtained with PA03 and RP02 random primers.	71
4.14	Total RNA extracted from HUVEC with (A) medium alone; (B) *C. albicans 14053* (ATCC strain); (C) *C. albicans* 2714; (D) *C. albicans* 2639; (E) *C. albicans* 2696 and (E) LPS (positive control).	72
4.15	PCR amplification of GM-CSF cytokine from (A) HUVEC with medium alone and HUVEC treated with *C. albicans* 14053 and 2714, respectively; (B) HUVEC treated with *C. albicans* 2639 and 2696, respectively; (C) HUVEC treated with LPS (positive control).	74
4.16	PCR amplification of β-actin mRNA transcripts from (A) HUVEC with medium alone and HUVEC treated with *C. albicans* 14053 and 2714, respectively; (B) HUVEC treated with *C. albicans* 2639 and 2696, respectively; (C) HUVEC treated with LPS (positive control).	75
4.17	GM-CSF concentrations in the cell culture supernatants collected from co-cultures of HUVEC with four different strains of *C. albicans*.	77
4.18(A)	Representative real time PCR cycling profiles of GM-CSF and β2M from the co-cultures of HUVEC with different strains of *C. albicans* after 8 hours of incubation.	79

4.18(B) Representative melting curves for GM-CSF and β2M mRNA amplification products from co-cultures of HUVEC with different strains of *C. albicans* after 8 hours of incubation. 80

4.19 Relative expression of GM-CSF mRNA transcript in HUVEC co-cultured with four different strains of *C. albicans* after 8 hours of incubation. 81

4.20 PGE_2 concentrations in the cell culture supernatants collected from co-cultures of HUVEC with four different strains of *C. albicans*. 84

4.21 Total RNA extracted from HUVEC with (A) medium alone; (B) *C. albicans 14053* (ATCC strain); (C) *C. dubliniensis* 1; (D) *C. dubliniensis 2* and (E) LPS (positive control). 86

4.22 PCR amplification of GM-CSF cytokine from (A) HUVEC with medium alone, HUVEC treated with *C. albicans* 14053 (ATCC strain) and *C. dubliniensis* 1, respectively; (B) HUVEC treated with *C. dubliniensis* 2 and LPS (positive control), respectively. 88

4.23 PCR amplification of β-actin mRNA transcripts from (A) HUVEC with medium alone, HUVEC treated with *C. albicans* 14053 (ATCC strain) and *C. dubliniensis* 1, respectively; (B) HUVEC treated with *C. dubliniensis* 2 and LPS (positive control), respectively. 89

4.24 GM-CSF concentrations in the cell culture supernatants collected from co-cultures of HUVEC with *C. albicans* and *C. dubliniensis*. 91

4.25(A) Representative real time PCR cycling profiles of GM-CSF and β2M from co-cultures of HUVEC treated with *C. albicans* and two *C. dubliniensis* strains, respectively after 8 hours of incubation. 93

4.25(B)	Representative melting curves for GM-CSF and β2M mRNA amplification products from co-cultures of HUVEC with *C. albicans* and two *C. dubliniensis* strains, respectively after 8 hours of incubation.	94
4.26	Relative expression of GM-CSF mRNA transcript in HUVEC co-cultured with *C. albicans* and *C. dubliniensis*, respectively.	95
4.27	PGE_2 concentration in cell culture supernatants collected from co-cultures of HUVEC with *C. albicans* and two *C. dubliniensis* strains, respectively.	98
4.28	Total RNA extracted from (A) HUVEC with medium alone, HUVEC treated with *C. albicans* 14053 in the absence or presence of cell culture insert, respectively; (B) HUVEC treated with LPS (positive control).	100
4.29	PCR amplification of GM-CSF cytokine from (A) HUVEC with medium alone, HUVEC treated with *C. albicans* 14053 in the absence or presence of cell culture insert, respectively; (B) HUVEC treated with LPS (positive control).	102
4.30	PCR amplification of β-actin housekeeping gene from (A) HUVEC with medium alone, HUVEC treated with *C. albicans* 14053 in the absence or presence of cell culture insert, respectively; (B) HUVEC treated with LPS (positive control).	103
4.31(A)	Representative real time PCR cycling profiles of GM-CSF and β2M from the co-cultures of HUVEC with *C. albicans* 14053 in the absence or presence of cell culture insert respectively after 4 and 8 hours of incubation.	105
4.31(B)	Representative melting curves for GM-CSF and β2M mRNA amplification products from the co-cultures of HUVEC with *C. albicans* 14053 in the absence or presence of cell culture insert respectively after 4 and 8 hours of incubation.	106

4.32	Relative expression of GM-CSF mRNA transcript in HUVEC co-cultured with *C. albicans* 14053 in the absence or presence of cell culture insert respectively after 4 and 8 hours of incubation.	107
4.33	Total RNA extracted from HUVEC with medium alone and HUVEC treated with *C. albicans* 2714 in the absence or presence of respective inhibitors after 4 hours of incubation.	110
4.34(A)	Representative real time PCR cycling profiles of GM-CSF and β2M from co-cultures of HUVEC with *C. albicans* 2714 in the absence or presence of respective inhibitors after 4 and 8 hours of incubation.	111
4.34(B)	Representative melting curves for GM-CSF and β2M mRNA amplification products from the co-cultures of HUVEC with *C. albicans* 2714 in the absence or presence of respective inhibitors after 4 and 8 hours of incubation.	112
4.35	Relative expression of GM-CSF mRNA transcript in HUVEC co-cultured with *C. albicans* 2714 in the absence or presence of respective inhibitors after 4 hours of incubation.	114

LIST OF ABBREVIATIONS

~	approximately
α	alpha
β	beta
γ	gamma
Δ	delta
κ	kappa
cm	centimeter
g	gram
μg	microgram
pg	picogram
μl	microliter
μm	micrometer
mg	milligram
mM	millimolar
ml	millimiter
nm	nanometer
°C	degree of Celsius
%	percentage
V	volt
bp	base-pair
kb	kilobase-pair

L	liter
ALS	Agglutinin-Like-Sequence
AP	Alkaline Phosphatase
Arg	Arginine
Asp	Aspartic acid
ATCC	American Type Culture Collection
β2M	beta-2-microglobulin
cDNA	complementary Deoxyribonucleic acid
CGM	completed growth medium
CO_2	carbon dioxide
CSF	colony-stimulating factor
C_T	threshold cycle
DEPC	diethyl pyrocarbonate
dNTPs	dideoxynucleotide triphosphates
ECGS	Endothelial Cells Growth Supplement
EDTA	ethylenediamine tetraacetic acid
ELISA	Enzyme-Linked Immunosorbent Assay
FBS	Foetal Bovine Serum
Gly	Glycine
GM-CSF	Granulocyte-Macrophage Colony-Stimulating Factor
HBSS	Hank's Balance Salts Solution
HUVEC	Human Umbilical Vein Endothelial Cells
HWP-1	Hyphal wall protein-1