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(54) **BIOLOGICAL MARKERS OF CHRONIC WOUND TISSUE AND METHODS OF USING FOR CRITERIA IN SURGICAL DEBRIDEMENT**

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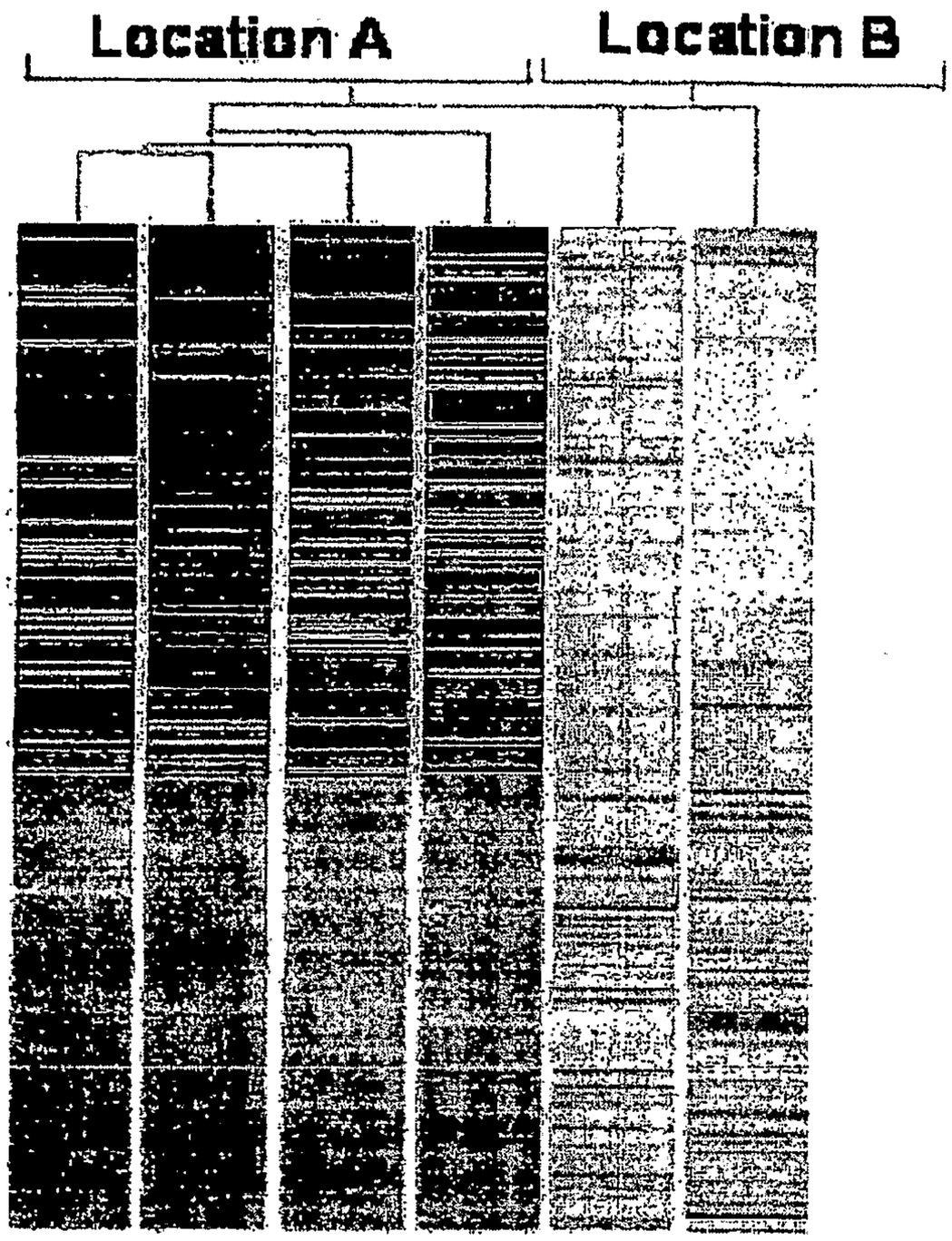
(57) **ABSTRACT**

The present invention relates to methods for identifying tissue sites in a chronic wound that are suitable for debridement and whether debridement procedure has been successful using particular biological markers of the cells within the tissue sites of the chronic wounds.



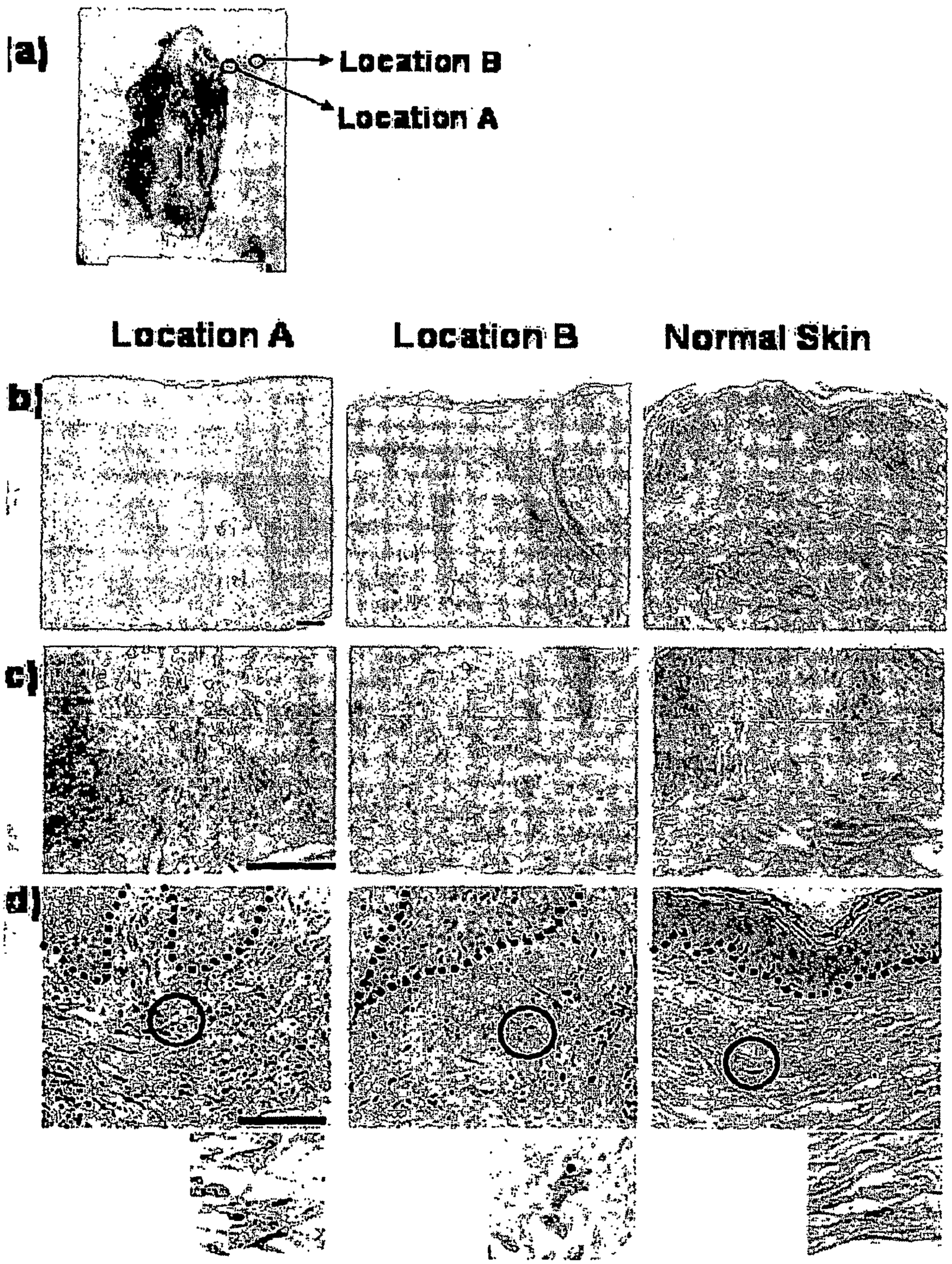

a)
Location B
Location A
Location A
Location B
Normal Skin
b)
c)
d)

