

### INTRODUCTION

Endometriosis is defined as the presence of endometrial tissues outside the uterine cavity. The molecular change of endometriosis in response to hormonal therapy remained to be elucidated. Women with endometriosis suffer from pelvic pain and infertility. These symptoms may be improved by hormone therapies. The gonadotropin-releasing hormone agonist (GnRHa) is effective in pain relieving and fertility. The aim of this study is investigating the proteomics of GnRHa treatment in women with endometriosis. The serum samples were collected from 7 patients to compare the expression levels of 200 human cytokines before and after GnRHa treatment.

### METHODS

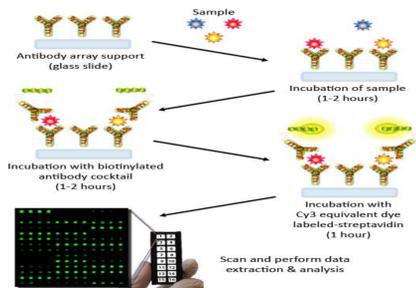
QAH-INF-3				QAH-GF-1			
Each antibody is printed in quadruplicate horizontally				Each antibody is printed in quadruplicate horizontally			
1	2	3	4	1	2	3	4
A	POS1	POS2	BLC (CXCL13)	A	POS1	POS2	Amphiregulin
B	Eotaxin-1 (CCL11)	Eotaxin-2 (MIPF-2)	GCSF	B	BDNF	bFGF	BMP-4
C	GM-CSF	I-309 (TCA-3/CCL1)	ICAM-1 (CD54)	C	BMP-5	BMP-7	beta-NGF
D	IFN-gamma	IL-1 alpha	IL-1 beta	D	EGF	EGFR	EG-VEGF (PK1)
E	IL-1 ra (IL-1 F3)	IL-2	IL-4	E	FGF4	FGF7 (KGF)	GDF-15
F	IL-5	IL-6	IL-6 R	F	GDNF	Growth Hormone	HB-EGF
G	IL-7	IL-8 (CXCL8)	IL-10	G	HGF	IGFBP-1	IGFBP-2
H	IL-11	IL-12 p40	IL-12 p70	H	IGFBP-3	IGFBP-4	IGFBP-6
I	IL-13	IL-15	IL-16	I	IGF-1	Insulin	M-CSF R
J	IL-17A	MCP-1 (CCL2)	M-CSF	J	NGFR (TNFRSF16)	NT-3	NT-4
K	MIG (CXCL9)	MIP-1 alpha (CCL3)	MIP-1 beta (CCL4)	K	Osteoprotegerin	PDGF-AA	PLGF
L	MIP-1 delta (CCL15)	PDGF-BB	RANTES (CCL5)	L	SCF	SCF R (CD117)	TGF alpha
M	TIMP-1	TIMP-2	TNF-alpha	M	TGF beta 1	TGF beta 3	VEGF-A
N	TNF beta	TNF RI	TNF RII	N	VEGFR2	VEGFR3	VEGF-D

