

行政院國家科學委員會專題研究計畫 成果報告

以雙色 cDNA 微陣列技術探討同半胱胺酸對血管內皮細胞之 作用

計畫類別： 個別型計畫

計畫編號： NSC91-2314-B-002-289-

執行期間： 91 年 08 月 01 日至 92 年 07 月 31 日

執行單位： 國立臺灣大學醫學院內科

計畫主持人： 黃瑞仁

報告類型： 精簡報告

處理方式： 本計畫可公開查詢

中 華 民 國 92 年 10 月 31 日

國科會

以雙色 DNA 微陣列技術探討同半胱胺酸對血管內皮細胞之作用

研究報告：精簡報告

進度報告：

本計畫主要是建立基因微陣列(cDNA microarray) 之數據分析篩選出差異表達基因，並選出血管新生相關之基因群。在人體不易取得之腫瘤組織開始實驗及分析前，我們先以細胞培養模式，使研究工作者熟習細胞培養技術，RNA 之抽取及 cDNA 微陣列晶片之實驗技術，取得影像資料後再加以統計分析，建立研究平台。

初步研究重點為藉由 cDNA 微陣列技術來探討在不同濃度及時間下同半胱胺酸(homocysteine, Hcy) 對培養血管內皮細胞的影響。因此，現階段著重分離臍帶靜脈血管內皮細胞 (HUVEC) 技術之建立和細胞培養技術之穩定。我們可成功地從新生嬰兒的臍帶分離出原始臍帶靜脈血管內皮細胞 (primary cells)，以完全培養液在 37°C 及 5% CO₂ 培養箱中培養，每隔二至三天，更換新的培養液。待細胞長滿後，繼代培養之。另外為了確定分離出的細胞是否為血管內皮細胞，而非纖維母細胞 (fibroblasts) 或是平滑肌細胞 (smooth muscle cells)，我們先利用光學顯微鏡觀察細胞，培養的 HUVEC 為同種的 (homogenous) 且單層地 (monolayer) 貼附生長；細胞外形呈多邊形，具有典型 'cobblestone' 外觀。同時也擬利用 immunocytochemistry 的方法來鑑定內皮細胞上特有的 marker 蛋白質，如 CD-31、factor VIII-related antigen，佐證我們所培養的細胞為內皮細胞。除此之外，在測試 Hcy 對 HUVEC 細胞的作用之前，我們欲先取第二代至第四代的正常 HUVEC 細胞，利用 RNA pure Kit (Genesis 代理) 萃取 RNA，檢查 RNA 濃度及純度；若 RNA 濃度或純度太低，考慮是否要增加細胞數目或改良萃取方法。以估算未來從事 cDNA microarray 所需的細胞數目及 RNA 濃度，決定要取多少細胞來進行 Hcy 測試較為恰當。

現階段主要是將 array hybridization 之強度影像，經過 scanner 得到，並藉由影像分析軟體將其強度數位化，並將數位值標準化之後比較。將數值變化幅度超過 10^2 或小於 10^{-2} 定義為顯著增加或抑制。

RNA array 分析結果如下：

1.以 100 μ m Hcy treat HUVEC 1 小時之後，顯著被抑制的基因：

RBCL	hepatocyte growth factor (hepapoietin A; scatter factor)	cyclin-dependent kinase 5
Cadherin 1, type 1, E-cadherin	MMP17,membrane-inserted	CD1D antigen, d polypeptide
fibroblast growth factor 1 (acidic)	DKFZP434C211 protein,TEM5	matrix metalloproteinase 12
retinoic acid receptor, beta	angiogenin, ribonuclease, RNase A family, 5	androgen receptor (dihydrotestosterone receptor; testicular feminization; spinal and bulbar muscular atrophy; Kennedy disease)
EGF	neuropilin 1	S-3C
PDGFC	TIMP3,(Sorsby fundus dystrophy, pseudoinflammatory)	Mucin 5B
lectomedin-2,KIAA0821	hyaluronan synthase 1	CD3Z antigen, zeta polypeptide (TiT3 complex)
MCF.2 cell line derived transforming sequence	ASAR	caveolin-3
V-Ki-ras2 Kirsten rat sarcoma 2 viral oncogene homolog	prostaglandin D2 synthase, hematopoietic	chemokine (C-C motif) receptor 7
MMP15,membrane-inserted	Mucin 1	S-3E
protein kinase C, eta	Mucin 8	Mucin 6
CD38 antigen (p45)	GA4	
ephrin-B1	collagen, type VI, alpha 2	
MMP26	MNRP	CD6 antigen
hyaluronan synthase 3	chemokine (C-C motif) receptor 5	unknow
colony stimulating factor 1 (macrophage)	NP-1	LHC
C-reactive protein, pentraxin-related	Mucin 3	endothelin 2
CD1C antigen, c polypeptide	unknow	cysteine-rich protein 2
cystatin S	chemokine (C-C motif) receptor-like 2	CD5 antigen-like (scavenger receptor cysteine rich family)
IGF1	IL17B	leukotriene C4 synthase
transforming growth factor, beta 3	Mucin 4	CD28 antigen (Tp44)
TIMP2	Mucin 5AC	colony stimulating factor 2 (granulocyte-macrophage)
Laminin, alpha 4	melanoma adhesion molecule,CD146	