Figure 1

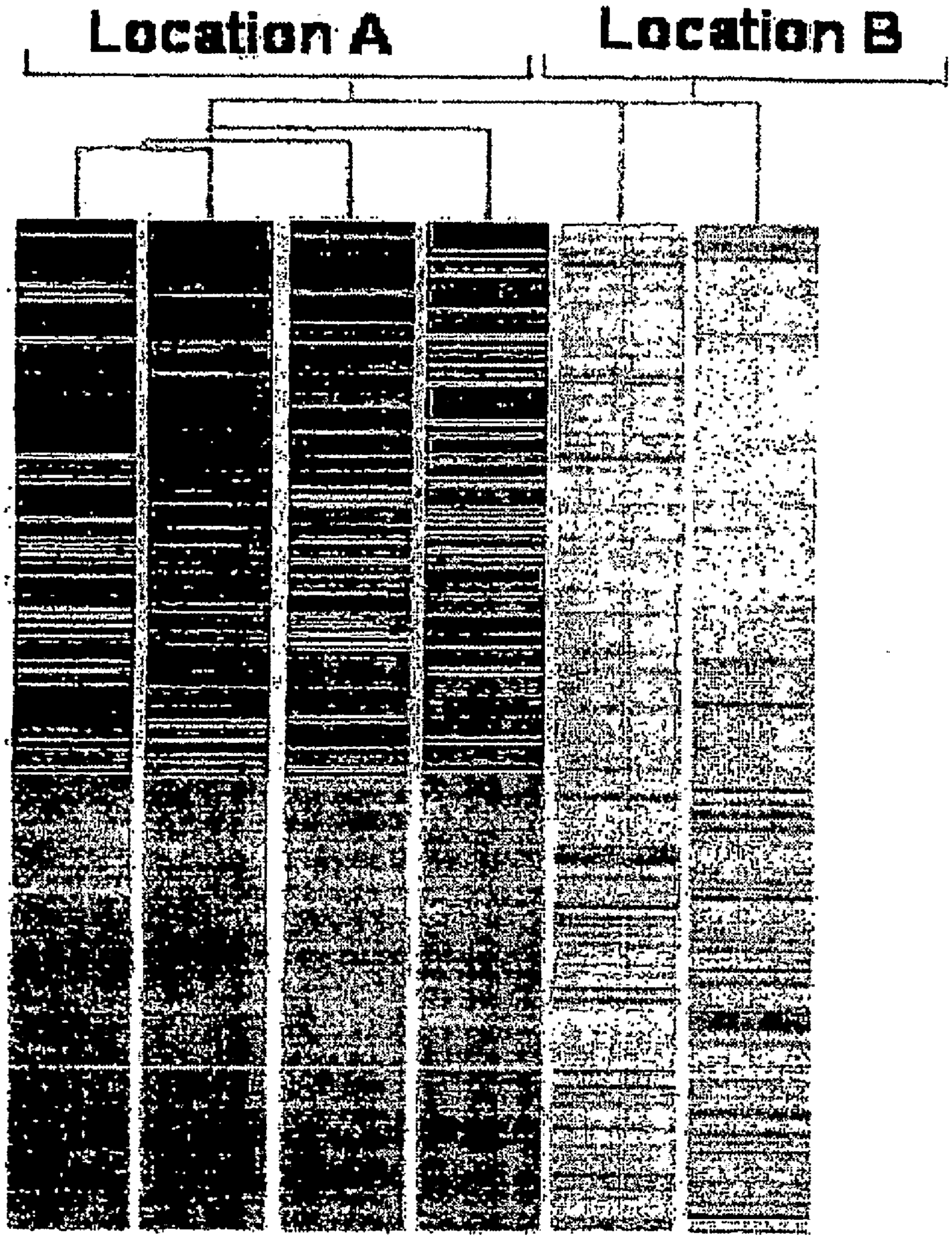


Figure 2

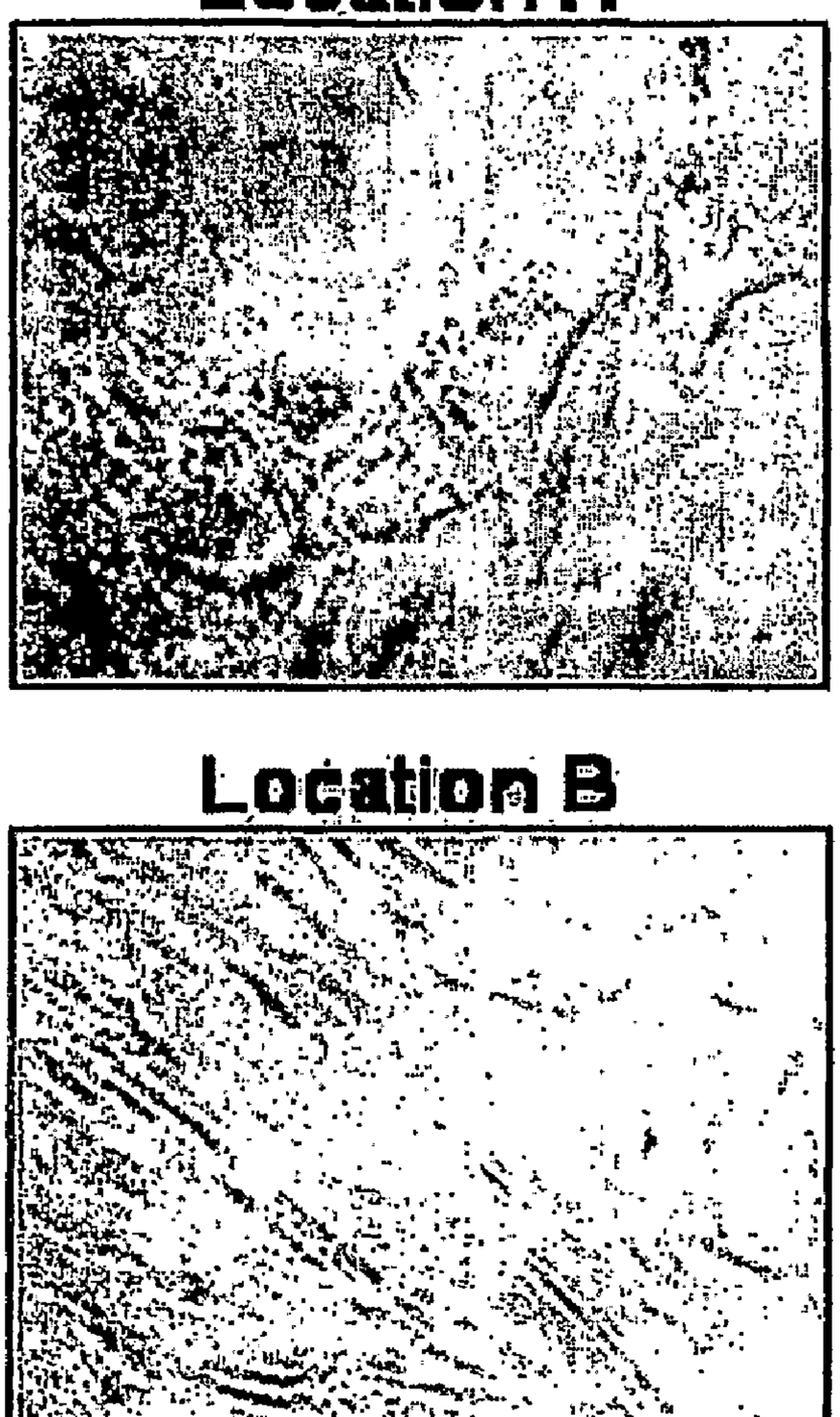


Figure 3

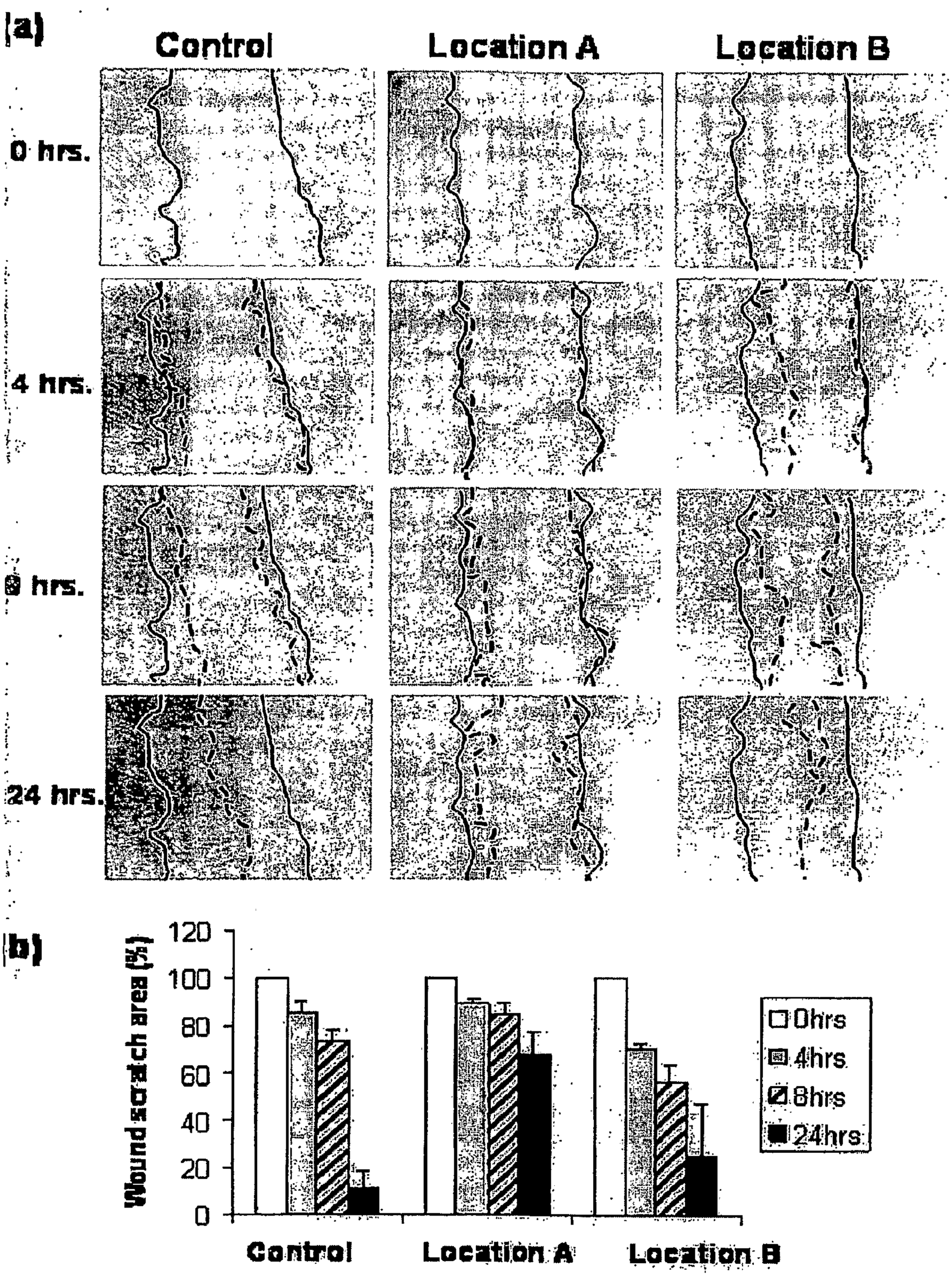

(a)
Control
Location A
Location B
0 hrs.
4 hrs.
8 hrs.
24 hrs.
(b)
Wound scratch area (%)
120
100
80
60
40
20
0
Control
Location A
Location B
0hrs
4hrs
8hrs
24hrs