QAH-CHE-1				QAH-REC-1			
Each antibody is printed in quadruplicate horizontally				Each antibody is printed in quadruplicate horizontally			
1	2	3	4	1	2	3	4
A	POS1	POS2	6CKine (CCL21)	A	POS1	POS2	4-1BB (CD137)
B	AI	Betacellulin (BTC)	CCL28 (MEC)	B	ALCAM (CD166)	CD80 (B7-1)	BCAM (TNFRSF17)
C	CTACK (CCL27)	CXCL16	ENA-78 (CXCL5)	C	CD14	CD30 (TNFRSF21)	CD40 Ligand
D	Eotaxin-3 (CCL26)	GCP-2 (CXCL6)	GRO	D	CEACAM-1	DR6 (TNFRSF21)	Dkk
E	HCC-1 (CCL14)	HCC-4 (CCL16)	IL-9	E	Endoglin (CD105)	ErbB3	E-Selectin
F	IL-17F	IL-18 BP alpha	IL-28A	F	Fas (Apo-1)	Fas Ligand	GITR (TNFRSF18)
G	IL-29	IL-31	IP-10 (CXCL10)	G	HVEM (TNFRSF14)	ICAM-3 (CD50)	IL-1 R4 (ST2)
H	I-TAC (CXCL11)	LIF	LIGHT (TNFSF14)	H	IL-1 R1	IL-2 R gamma	IL-1 R beta
I	Lymphotactin	MCP-2 (CCL8)	MCP-3 (CCL7)	I	IL-17 RA	IL-21 R	LIMPII
J	MCP-4 (CCL13)	MDC (CCL22)	MIF	J	Lipocalin-2 (NGAL)	L-Selectin (CD62L)	LYVE-1
K	MIP-3 alpha	MIP-3 beta	MIPF-1 (CCL23)	K	MICA	MICB	NRG1-beta 1
L	MSP alpha/beta	NAP-2 (CXCL7)	Osteopontin	L	PDGF R beta	PECAM-1 (CD31)	RAGE
M	PARC (CCL18)	Platelet Factor 4	SDF-1 alpha	M	TIM-1 (KIM-1)	TRAIL R3	Trappin-2
N	TARC (CCL17)	TECK (CCL25)	TSLP	N	uPAR	VCAM-1	XEDAR

QAH-CYT-4			
Each antibody is printed in quadruplicate horizontally			
1	2	3	4
A	POS1	POS2	Activin A
B	AgRP	Angiogenin	Angiopoietin-1
C	Angiostatin	Cathepsin S	CD40
D	Cripto-1	DAN	DKK-1
E	E-Cadherin	TROP1 (EpCam)	Fas Ligand (TNFSF8)
F	Fc gamma RIIB/C	Follistatin	Galectin-7
G	IGAHF-2 (CD102)	IL-13 R1	IL-13 R alpha 2
H	IL-17B	IL-2 R alpha	IL-2 R beta
I	IL-23	LAP/TGF beta 1	NrCAM
J	PAI-1	PDGF-AB	Resistin
K	SDF-1 beta	gp130	Sih-N
L	Siglec-5 (CD170)	IL-1 R4 (ST2)	TGF beta2
M	Tie-2	Thrombopoietin (TPO)	TRAIL R4
N	TREM-1	VEGF-C	VEGFR1



### RESULTS

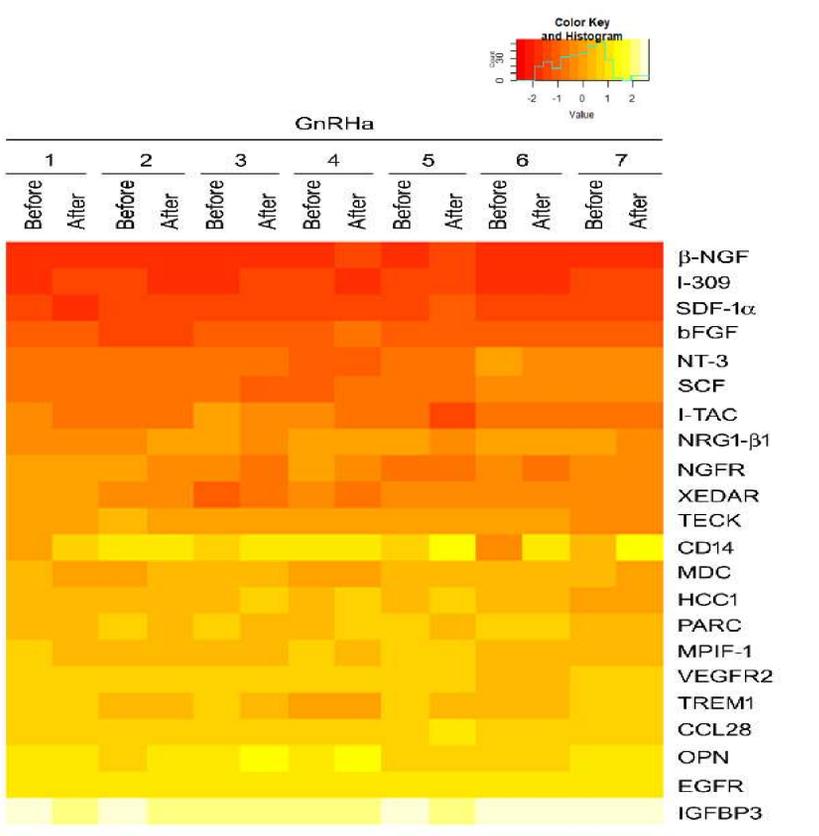


Figure 1. The heatmap analysis of 200 human cytokines. Based on cytokine expression profile, suggesting specific protein networks and molecular pathways are altered after GnRHa treatment.