CD14 antigen	hypoxia-inducible factor 1, alpha subunit (basic helix-loop-helix transcription factor)	
--------------	---	--

2.以 200 μ m Hcy treat HUVEC 1 小時之後，顯著表達的基因：

breakpoint cluster region	tumor endothelial marker 8
cytochrome P450, subfamily I (aromatic compound-inducible), polypeptide 1	v-myb avian myeloblastosis viral oncogene homolog
neurofibromin 2 (bilateral acoustic neuroma)	ASAR
tenascin XB	Mucin 2
	PDGFA

3.以 1000 μ m Hcy treat HUVEC 1 小時之後，顯著被抑制表達的基因：

hyaluronan synthase 1	eukaryotic translation initiation factor 3, subunit 4 (delta, 44kD)	RBCL
CD14 antigen	lectomedin-2, KIAA0821	neuropilin 1
cystatin S	collagen, type VI, alpha 3	dickkopf (Xenopus laevis) homolog 3, DKK3
v-erb-b2 avian erythroblastic leukemia viral oncogene homolog 2 (neuro/glioblastoma derived oncogene homolog)	SYN2	chemokine (C-C motif) receptor 1
Ephrin-B2	angiogenin, ribonuclease, RNase A family, 5	leukotriene C4 synthase
S-3B	interleukin 7	CD6 antigen
CD28 antigen (Tp44)	GA4	colony stimulating factor 1 (macrophage)
MNRP	Mucin 7	matrix metalloproteinase 12
TIMP2	TGF-alpha	ASAR
angiopoietin 1	ephrin-B1	DKFZP434C211 protein, TEM5
transforming growth factor, beta receptor II (70-80kD)	S-3E	
RCA	MMP17, membrane-inserted	

顯著表達的基因:

breakpoint cluster region	tenascin XB
neurofibromin 2 (bilateral acoustic neuroma)	tumor endothelial marker 8
PDGFA	ASAR

4.以 100µm Hcy treat HUVEC 4 小時之後，顯著被抑制表達的基因：

hyaluronan synthase 1	Mucin 5B	CD6 antigen	insulin-like growth factor binding protein 6
cystatin S	IL19	colony stimulating factor 1 (macrophage)	melanoma adhesion molecule,CD146
Ephrin-B2	interleukin 1, alpha	matrix metalloproteinase 12	Colony stimulating factor 1 receptor, formerly McDonough feline sarcoma viral (v-fms) oncogene homolog
S-3B	neurofibromin 1 (neurofibromatosis, von Recklinghausen disease, Watson disease)	ASAR	endothelin 2
CD28 antigen (Tp44)	LHC	DKFZP434C211 protein,TEM5	Laminin, alpha 4
MNRP	epidermal growth factor (beta-urogastrone)	SPARC-like 1 (mast9, hevin)	caveolin-1
TIMP2	Mucin 3	IGF1	CD4 antigen (p55)
angiopoietin 1	spleen focus forming virus (SFFV) proviral integration oncogene spi1	Mucin 5AC	NS54
transforming growth factor, beta receptor II (70-80kD)	Wilms tumor 1	retinoic acid receptor, beta	deleted in colorectal carcinoma
RCA	MMP19,	Mucin 6	Cadherin 1, type 1, E-cadherin (epithelial
collagen, type VI, alpha 3	caveolin-3	BCL2-associated X protein	hepatocyte growth factor (hepapoietin A; scatter factor)
angiogenin, ribonuclease, RNase A family, 5	V-Ki-ras2 Kirsten rat sarcoma 2 viral oncogene homolog	Mucin 8	CD1A antigen, a polypeptide
interleukin 7	ephrin-A1	IL17B	insulin-like growth factor-binding protein 4
GA4	MCF.2 cell line derived transforming sequence-like	TGF-B2	MMP11,stromelysin 3
Mucin 7	RCA	RBCL	CD8 antigen, alpha polypeptide (p32)
TGF-alpha	v-src avian sarcoma (Schmidt-Ruppin A-2) viral oncogene homolog	CD1C antigen, c polypeptide	