Figure 4

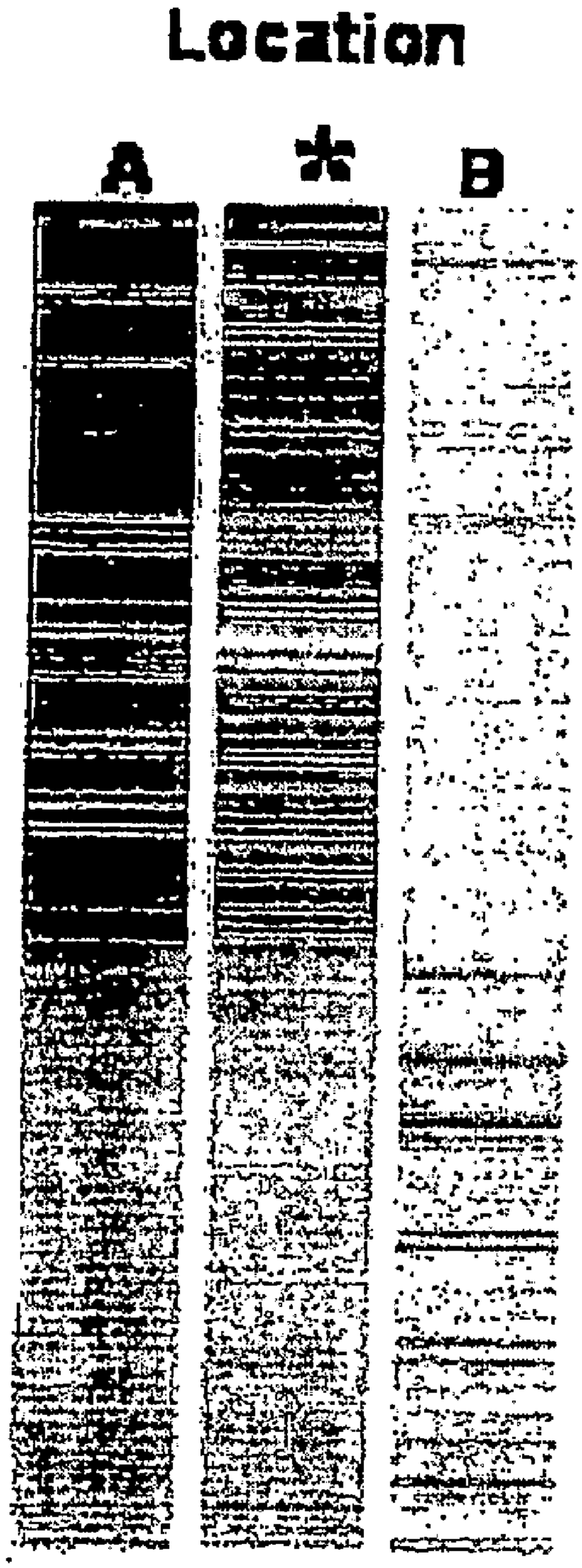


Figure 5

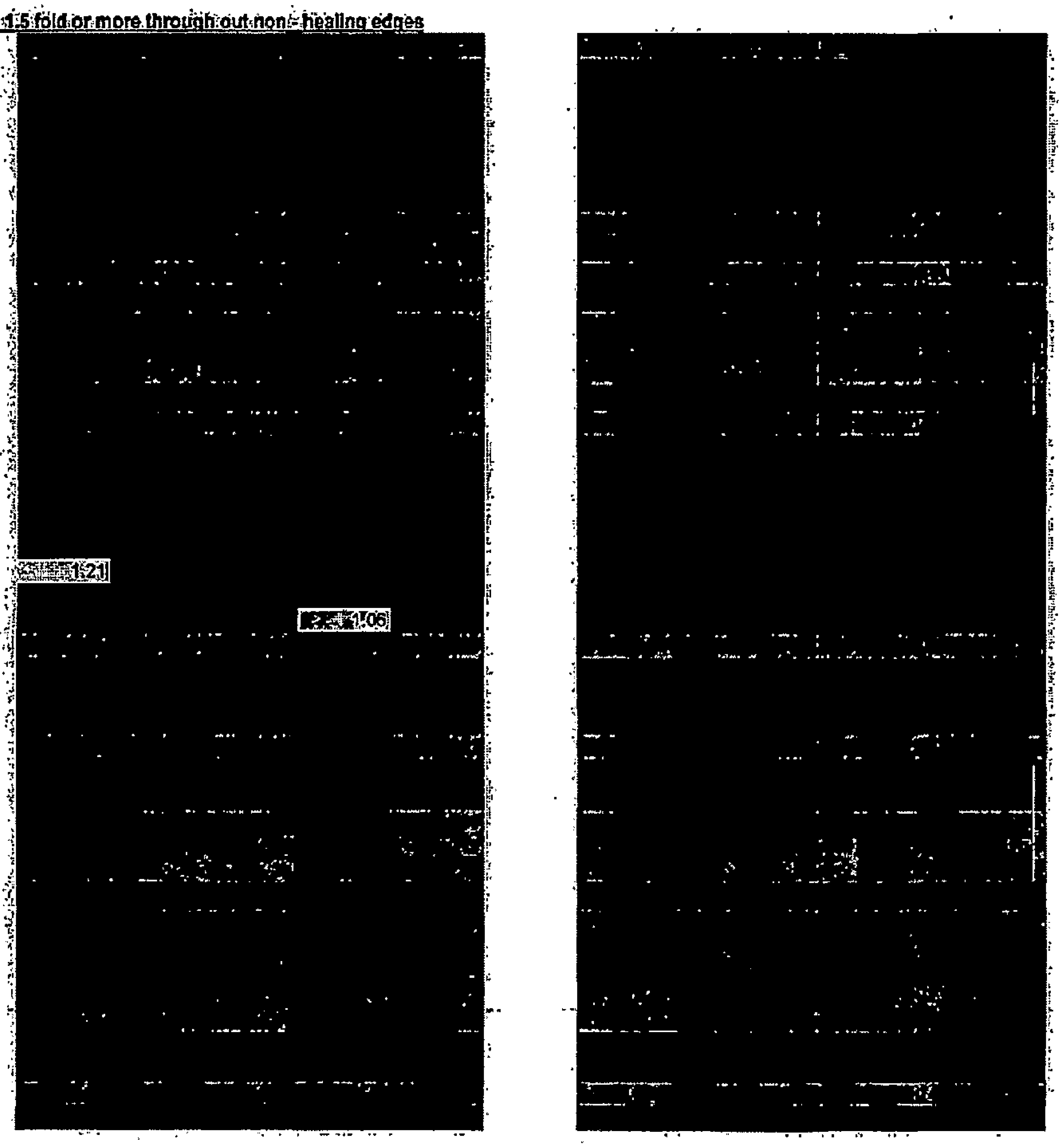
Figure 6A

Raw Data between 100 and 9,089 (3559)
Chronic wounds

upregulated more than 1.5 folds
upregulated less than 1.5 folds

Systematic | Fold chan | Fold chan | Fold chan | Fold chan | Fold change | Fold chan | Fold chan | Fold chan | Fold chan | Fold change

Genes upregulated 1.5 fold or more through out non-healing edges



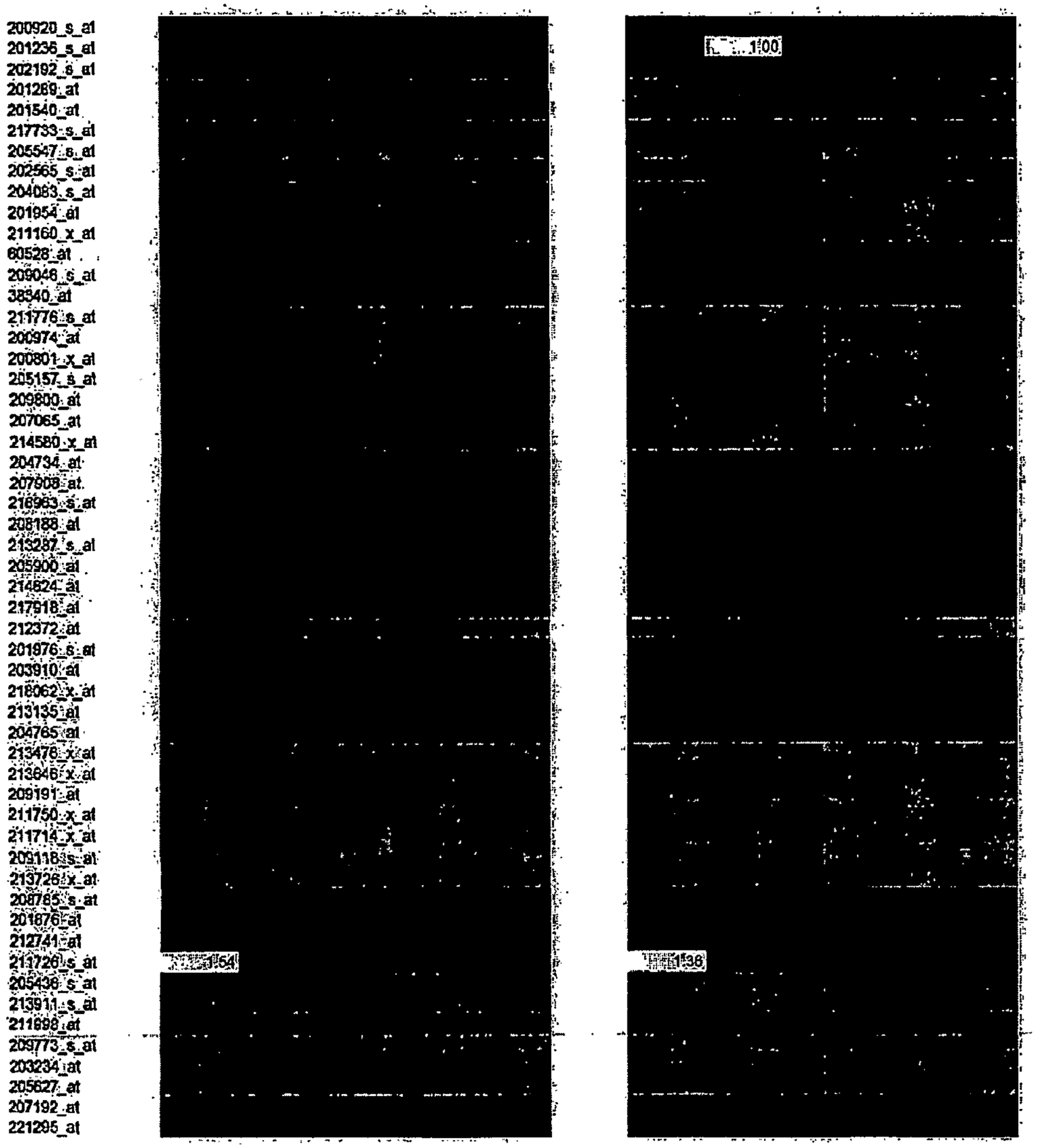
Systematic	Function	SYMBOL
201645_at	Adhesion	TNC
201581_s_at	Adhesion	CLSTN1
201681_s_at	Adhesion	DLG5
213369_at	Adhesion, cadherin	PCDH21
208153_s_at	Adhesion, cadherin	FAT2
208407_s_at	Adhesion, cadherin	CTNND1
204029_at	Adhesion, cadherin	CELSR2
204750_s_at	Adhesion, desmosomal	DSC2
207324_s_at	Adhesion, desmosomal	DSC1
211075_s_at	Adhesion, integrin	CD47
204455_at	Adhesion, integrin	BPAG1
210869_s_at	Adhesion, junctional	MCAM
203757_s_at	Adhesion, junctional	CEACAM6
201615_x_at	Adhesion, junctional	CALD1
205480_x_at	Adhesion, junctional	GJB3
201470_at	Antioxidant	GSTO1
202967_at	Antioxidant	GSTA4
201427_s_at	Antioxidant	SEPP1
204168_at	Antioxidant	MGST2
209276_s_at	Antioxidant	GLRX
201432_at	Antioxidant	CAT
202831_at	Antioxidant	GPX2
206662_at	Antioxidant	GLRX
211922_s_at	Antioxidant	CAT
218856_at	Apoptosis	TNFRSF21
209230_s_at	Apoptosis	P8
212593_s_at	Apoptosis	PDCD4
204004_at	Apoptosis	PAWR
201631_s_at	Apoptosis inhibitor	IER3
202037_s_at	Apoptosis inhibitor	SFRP1
203528_at	Apoptosis inhibitor	SEMA4D
219454_at	Ca binding	EGFL6
61734_at	Ca binding	RCN3
200755_s_at	Ca binding	CALU
219197_s_at	Ca binding	SCUBE2
202870_s_at	Cell cycle	CDC20
204170_s_at	Cell cycle	CKS2
204026_s_at	Cell cycle	ZWINT
202388_at	Cell cycle	RGS2
201853_s_at	Cell cycle	CDC25B
201371_s_at	Cell cycle	CUL3
211382_s_at	Cell cycle	TACC2
201482_at	Cell cycle inhibitor	QSCN6
218346_s_at	Cell cycle inhibitor	SESN1

Figure 6B

GENENAME

tenascin C (hexabrachion)
calsyntenin 1
discs, large homolog 5 (Drosophila)
protocadherin 21
FAT tumor suppressor homolog 2 (Drosophila)
catenin (cadherin-associated protein), delta 1
cadherin, EGF LAG seven-pass G-type receptor 2 (flamingo homolog, Drosophila)
desmocollin 2
desmocollin 1
CD47 antigen (Rh-related antigen, integrin-associated signal transducer)
bullous pemphigoid antigen 1, 230/240kDa
melanoma cell adhesion molecule
carcinoembryonic antigen-related cell adhesion molecule 6 (non-specific cross reacting antigen)
caldesmon 1
gap junction protein, beta 3, 31kDa (connexin 31)
glutathione S-transferase omega 1
glutathione S-transferase A4
selenoprotein P, plasma, 1
microsomal glutathione S-transferase 2
glutaredoxin (thioltransferase)
catalase
glutathione peroxidase 2 (gastrointestinal)
glutaredoxin (thioltransferase)
catalase
tumor necrosis factor receptor superfamily, member 21
p8 protein (candidate of metastasis 1)
programmed cell death 4 (neoplastic transformation inhibitor)
PRKC, apoptosis, WT1, regulator
immediate early response 3
secreted frizzled-related protein 1
sema domain, immunoglobulin domain (Ig), transmembrane domain (TM) and short cytoplasmic domain, (semaphorin) 4D
EGF-like-domain, multiple 6
reticulocalbin 3, EF-hand calcium binding domain
calumenin
signal peptide, CUB domain, EGF-like 2
CDC20 cell division cycle 20 homolog (S. cerevisiae)
CDC28 protein kinase regulatory subunit 2
ZW10 interactor
regulator of G-protein signalling 2, 24kDa
cell division cycle 25B
cullin 3
transforming, acidic coiled-coil containing protein 2
quiescin Q6
sestrin 1