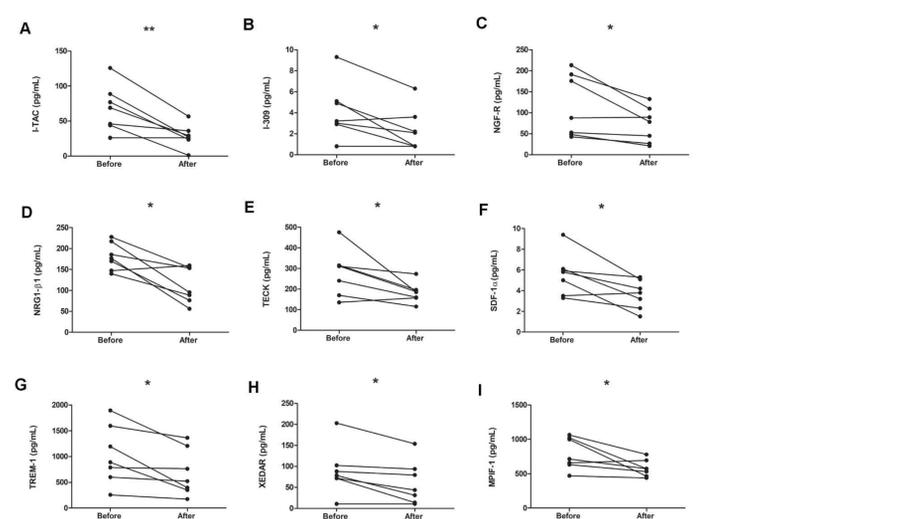


Figure 2. Serum cytokine levels of endometriosis patients treated with or without GnRH agonist. Serum levels of Interferon-inducible T-cell alpha chemoattractant (I-TAC) (A), I-309 (B), Nerve growth factor receptor (NGFR) (C), Neuregulin-1 beta 1 (NRG1-beta 1) (D), thymus-expressed chemokine (TECK) (E), Stromal Cell-Derived Factor-1 alpha (SDF-1α) (F), Triggering receptor expressed on myeloid cells 1 (TREM-1) (G), X-linked ectodysplasin-A2 receptor (XEDAR) (H), and Myeloid progenitor inhibitory factor 1 (MIPF-1) (I) were measure in 7 women with endometriosis. \* indicates p<0.05 and \*\* indicates p<0.01.