CD3E antigen, epsilon polypeptide (TiT3 complex)ephrin-B1	MCF.2 cell line derived transforming sequence	Lymphocyte-specific protein tyrosine kinase	
S-3E	PDGFC	Flap structure-specific endonuclease 1	interleukin 15
MMP17,membrane-inserted	hyaluronan synthase 3	ATPS	Bcl-2
RBCL	cyclin E1	interleukin 16 (lymphocyte chemoattractant factor)	CCAAT/enhancer binding protein (C/EBP), beta
neuropilin 1	endothelin 3	leukotriene A4 hydrolase	t-PA
chemokine (C-C motif) receptor 1	transforming growth factor, beta 3	plasminogen activator, tissue	GA4
leukotriene C4 synthase	hypoxia-inducible factor 1, alpha subunit (basic helix-loop-helix transcription factor)	ephrin-B3	B-cell CLL/lymphoma 2
chemokine (C-C motif) receptor-like 2	Mucin 1	CD3G antigen, gamma polypeptide (TiT3 complex)	NP-1
slit (Drosophila) homolog 3	LHC	v-rel avian reticuloendotheliosis viral oncogene homolog B (nuclear factor of kappa light polypeptide gene enhancer in B-cells 3)	eukaryotic translation initiation factor 2B, subunit 1 (alpha, 26kD)
tumor endothelial marker 1 precursor,TEM1	eukaryotic translation initiation factor 3, subunit 5 (epsilon, 47kD)	pro-platelet basic protein (includes platelet basic protein, beta-thromboglobulin, connective tissue-activating peptide III, neutrophil-activating peptide-2)	TIMP3,(Sorsby fundus dystrophy, pseudoinflammatory)
ornithine decarboxylase 1	MMP15,membrane-inserted	CD1D antigen, d polypeptide	insulin-like growth factor 1 (somatomedin C)
collagen, type VI, alpha 2	CD5 antigen-like (scavenger receptor cysteine rich family)	cyclin-dependent kinase 5	cyclinD1
MMP20, enamelysin	Thrombospondin-2	cysteine-rich protein 2	eukaryotic translation initiation factor 2 alpha kinase 3
colony stimulating factor 2 (granulocyte-macrophage)	thrombospondin 4	S-3C	Mucin 4

CD8 antigen, (p37)	MMP13,(collagenase 3)		GAPDH
androgen receptor (dihydrotestosterone receptor; testicular feminization; spinal and bulbar muscular atrophy; Kennedy disease)	endocytic receptor (macrophage mannose receptor family)		
MMP26	protein kinase C, eta		

顯著表達者

ASAR PDGFA

4.以 200µm Hcy treat HUVEC 4 小時之後，顯著被抑制表達的基因：

hyaluronan synthase 1	Mucin 3	Mucin 5B
cystatin S	Wilms tumor 1	Thrombospondin-2
S-3B	MMP19,	CD1A antigen, a polypeptide
CD28 antigen (Tp44)	V-Ki-ras2 Kirsten rat sarcoma 2 viral oncogene homolog	CD8 antigen, alpha polypeptide (p32)
TIMP2	ephrin-A1	caveolin-1
angiopoietin 1	MCF.2 cell line derived transforming sequence-like	NS54
collagen, type VI, alpha 3	v-src avian sarcoma (Schmidt-Ruppin A-2) viral oncogene homolog	B-cell CLL/lymphoma 2
interleukin 7	cyclin E1	Laminin, alpha 4
GA4	transforming growth factor, beta 3	NP-1
Mucin 7	Mucin 6	TIMP3,(Sorsby fundus dystrophy, pseudoinflammatory)
TGF-alpha	BCL2-associated X protein	insulin-like growth factor 1 (somatomedin C)
ephrin-B1	Mucin 8	cyclinD1
S-3E	IL17B	eukaryotic translation initiation factor 2 alpha kinase 3
MMP17,membrane-inserted	RBCL	IL19
RBCL	CD1C antigen, c polypeptide	CD5 antigen-like (scavenger receptor cysteine rich family)
neuropilin 1	Lymphocyte-specific protein tyrosine kinase	IGF1
chemokine (C-C motif) receptor 1	interleukin 1, alpha	Mucin 5AC
leukotriene C4 synthase	LHC	retinoic acid receptor, beta
CD6 antigen	epidermal growth factor (beta-urogastrone)	interleukin 15
colony stimulating factor 1 (macrophage)	Flap structure-specific endonuclease 1	Bcl-2
matrix metalloproteinase 12	interleukin 16 (lymphocyte chemoattractant factor)	Mucin 1