Figure 6C

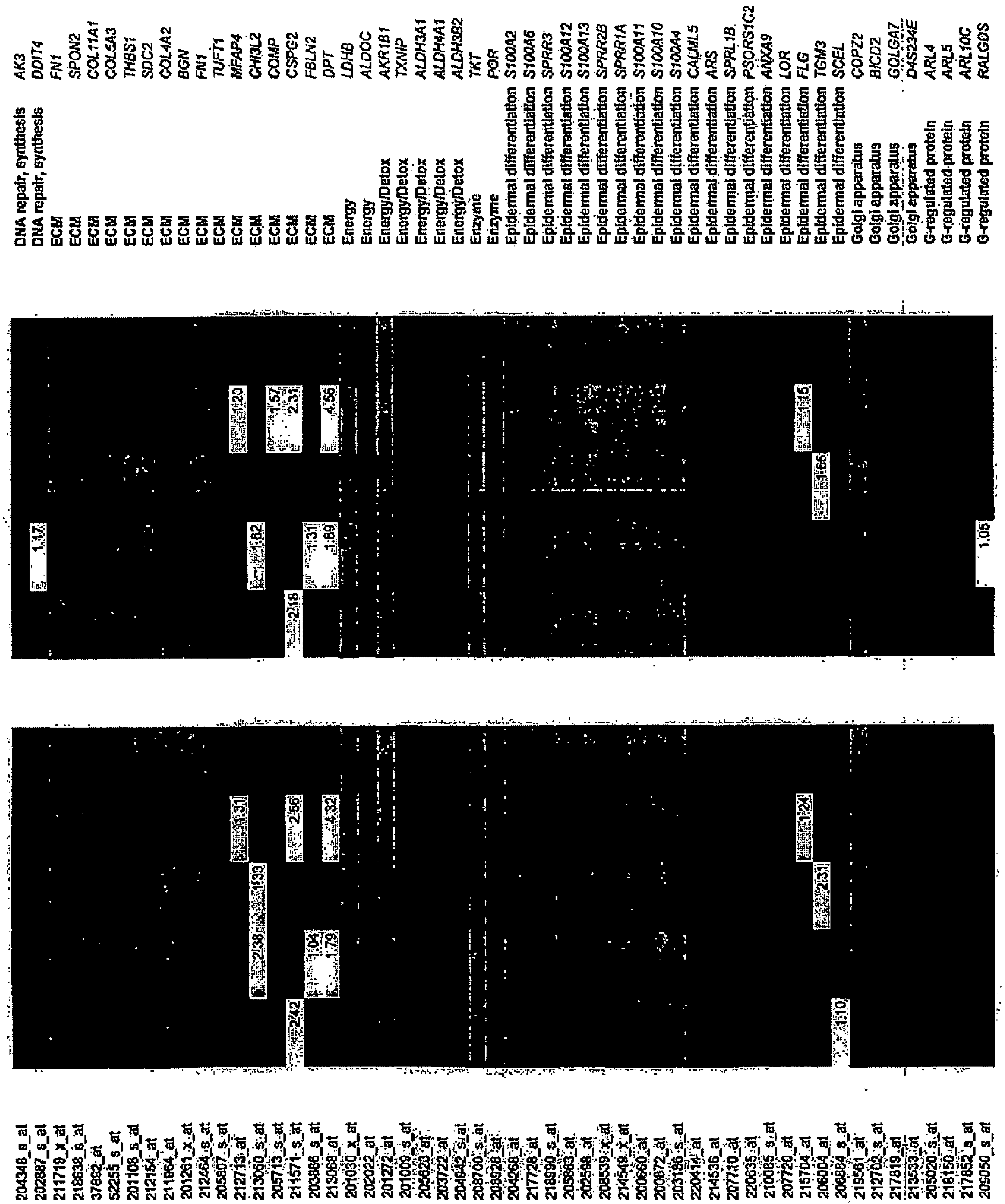


200920_s_at	Cell cycle inhibitor	*BTG1*
201236_s_at	Cell cycle inhibitor	*BTG2*
202192_s_at	Cell cycle inhibitor	*GAS7*
201289_at	Cell growth, proliferation	*CYR61*
201540_at	Cell growth, proliferation	*FHL1*
217733_s_at	Cytoskeletal	*TMSB10*
205547_s_at	Cytoskeletal	*TAGLN*
202565_s_at	Cytoskeletal	*SVIL*
204083_s_at	Cytoskeletal, actin	*TPM2*
201954_at	Cytoskeletal, actin	*ARPC1B*
211160_x_at	Cytoskeletal, actin	*ACTN1*
60528_at	Cytoskeletal, actin	*SPTBN5*
209046_s_at	Cytoskeletal, actin	*GABARAPL2*
38540_at	Cytoskeletal, actin	*HIP1R*
211776_s_at	Cytoskeletal, actin, memb	*EPB41L3*
200974_at	Cytoskeletal, actin, motilit	*ACTA2*
200801_x_at	Cytoskeletal, actin, motilit	*ACTB*
205157_s_at	Cytoskeletal, keratin	*KRT17*
209800_at	Cytoskeletal, keratin	*KRT16*
207065_at	Cytoskeletal, keratin	*K6HF*
214580_x_at	Cytoskeletal, keratin	*KRT6A*
204734_at	Cytoskeletal, keratin	*KRT15*
207908_at	Cytoskeletal, keratin	*KRT2A*
216963_s_at	Cytoskeletal, keratin	*KRT23*
208168_at	Cytoskeletal, keratin	*KRT9*
213287_s_at	Cytoskeletal, keratin	*KRT10*
205900_at	Cytoskeletal, keratin	*KRT1*
214824_at	Cytoskeletal, membrane	*UPK1A*
217918_at	Cytoskeletal, motility	*DNCL2A*
212372_at	Cytoskeletal, myosin	*MYH10*
201876_s_at	Cytoskeletal, myosin	*MYO10*
203910_at	Cytoskeletal, Rho,Cdc42	*PARG1*
218062_x_at	Cytoskeletal, Rho,Cdc42	*CDC42EP4*
213135_at	Cytoskeletal, Rho,Cdc42	*TIAM1*
204765_at	Cytoskeletal, Rho,Cdc42	*ARHGEF5*
213476_x_at	Cytoskeletal, tubulin	*TUBB4*
213646_x_at	Cytoskeletal, tubulin	*K-ALPHA-1*
209191_at	Cytoskeletal, tubulin	*MGC4083*
211750_x_at	Cytoskeletal, tubulin	*TUBA6*
211714_x_at	Cytoskeletal, tubulin	*OK/SW-cl.56*
209118_s_at	Cytoskeletal, tubulin	*TUBA3*
213726_x_at	Cytoskeletal, tubulin	*TUBB2*
208785_s_at	Cytoskeletal, tubulin	*MAP1LC3B*
201876_at	Detoxification	*PON2*
212741_at	Detoxification	*MAOA*
211726_s_at	Detoxification	*FMO2*
205436_s_at	DNA binding, histone	*H2AFX*
213911_s_at	DNA binding, histone	*H2AFZ*
211698_at	DNA binding, histone	*H3F3B*
209773_s_at	DNA repair, synthesis	*RRM2*
203234_at	DNA repair, synthesis	*UPP1*
205627_at	DNA repair, synthesis	*CDA*
207192_at	DNA repair, synthesis	*DNASE1L2*
221295_at	DNA repair, synthesis	*CIDEA*

Figure 6D

B-cell translocation gene 1, anti-proliferative
BTG family, member 2
growth arrest-specific 7
cysteine-rich, angiogenic inducer, 61
four and a half LIM domains 1
thymosin, beta 10
transgelin
supervillin
tropomyosin 2 (beta)
actin related protein 2/3 complex, subunit 1B, 41kDa
actinin, alpha 1
spectrin, beta, non-erythrocytic 5
GABA(A) receptor-associated protein-like 2
huntingtin interacting protein-1-related
erythrocyte membrane protein band 4.1-like 3
actin, alpha 2, smooth muscle, aorta
actin, beta
keratin 17
keratin 16 (focal non-epidermolytic palmoplantar keratoderma)
cytokeratin type II
keratin 6A
keratin 15
keratin 2A (epidermal ichthyosis bullosa of Siemens)
keratin 23 (histone deacetylase inducible)
keratin 9 (epidermolytic palmoplantar keratoderma)
keratin 10 (epidermolytic hyperkeratosis; keratosis palmaris et plantaris)
keratin 1 (epidermolytic hyperkeratosis)
uroplakin 1A
dynein, cytoplasmic, light polypeptide 2A
myosin, heavy polypeptide 10, non-muscle
myosin X
PTPL1-associated RhoGAP 1
CDC42 effector protein (Rho GTPase binding) 4
T-cell lymphoma invasion and metastasis 1
Rho guanine nucleotide exchange factor (GEF) 5
tubulin, beta, 4
tubulin, alpha, ubiquitous
tubulin beta MGC4083
tubulin alpha 6
beta-5-tubulin
tubulin, alpha 3
tubulin, beta, 2
microtubule-associated protein 1 light chain 3 beta
paraoxonase 2
monoamine oxidase A
flavin containing monooxygenase 2
H2A histone family, member X
H2A histone family, member Z
H3 histone, family 3B (H3.3B)
ribonucleotide reductase M2 polypeptide
uridine phosphorylase 1
cytidine deaminase
deoxyribonuclease I-like 2
cell death-inducing DFFA-like effector a

Figure 6E


DNA repair, synthesis AK3
DNA repair, synthesis DDIT4
ECM FN1
ECM SPON2
ECM COL11A1
ECM COL5A3
ECM THBS1
ECM SDC2
ECM COL4A2
ECM BGN
ECM FN1
ECM TUFT1
ECM MFAP4
ECM CHI3L2
ECM COMP
ECM CSPG2
ECM FBLN2
ECM DPT
Energy LDHB
Energy ALDOC
Energy/Detox AKR1B1
Energy/Detox TXNIP
Energy/Detox ALDH3A1
Energy/Detox ALDH4A1
Energy/Detox ALDH3B2
Enzyme TKT
Enzyme POR
Epidermal differentiation S100A2
Epidermal differentiation S100A6
Epidermal differentiation SPRR3
Epidermal differentiation S100A12
Epidermal differentiation S100A13
Epidermal differentiation SPRR2B
Epidermal differentiation SPRR1A
Epidermal differentiation S100A11
Epidermal differentiation S100A10
Epidermal differentiation S100A4
Epidermal differentiation CALML5
Epidermal differentiation ARS
Epidermal differentiation SPRL1B
Epidermal differentiation PSORS1C2
Epidermal differentiation ANXA9
Epidermal differentiation LOR
Epidermal differentiation FLG
Epidermal differentiation TGM3
Epidermal differentiation SCEL
Golgi apparatus COPZ2
Golgi apparatus BICD2
Golgi apparatus GOLGA7
Golgi apparatus D4S234E
G-regulated protein ARL4
G-regulated protein ARL5
G-regulated protein ARL10C
G-regulated protein RALGDS
1.37
1.20
1.82
1.57
2.31
2.18
1.31
1.89
4.56
1.15
1.66
1.05
1.31
2.38
1.33
2.56
2.42
1.04
1.79
4.32
1.24
2.31
1.10
204348_s_at
202887_s_at
211719_x_at
218638_s_at
37892_at
52255_s_at
201108_s_at
212154_at
211864_at
201261_x_at
212464_s_at
205807_s_at
212713_at
213060_s_at
205713_s_at
211571_s_at
203886_s_at
219068_at
201030_x_at
202022_at
201272_at
201009_s_at
205623_at
203722_at
204942_s_at
208700_s_at
208928_at
204268_at
217728_at
218990_s_at
205863_at
202588_at
208539_x_at
214549_x_at
200660_at
200872_at
203186_s_at
220414_at
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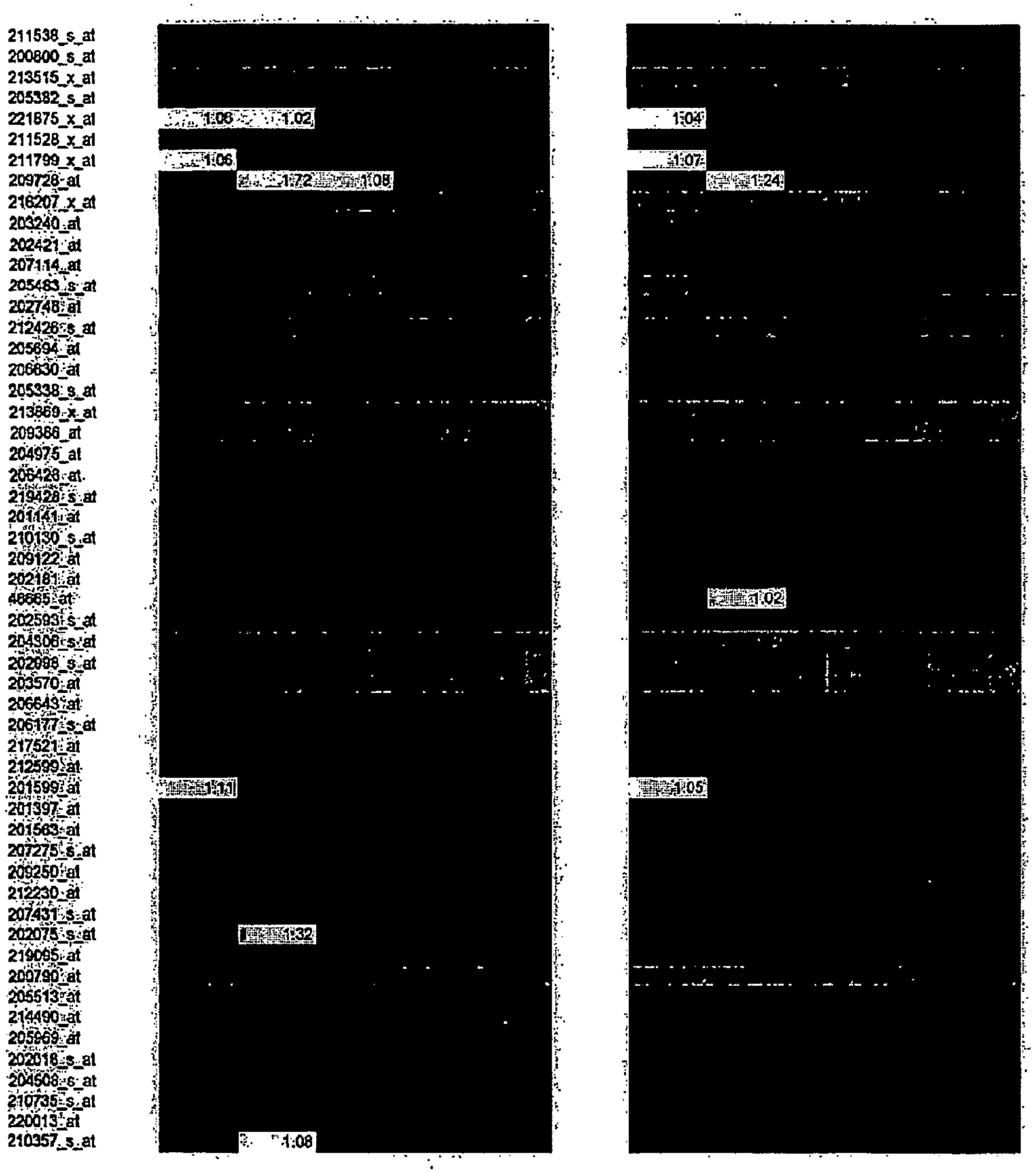
207710_at
220635_at
210085_s_at
207720_at
215704_at
206004_at
206884_s_at
219561_at
212702_s_at
217819_at
213533_at
205020_s_at
218150_at
217852_s_at
209050_s_at