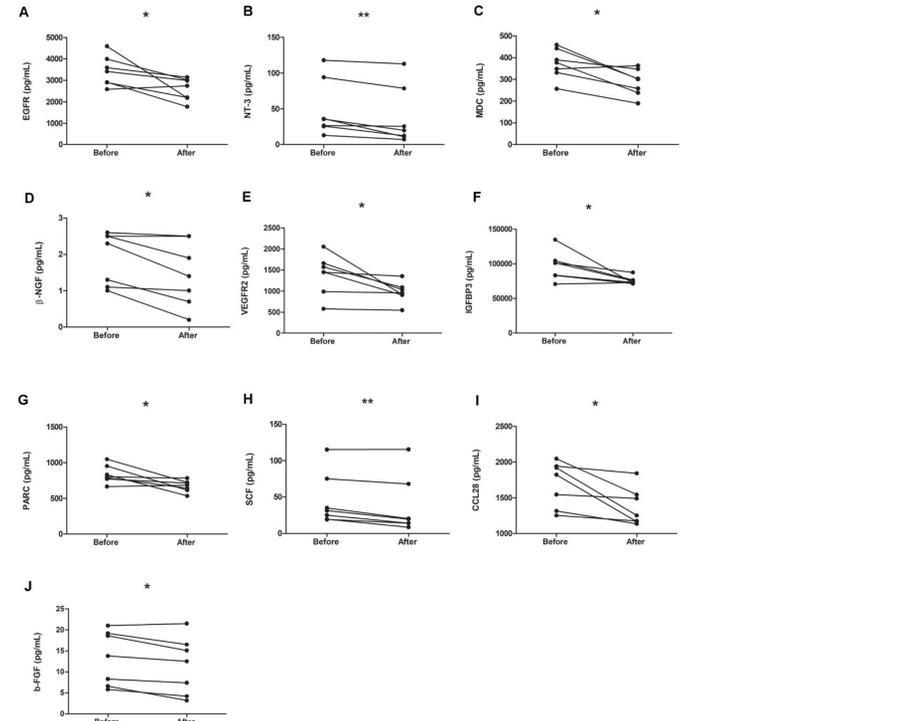


Figure 3. Serum cytokine levels of endometriosis patients treated with or without GnRH agonist. Serum levels of Epidermal growth factor receptor (EGFR) (A), Neurotrophin-3 (NT-3) (B), macrophage-derived chemokine (MDC) (C), β-nerve growth factor (β-NGF) (D), Vascular endothelial growth factor receptor 2 (VEGF R2) (E), Insulin-like growth factor-binding protein 3 (IGFBP-3) (F), Pulmonary and activation-regulated chemokine (PARC) (G), stem cell factor (SCF) (H), CCL28 (I), and Basic fibroblast growth factor (bFGF) (J) were measure in 7 women with endometriosis. \* indicates p<0.05 and \*\* indicates p<0.01.

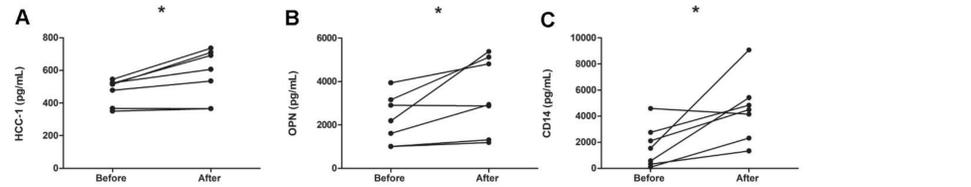


Figure 4. Serum cytokine levels of endometriosis patients treated with or without GnRH agonist. Hemofiltrate CC Chemokine-1 (HCC-1)(A), osteopontin (OPN)(B), and Cluster of differentiation 14 (CD14) (C) were measured in 7 paired endometriosis. \* indicates p<0.05 and \*\* indicates p<0.01.

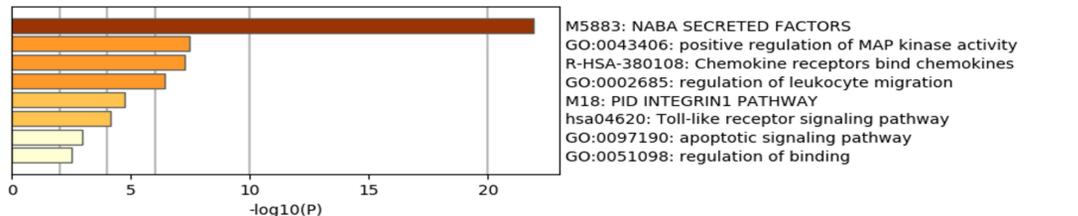


Figure 5. The metascape pathway analysis of 200 human cytokines. From metascape pathway analysis, these cytokine signature play important pathways, including MAP kinase activity, regulation of leukocyte migration, integrin pathway, Toll-like receptor signaling pathway, and apoptosis pathway.

### CONCLUSION

The analysis identifies a common cytokine signature for endometriosis after treatment of GnRHa. The personalized treatment revealed cytokine signature may be a biomarker response to the effect of GnRHa treatment in women with endometriosis.

CONTACT: ycchou1@nctu.edu.tw, tzengcr@tmu.edu.tw