DKFZP434C211 protein,TEM5	plasminogen activator, tissue	
CCAAT/enhancer binding protein (C/EBP), beta	CD36 antigen (collagen type I receptor, thrombospondin receptor)-like 1	interleukin 6 (interferon, beta 2)
chemokine (C-C motif) receptor-like 2	tensin, DKFZp434G162	unknow
ornithine decarboxylase 1	matrix metalloproteinase 12 (macrophage elastase)	secreted protein, acidic, cysteine-rich (osteonectin),SPARC
collagen, type VI, alpha 2	unknow	unknow
MMP20, enamelysin	chemokine (C-C motif) receptor 5	lyphotoxin alpha (TNF superfamily, member 1)
unknow	endothelin 1	CD3Z antigen, zeta polypeptide (TiT3 complex)
colony stimulating factor 2 (granulocyte-macrophage)	CD14 antigen	androgen receptor (dihydrotestosterone receptor; testicular feminization; spinal and bulbar muscular atrophy; Kennedy disease)
CD8 antigen, beta polypeptide 1 (p37)	MMP23A	TIMP4
CD3E antigen, epsilon polypeptide (TiT3 complex)	unknow	C-reactive protein, pentraxin-related
Cadherin 1, type 1, E-cadherin (epithelial)	CD3D antigen, delta polypeptide (TiT3 complex)	thrombospondin 3
hepatocyte growth factor (hepapoietin A; scatter factor)	interleukin 18 (interferon-gamma-inducing factor)	Non-metastatic cells 1, protein (NM23A) expressed in
unknow	prostaglandin D2 synthase, hematopoietic	intercellular adhesion molecule 4, Landsteiner-Wiener blood group
insulin-like growth factor binding protein 6	monokine induced by gamma interferon	EphB3
melanoma adhesion molecule,CD146	cyclin E2	insulin-like growth factor binding protein 7,IGFBP7
Colony stimulating factor 1 receptor, formerly McDonough feline sarcoma viral (v-fms) oncogene homolog	chemokine (C-X-C motif), receptor 4 (fusin)	CD2 antigen (p50), sheep red blood cell receptor
endothelin 2	MMP9,gelatinase B, 92kD gelatinase, 92kD type IV collagenase)	NP-2
endocytic receptor (macrophage mannose receptor family)	chemokine (C-C motif) receptor 7	interleukin 13
unknow	chemokine (C-X3-C) receptor 1	protein kinase C, eta
GA4	collagen, type III, alpha 1 (Ehlers-Danlos syndrome type IV, autosomal dominant)	lectomedin-2,KIAA0821
pro-platelet basic protein (includes platelet basic protein, beta-thromboglobulin, connective tissue-activating peptide III,	v-erb-b2 avian erythroblastic leukemia viral oncogene homolog 2 (neuro/glioblastoma derived oncogene homolog)	mucosal vascular addressin cell adhesion molecule 1

neutrophil-activating peptide-2)		
CD1D antigen, d polypeptide		SYN2
HAT22	fibroblast growth factor 1 (acidic)	
chemokine (C-C motif) receptor 2	tachykinin, precursor 1 (substance K, substance P, neurokinin 1, neurokinin 2, neuromedin L, neurokinin alpha, neuropeptide K,neuropeptide gamma)	
hepatocyte growth factor (hepapoietin A; scatter factor)	EGF	

顯著表達者

ASAR	ASAR
neurofibromin 2 (bilateral acoustic neuroma)	PDGFA

4.以 200 μ m Hcy treat HUVEC 4 小時之後，顯著被抑制表達的基因：

hyaluronan synthase 1	androgen receptor (dihydrotestosterone receptor; testicular feminization; spinal and bulbar muscular atrophy; Kennedy disease)	ASAR
CD28 antigen (Tp44)	chemokine (C-C motif) receptor 5	hypoxia-inducible factor 1, alpha subunit (basic helix-loop-helix transcription factor)
Mucin 7	CD14 antigen	S-3C
S-3E	prostaglandin D2 synthase, hematopoietic	met proto-oncogene (hepatocyte growth factor receptor)
MMP17,membrane-inserted	cyclin E2	angiogenin, ribonuclease, RNase A family, 5
CD6 antigen	C-reactive protein, pentraxin-related	Laminin, alpha 4
retinoic acid receptor, beta	platelet/endothelial cell adhesion molecule (CD31 antigen)	Colony stimulating factor 1 receptor, formerly McDonough feline sarcoma viral (v-fms) oncogene homolog
Mucin 5B	melanoma adhesion molecule,CD146	S-3F
IL19	CD4 antigen (p55)	MMP11,stromelysin 3
Mucin 6	CD1A antigen, a polypeptide	Fas-activated serine/threonine kinase
Thrombospondin-2	B-cell CLL/lymphoma 2	

顯著表達者

v-myb avian myeloblastosis viral oncogene homolog	tumor endothelial marker 8	PDGFA
Mucin 2	neurofibromin 2 (bilateral acoustic neuroma)	tenascin XB