Figure 6F

adenylate kinase 3
DNA-damage-inducible transcript 4
fibronectin 1
spondin 2, extracellular matrix protein
collagen, type XI, alpha 1
collagen, type V, alpha 3
thrombospondin 1
syndecan 2 (heparan sulfate proteoglycan 1, cell surface-associated, fibroglycan)
collagen, type IV, alpha 2
biglycan
fibronectin 1
tuftelin 1
microfibrillar-associated protein 4
chitinase 3-like 2
cartilage oligomeric matrix protein
chondroitin sulfate proteoglycan 2 (versican)
fibulin 2
dermatopontin
lactate dehydrogenase B
aldolase C, fructose-bisphosphate
aldo-keto reductase family 1, member B1 (aldose reductase)
thioredoxin interacting protein
aldehyde dehydrogenase 3 family, memberA1
aldehyde dehydrogenase 4 family, member A1
aldehyde dehydrogenase 3 family, member B2
transketolase (Wernicke-Korsakoff syndrome)
P450 (cytochrome) oxidoreductase
S100 calcium binding protein A2
S100 calcium binding protein A6 (calcyclin)
small proline-rich protein 3
S100 calcium binding protein A12 (calgranulin C)
S100 calcium binding protein A13
small proline-rich protein 2B
small proline-rich protein 1A
S100 calcium binding protein A11 (calgizzarin)
S100 calcium binding protein A10 (annexin II ligand, calpactin I, light polypeptide (p11))
S100 calcium binding protein A4 (calcium protein, calvasculin, metastasin, murine placental homolog)
calmodulin-like 5
ARS component B
small proline rich-like (epidermal differentiation complex) 1B
psoriasis susceptibility 1 candidate 2
annexin A9
loricrin
filaggrin
transglutaminase 3 (E polypeptide, protein-glutamine-gamma-glutamyltransferase)
sciellin
coatomer protein complex, subunit zeta 2
bicaudal D homolog 2 (Drosophila)
golgi autoantigen, golgin subfamily a, 7
DNA segment on chromosome 4 (unique) 234 expressed sequence
ADP-ribosylation factor-like 4
ADP-ribosylation factor-like 5
ADP-ribosylation factor-like 10C
rat guanine nucleotide dissociation stimulator

Figure 6G

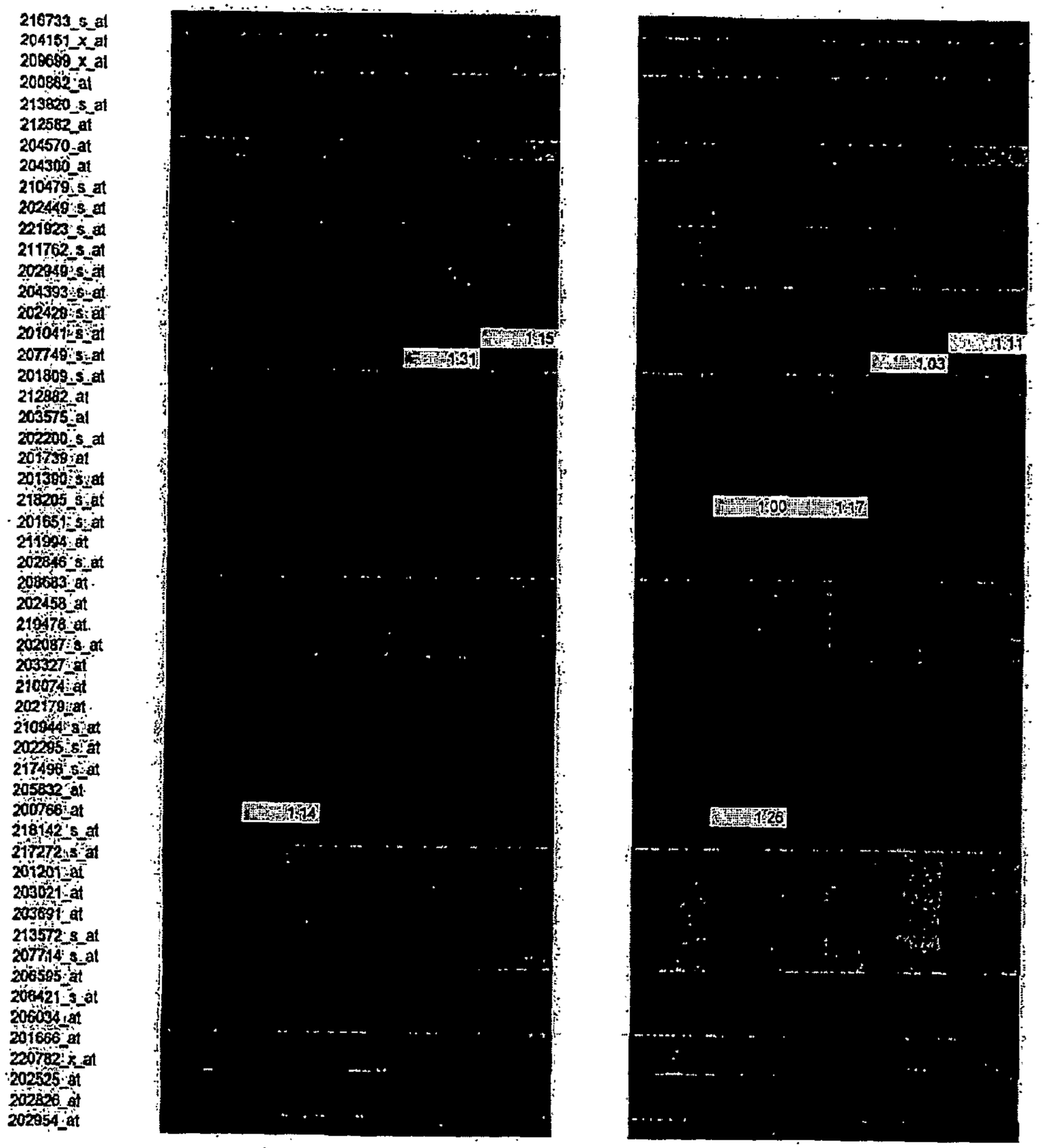


Heat shock, chaperone	*HSPA2*
Heat shock, chaperone	*HSPA1A*
Hemoglobin	*HBG2*
Immune response, compl	*DF*
Immune response, HLA r	*HLA-F*
Immune response, HLA r	*HLA-A*
Immune response, HLA r	*HLA-C*
Immune response, HLA r	*HLA-DRB4*
Immunoglobulin	*IGKV1D-13*
Immunoglobulin	*FCGBP*
Immunoglobulin	*IGSF3*
Immunoglobulin	*LY6G6C*
Interferon-regulated	*G1P2*
Interferon-regulated	*GBP2*
Melanogenesis	*YWHAQ*
Melanogenesis	*TYRP1*
Melanogenesis	*TYR*
Melanogenesis	*DCT*
Membrane protein	*THY1*
Membrane protein	*TM4SF1*
Membrane protein	*EMP2*
Membrane protein	*MLANA*
Membrane protein	*PXMP4*
Membrane protein	*GPNMB*
Membrane protein	*TM7SF2*
Membrane protein	*ADFP*
Membrane protein	*KIAA0247*
Membrane protein	*SEMA4C*
Membrane protein	*MIR16*
Membrane/cell surface	*CD151*
Metabolism, amino acid	*LOXL2*
Metabolism, amino acid	*LOXL1*
Metabolism, amino acid	*HAL*
Metabolism, amino acid	*ARG1*
Metabolism, amino acid	*HAL*
Metabolism, amino acid	*AUTS2*
Metabolism, amino acid	*OAT*
Metabolism, amino acid	*PHGDH*
Metabolism, carbohydrat	*SORD*
Metabolism, lipid	*ACSL1*
Metabolism, lipid	*DEGS*
Metabolism, lipid	*PPAP2B*
Metabolism, lipid	*DEGS*
Metabolism, lipid	*PLTP*
Metabolism, lipid	*PLA2G4B*
Metabolism, other	*ODC1*
Metabolism, other	*TCN1*
Metabolism, other	*ARSF*
Metabolism, other	*AADAC*
Metabolism, other	*LTF*
Metabolism, other	*CA12*
Metabolism, other	*CA12*
Metabolism, other	*ABHD9*
Metabolism, other	*SMOX*

Figure 6H

heat shock 70kDa protein 2
heat shock 70kDa protein 1A
hemoglobin, gamma G
D component of complement (adipsin)
major histocompatibility complex, class I, F
major histocompatibility complex, class I, A
major histocompatibility complex, class I, C
major histocompatibility complex, class II, DR beta 4
Immunoglobulin kappa variable 1D-13
Fc fragment of IgG binding protein
immunoglobulin superfamily, member 3
lymphocyte antigen 6 complex, locus G6C
interferon, alpha-inducible protein (clone IFI-15K)
guanylate binding protein 2, interferon-inducible
tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, theta polypeptide
tyrosinase-related protein 1
tyrosinase (oculocutaneous albinism IA)
dopachrome tautomerase (dopachrome delta-isomerase, tyrosine-related protein 2)
Thy-1 cell surface antigen
transmembrane 4 superfamily member 1
epithelial membrane protein 2
melan-A
peroxisomal membrane protein 4, 24kDa
glycoprotein (transmembrane) nmb
transmembrane 7 superfamily member 2
adipose differentiation-related protein
KIAA0247
sema domain, immunoglobulin domain (Ig), transmembrane domain (TM) and short cytoplasmic domain, (semaphorin) 4C
membrane interacting protein of RGS16
CD151 antigen
lysyl oxidase-like 2
lysyl oxidase-like 1
histidine ammonia-lyase
arginase, liver
histidine ammonia-lyase
autism susceptibility candidate 2
ornithine aminotransferase (gyrate atrophy)
phosphoglycerate dehydrogenase
sorbitol dehydrogenase
acyl-CoA synthetase long-chain family member 1
degenerative spermatocyte homolog, lipid desaturase (Drosophila)
phosphatidic acid phosphatase type 2B
degenerative spermatocyte homolog, lipid desaturase (Drosophila)
phospholipid transfer protein
phospholipase A2, group IVB (cytosolic)
ornithine decarboxylase 1
transcobalamin I (vitamin B12 binding protein, R binder family)
arylsulfatase F
arylacetamide deacetylase (esterase)
lactotransferrin
carbonic anhydrase XII
carbonic anhydrase XII
abhydrolase domain containing 9
spermine oxidase

Figure 6I



Metabolism, other	*GATM*
Metabolism, steroid	*AKR1C1*
Metabolism, steroid	*AKR1C2*
Metabolism, steroid	*DHCR24*
Metabolism, steroid	*STARD5*
Metabolism, steroid	*OSBPL8*
Mitochondrial	*COX7A1*
Mitochondrial	*PET112L*
Nuclear receptor/RA	*RORA*
Nuclear receptor/RA	*RXRA*
Nucleoskeletal	*NPM1*
Nucleoskeletal	*KPNA2*
Oncogenesis	*FHL2*
Phosphatase	*ACPP*
Phosphatase	*PPP3CA*
Phosphatase	*DUSP1*
Phosphatase	*PPP2R3A*
Protein binding	*ENG*
Protein binding	*KIAA0795*
Protein kinase	*CSNK2A2*
Protein kinase	*SRPK1*
Protein kinase	*SGK*
Protein kinase	*CSNK2B*
Protein kinase	*MKNK2*
Protein kinase	*PACSIN2*
Protein kinase inhibitor	*PRKWNK1*
Protein modification	*PIGC*
Proteolysis	*CAPN2*
Proteolysis	*SPUVE*
Proteolysis	*WFDC1*
Proteolysis	*CTSL*
Proteolysis	*IDE*
Proteolysis	*CTSL2*
Proteolysis	*BLMH*
Proteolysis	*CAPN3*
Proteolysis	*CTSH*
Proteolysis	*IDE*
Proteolysis	*CPA4*
Proteolysis	*CTSD*
Proteolysis	*LOC51185*
Proteolysis inhibitor	*SERPINB13*
Proteolysis inhibitor	*CSTB*
Proteolysis inhibitor	*SLPI*
Proteolysis inhibitor	*PI3*
Proteolysis inhibitor	*SERPINB1*
Proteolysis inhibitor	*SERPINH1*
Proteolysis inhibitor	*CST6*
Proteolysis inhibitor	*SERPINB7*
Proteolysis inhibitor	*SERPINB8*
Proteolysis inhibitor, extr.	*TIMP1*
Proteolysis, extracellular	*KLK12*
Proteolysis, extracellular	*PRSS8*
Proteolysis, extracellular	*SPINT1*
Proteolysis, ubiquitin	*UBE2C*

Figure 6J

glycine amidinotransferase (L-arginine:glycine amidinotransferase)
aldo-keto reductase family 1, member C1 (dihydrodiol dehydrogenase 1; 20-alpha (3-alpha)-hydroxysteroid dehydrogenase)
aldo-keto reductase family 1, member C2 (dihydrodiol dehydrogenase 2; bile acid binding protein; 3-alpha hydroxysteroid dehydrogenase, type III)
24-dehydrocholesterol reductase
START domain containing 5
oxysterol binding protein-like 8
cytochrome c oxidase subunit VIIa polypeptide 1 (muscle)
PET112-like (yeast)
RAR-related orphan receptor A
retinoid X receptor, alpha
nucleophosmin (nucleolar phosphoprotein B23, numatrin)
karyopherin alpha 2 (RAG cohort 1, importin alpha 1)
four and a half LIM domains 2
acid phosphatase, prostate
protein phosphatase 3 (formerly 2B), catalytic subunit, alpha isoform (calcineurin A alpha)
dual specificity phosphatase 1
protein phosphatase 2 (formerly 2A), regulatory subunit B", alpha
endoglin (Osler-Rendu-Weber syndrome 1)
KIAA0795 protein
casein kinase 2, alpha prime polypeptide
SFRS protein kinase 1
serum/glucocorticoid regulated kinase
casein kinase 2, beta polypeptide
MAP kinase-interacting serine/threonine kinase 2
protein kinase C and casein kinase substrate in neurons 2
protein kinase, lysine deficient 1
phosphatidylinositol glycan, class C
calpain 2, (m/II) large subunit
protease, serine, 23
WAP four-disulfide core domain 1
cathepsin L
insulin-degrading enzyme
cathepsin L2
bleomycin hydrolase
calpain 3, (p94)
cathepsin H
insulin-degrading enzyme
carboxypeptidase A4
cathepsin D (lysosomal aspartyl protease)
protein x 0001
serine (or cysteine) proteinase inhibitor, clade B (ovalbumin), member 13
cystatin B (stefin B)
secretory leukocyte protease inhibitor (antileukoproteinase)
protease inhibitor 3, skin-derived (SKALP)
serine (or cysteine) proteinase inhibitor, clade B (ovalbumin), member 1
serine (or cysteine) proteinase inhibitor, clade H (heat shock protein 47), member 1, (collagen binding protein 1)
cystatin E/M
serine (or cysteine) proteinase inhibitor, clade B (ovalbumin), member 7
serine (or cysteine) proteinase inhibitor, clade B (ovalbumin), member 8
tissue inhibitor of metalloproteinase 1 (erythroid potentiating activity, collagenase inhibitor)
kallikrein 12
protease, serine, 8 (prostasin)
serine protease inhibitor, Kunitz type 1
ubiquitin-conjugating enzyme E2C

Figure 6K

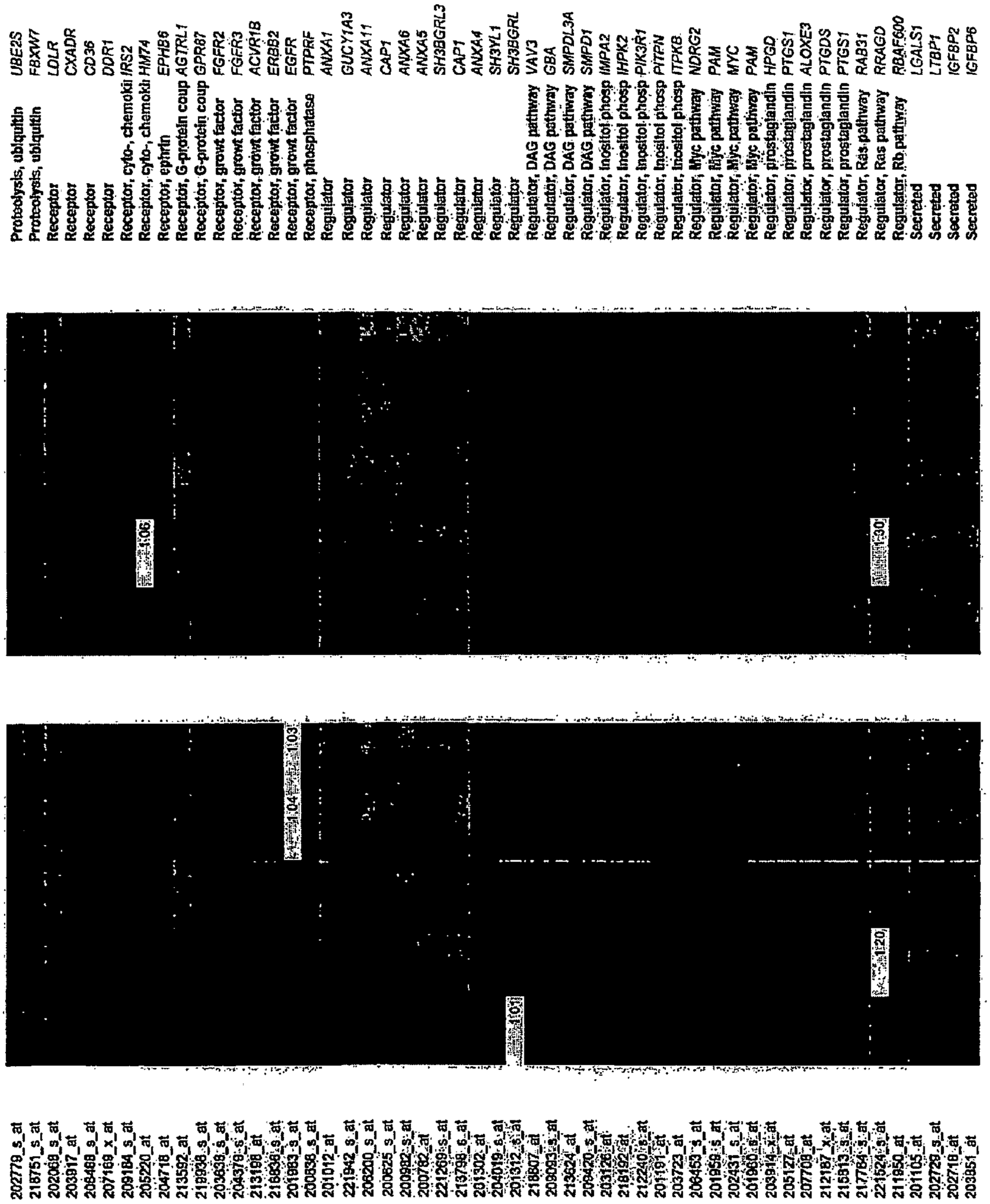
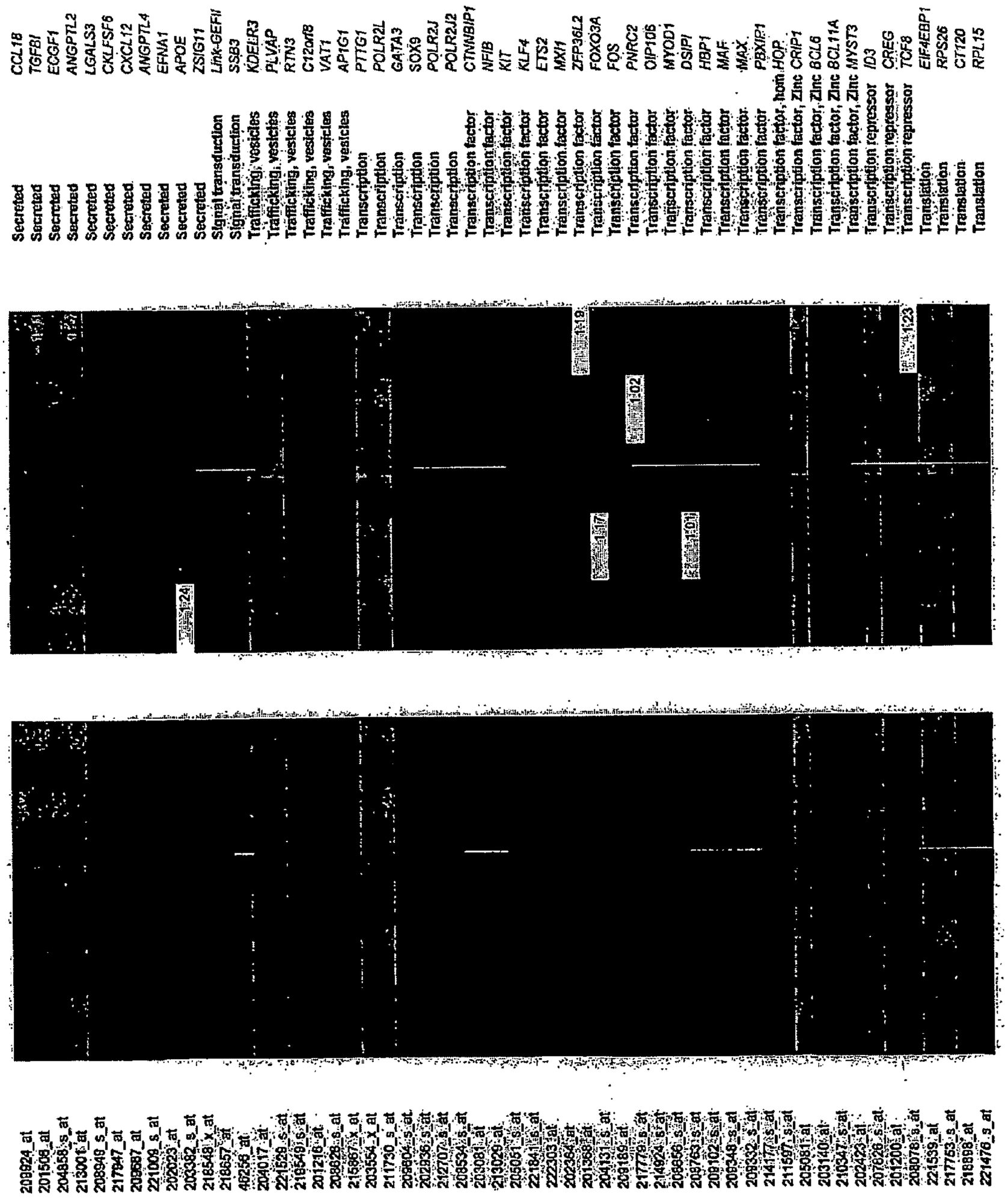

Proteolysis, ubiquitin UBE2S
Proteolysis, ubiquitin FBXW7
Receptor LDLR
Receptor CXADR
Receptor CD36
Receptor DDR1
Receptor, cyto-, chemokii IRS2
Receptor, cyto-, chemokii HM74
Receptor, ephrin EPHB6
Receptor, G-protein coup AGTRL1
Receptor, G-protein coup GPR87
Receptor, growt factor FGFR2
Receptor, growt factor FGFR3
Receptor, growt factor ACVR1B
Receptor, growt factor ERBB2
Receptor, growt factor EGFR
Receptor, phosphatase PTPRF
Regulator ANXA1
Regulator GUCY1A3
Regulator ANXA11
Regulator CAP1
Regulator ANXA6
Regulator ANXA5
Regulator SH3BGRL3
Regulator CAP1
Regulator ANXA4
Regulator SH3YL1
Regulator SH3BGRL
Regulator, DAG pathway VAV3
Regulator, DAG pathway GBA
Regulator, DAG pathway SMPDL3A
Regulator, DAG pathway SMPD1
Regulator, inositol phosp IMPA2
Regulator, inositol phosp IHPK2
Regulator, inositol phosp PIK3R1
Regulator, inositol phosp PITPN
Regulator, inositol phosp ITPK8
Regulator, Myc pathway NDRG2
Regulator, Myc pathway PAM
Regulator, Myc pathway MYC
Regulator, Myc pathway PAM
Regulator, prostaglandin HPGD
Regulator, prostaglandin PTGS1
Regulator, prostaglandin ALOXE3
Regulator, prostaglandin PTGDS
Regulator, prostaglandin PTGS1
Regulator, Ras pathway RAB31
Regulator, Ras pathway RRAGD
Regulator, Rb pathway RBAF600
Secreted LGALS1
Secreted LTBP1
Secreted IGFBP2
Secreted IGFBP6
106
103
104
101
120
130
202779_s_at
218751_s_at
202068_s_at
203917_at
208488_s_at
207169_x_at
209184_s_at
205220_at
204718_at
213592_at
219308_s_at
203638_s_at
204379_s_at
213198_at
218836_s_at
201983_s_at
200838_s_at
201012_at
221942_s_at
206200_s_at
200625_s_at
200992_s_at
200782_at
221269_s_at
213798_s_at
201302_at
204019_s_at
201312_s_at
218807_at
208093_s_at
213624_at
209420_s_at
203126_at
218192_at
212240_s_at
201101_at
203723_at
208453_s_at
201959_s_at
202431_s_at
201960_s_at
203914_x_at
205127_at
207708_at
212187_x_at
215313_s_at
217784_s_at
221524_s_at
211950_at
201105_at
202729_s_at
202718_at
203851_at

Figure 6L

ubiquitin-conjugating enzyme E2S
F-box and WD-40 domain protein 7 (archipelago homolog, Drosophila)
low density lipoprotein receptor (familial hypercholesterolemia)
coxsackie virus and adenovirus receptor
CD38 antigen (collagen type I receptor, thrombospondin receptor)
discoidin domain receptor family, member 1
insulin receptor substrate 2
putative chemokine receptor
EphB6
angiotensin II receptor-like 1
G protein-coupled receptor 87
fibroblast growth factor receptor 2 (bacteria-expressed kinase, keratinocyte growth factor receptor, craniofacial dysostosis 1, Crouzon syndrome, Pfeiffer syndrome, Jackson-Weiss syndrome)
fibroblast growth factor receptor 3 (achondroplasia, thanatophoric dwarfism)
activin A receptor, type IB
v-erb-b2 erythroblastic leukemia viral oncogene homolog 2, neuro/glioblastoma derived oncogene homolog (avian)
epidermal growth factor receptor (erythroblastic leukemia viral (v-erb-b) oncogene homolog, avian)
protein tyrosine phosphatase, receptor type, F
annexin A1
guanylate cyclase 1, soluble, alpha 3
annexin A11
CAP, adenylate cyclase-associated protein 1 (yeast)
annexin A6
annexin A5
SH3 domain binding glutamic acid-rich protein like 3
CAP, adenylate cyclase-associated protein 1 (yeast)
annexin A4
SH3 domain containing, Ysc84-like 1 (S. cerevisiae)
SH3 domain binding glutamic acid-rich protein like
vav 3 oncogene
glucosidase, beta; acid (includes glucosylceramidase)
sphingomyelin phosphodiesterase, acid-like 3A
sphingomyelin phosphodiesterase 1, acid lysosomal (acid sphingomyelinase)
inositol(myo)-1(or 4)-monophosphatase 2
inositol hexaphosphate kinase 2
phosphoinositide-3-kinase, regulatory subunit, polypeptide 1 (p85 alpha)
phosphatidylinositol transfer protein
inositol 1,4,5-trisphosphate 3-kinase B
NDRG family member 2
protein associated with Myc
v-myc myelocytomatosis viral oncogene homolog (avian)
protein associated with Myc
hydroxyprostaglandin dehydrogenase 15-(NAD)
prostaglandin-endoperoxide synthase 1 (prostaglandin G/H synthase and cyclooxygenase)
arachidonate lipoxygenase 3
prostaglandin D2 synthase 21kDa (brain)
prostaglandin-endoperoxide synthase 1 (prostaglandin G/H synthase and cyclooxygenase)
RAB31, member RAS oncogene family
Ras-related GTP binding D
retinoblastoma-associated factor 600
lectin, galactoside-binding, soluble, 1 (galectin 1)
latent transforming growth factor beta binding protein 1
insulin-like growth factor binding protein 2, 36kDa
insulin-like growth factor binding protein 6

Figure 6M


Secreted CCL18
Secreted TGFBI
Secreted ECGF1
Secreted ANGPTL2
Secreted LGALS3
Secreted CKLFSF6
Secreted CXCL12
Secreted ANGPTL4
Secreted EFNA1
Secreted APOE
Secreted ZSIG11
Signal transduction Link-GEFII
Signal transduction SSB3
Trafficking, vesicles KDELR3
Trafficking, vesicles PLVAP
Trafficking, vesicles RTN3
Trafficking, vesicles C12orf8
Trafficking, vesicles VAT1
Trafficking, vesicles AP1G1
Transcription PTTG1
Transcription POLR2L
Transcription GATA3
Transcription SOX9
Transcription POLR2J
Transcription POLR2J2
Transcription factor CTNNBIP1
Transcription factor NFIB
Transcription factor KIT
Transcription factor KLF4
Transcription factor ETS2
Transcription factor MXI1
Transcription factor ZFP36L2
Transcription factor FOXO3A
Transcription factor FOS
Transcription factor PNRC2
Transcription factor OIP106
Transcription factor MYOD1
Transcription factor DSIPI
Transcription factor HBP1
Transcription factor MAF
Transcription factor MAX
Transcription factor PBXIP1
Transcription factor, hom HOP
Transcription factor, Zinc CRIP1
Transcription factor, Zinc BCL6
Transcription factor, Zinc BCL11A
Transcription factor, Zinc MYST3
Transcription repressor ID3
Transcription repressor CREG
Transcription repressor TCF8
Translation EIF4EBP1
Translation RPS26
Translation CT120
Translation RPL15
1:24
1:19
1:02
1:17
1:01
1:23
209924_at
201506_at
204858_s_at
213001_at
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221009_s_at
202023_at
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46256_at
204017_at
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201216_at
208828_s_at
215867_x_at
203554_x_at
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202936_s_at
212707_s_at
208534_s_at
203081_at
213029_at
205051_s_at
221841_s_at
222303_at
202364_at
201368_at
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209189_at
217778_s_at
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208856_s_at
208763_s_at
209102_s_at
209348_s_at
209332_s_at
214177_s_at
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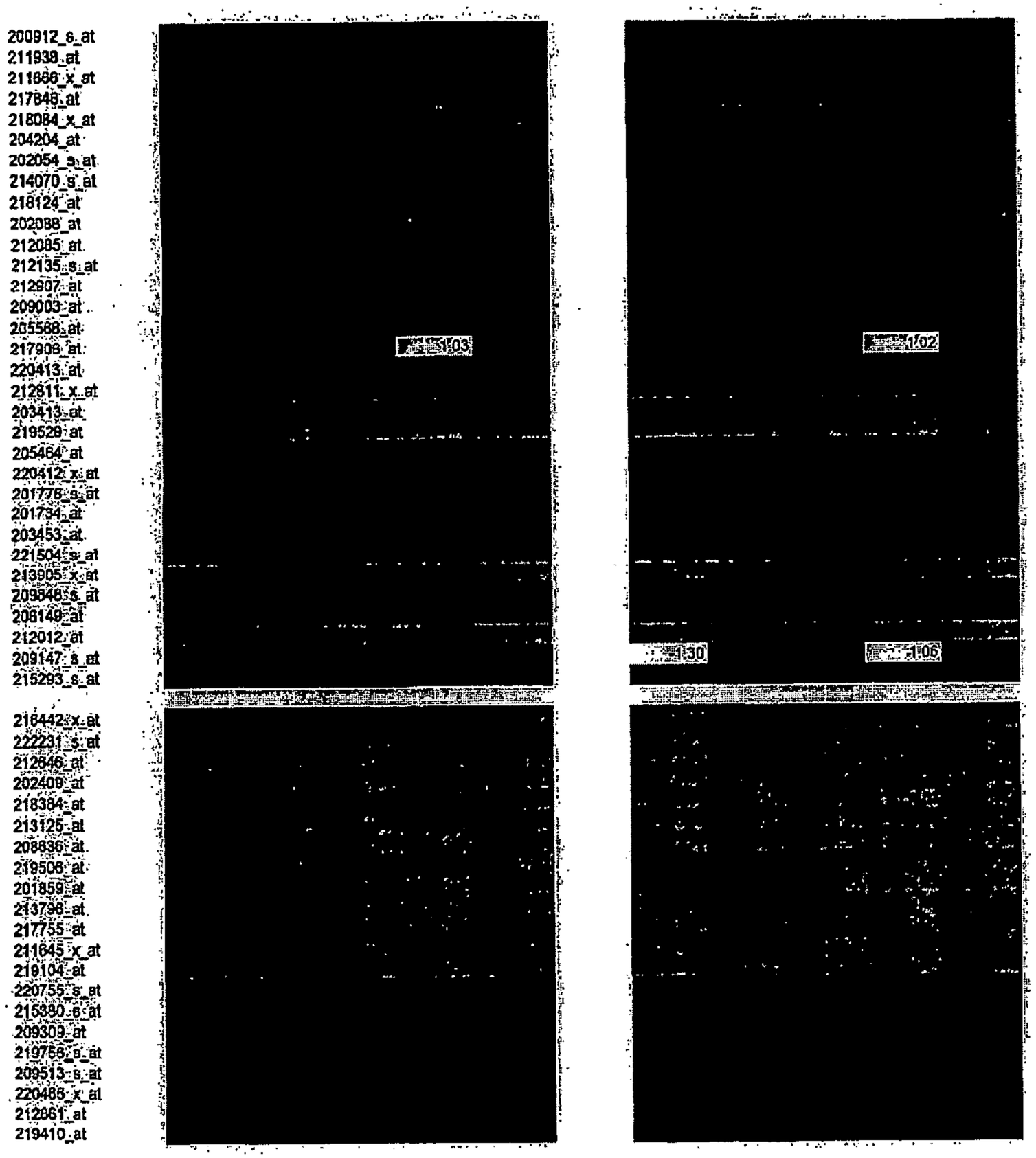
205081_at
203140_at
210347_s_at
202423_at
207626_s_at
201200_at
208078_s_at
221539_at
217763_s_at
218898_at
221476_s_at

Figure 6N

chemokine (C-C motif) ligand 18 (pulmonary and activation-regulated)
transforming growth factor, beta-induced, 68kDa
endothelial cell growth factor 1 (platelet-derived)
angiopoietin-like 2
lectin, galactoside-binding, soluble, 3 (galectin 3)
chemokine-like factor super family 6
chemokine (C-X-C motif) ligand 12 (stromal cell-derived factor 1)
angiopoietin-like 4
ephrin-A1
apolipoprotein E
putative secreted protein ZSIG11
Link guanine nucleotide exchange factor II
SPRY domain-containing SOCS box protein SSB-3
KDEL (Lys-Asp-Glu-Leu) endoplasmic reticulum protein retention receptor 3
plasmalemma vesicle associated protein
reticulon 3
chromosome 12 open reading frame 8
vesicle amine transport protein 1 homolog (T californica)
adaptor-related protein complex 1, gamma 1 subunit
pituitary tumor-transforming 1
polymerase (RNA) II (DNA directed) polypeptide L, 7.6kDa
GATA binding protein 3
SRY (sex determining region Y)-box 9 (campomelic dysplasia, autosomal sex-reversal)
polymerase (RNA) II (DNA directed) polypeptide J, 13.3kDa
DNA directed RNA polymerase II polypeptide J-related gene
catenin, beta interacting protein 1
nuclear factor I/B
v-kit Hardy-Zuckerman 4 feline sarcoma viral oncogene homolog
Kruppel-like factor 4 (gut)
v-ets erythroblastosis virus E26 oncogene homolog 2 (avian)
MAX interacting protein 1
zinc finger protein 36, C3H type-like 2
forkhead box O3A
v-fos FBJ murine osteosarcoma viral oncogene homolog
proline-rich nuclear receptor coactivator 2
OGT(O-Glc-NAc transferase)-interacting protein 106 KDa
myogenic factor 3
delta sleep inducing peptide, immunoreactor
HMG-box transcription factor 1
v-maf musculoaponeurotic fibrosarcoma oncogene homolog (avian)
MAX protein
pre-B-cell leukemia transcription factor interacting protein 1
homeodomain only protein
cysteine-rich protein 1 (intestinal)
B-cell CLL/lymphoma 6 (zinc finger protein 51)
B-cell CLL/lymphoma 11A (zinc finger protein)
MYST histone acetyltransferase (monocytic leukemia) 3
inhibitor of DNA binding 3, dominant negative helix-loop-helix protein
cellular repressor of E1A-stimulated genes
transcription factor 8 (represses interleukin 2 expression)
eukaryotic translation initiation factor 4E binding protein 1
ribosomal protein S26
membrane protein expressed in epithelial-like lung adenocarcinoma
ribosomal protein L15

Figure 6O



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211866_x_at	Translation	RPL3
217848_at	Translation	QARS
218084_x_at	Transporter	FXYD5
204204_at	Transporter	SLC31A2
202054_s_at	Transporter	ALDH3A2
214070_s_at	Transporter	ATP10B
218124_at	Transporter	FLJ20296
202088_at	Transporter	SLC39A6
212085_at	Transporter	SLC25A6
212135_s_at	Transporter	ATP2B4
212907_at	Transporter	SLC30A1
209003_at	Transporter	SLC25A11
205568_at	Transporter	AQP9
217909_at	Transporter	KLHDC2
220413_at	Transporter	SLC39A2
212811_x_at	Transporter	SLC1A4
203413_at	Transporter, channel	NELL2
219528_at	Transporter, channel	CLIC3
205464_at	Transporter, channel	SCNN1B
220412_x_at	Transporter, channel	KCNK7
201776_s_at	Transporter, channel	KIAA0494
201734_at	Transporter, channel	CLCN3
203453_at	Transporter, channel	SCNN1A
221504_s_at	Transporter, lysosome	ATP6V1H
213905_x_at	Tumor antigen	SDCCAG33
209848_s_at	Tumor antigen	SILV
208149_at	Tumor antigen	LOC63928
212012_at	Tumor antigen, melanom	D2S448
209147_s_at	Tumor suppressor	PPAP2A
215293_s_at	Tumor suppressor	FRAG1
	Unknown/Hypothetical	
216442_x_at	Unknown/Hypothetical	PRO1855
222231_s_at	Unknown/Hypothetical	RAFTLIN
212646_at	Unknown/Hypothetical	
202409_at	Unknown/Hypothetical	CARHSP1
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208836_at	Unknown/Hypothetical	FLJ23221
219506_at	Unknown/Hypothetical	PRG1
201859_at	Unknown/Hypothetical	
213796_at	Unknown/Hypothetical	HN1
217755_at	Unknown/Hypothetical	
211645_x_at	Unknown/Hypothetical	RNF141
219104_at	Unknown/Hypothetical	C6orf48
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209309_at	Unknown/Hypothetical	POF1B
219755_s_at	Unknown/Hypothetical	MGC10940
209513_s_at	Unknown/Hypothetical	FLJ22679
220485_x_at	Unknown/Hypothetical	MGC11308
212861_at	Unknown/Hypothetical	FLJ10134
219410_at		

Figure 6P

eukaryotic translation initiation factor 4A, isoform 2
eukaryotic translation initiation factor 4B
ribosomal protein L3
glutaminyl-tRNA synthetase
FXYD domain containing ion transport regulator 5
solute carrier family 31 (copper transporters), member 2
aldehyde dehydrogenase 3 family, member A2
ATPase, Class V, type 10B
hypothetical protein FLJ20298
solute carrier family 39 (zinc transporter), member 6
solute carrier family 25 (mitochondrial carrier; adenine nucleotide translocator), member 6
ATPase, Ca++ transporting, plasma membrane 4
solute carrier family 30 (zinc transporter), member 1
solute carrier family 25 (mitochondrial carrier; oxoglutarate carrier), member 11
aquaporin 9
kelch domain containing 2
solute carrier family 39 (zinc transporter), member 2
solute carrier family 1 (glutamate/neutral amino acid transporter), member 4
NEL-like 2 (chicken)
chloride intracellular channel 3
sodium channel, nonvoltage-gated 1, beta (Liddle syndrome)
potassium channel, subfamily K, member 7
KIAA0494 gene product
chloride channel 3
sodium channel, nonvoltage-gated 1 alpha
ATPase, H+ transporting, lysosomal 50/57kDa, V1 subunit H
serologically defined colon cancer antigen 33
silver homolog (mouse)
hepatocellular carcinoma antigen gene 520
Melanoma associated gene
phosphatidic acid phosphatase type 2A
FGF receptor activating protein 1

hypothetical protein PRO1855
raft-linking protein

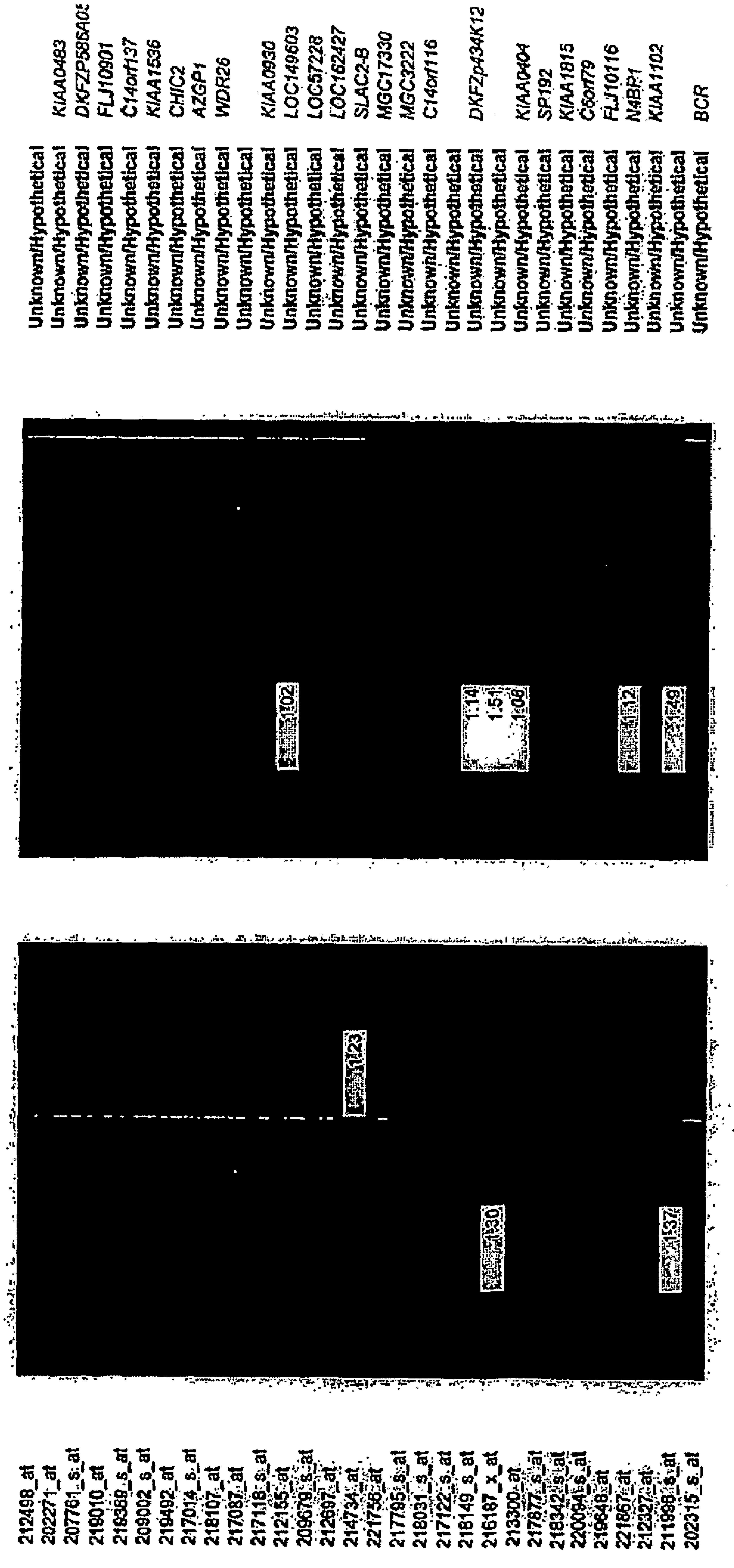
calcium regulated heat stable protein 1, 24kDa
DKFZP586L151 protein

hypothetical protein FLJ23221
proteoglycan 1, secretory granule

hematological and neurological expressed 1

ring finger protein 141
chromosome 6 open reading frame 48
chromosome 7 open reading frame 24
alpha-2-glycoprotein 1, zinc
premature ovarian failure 1B
hypothetical protein MGC10940
hypothetical protein FLJ22679
hypothetical protein MGC11308
hypothetical protein FLJ10134

Figure 6Q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217118_s_at
212155_at
209679_s_at
212697_at
214734_at
221756_at
217795_s_at
218031_s_at
217122_s_at
218149_s_at
216187_x_at
213300_at
217877_s_at
218342_s_at
220094_s_at
219648_at
221867_at
212327_at
211998_s_at
202315_s_at
1.02
1.14
1.51
1.08
1.12
1.49
1.23
1.30
1.37
Unknown/Hypothetical
Unknown/Hypothetical KIAA0483
Unknown/Hypothetical DKFZP586A05
Unknown/Hypothetical FLJ10901
Unknown/Hypothetical C14orf137
Unknown/Hypothetical KIAA1536
Unknown/Hypothetical CHIC2
Unknown/Hypothetical AZGP1
Unknown/Hypothetical WDR26
Unknown/Hypothetical
Unknown/Hypothetical KIAA0930
Unknown/Hypothetical LOC149603
Unknown/Hypothetical LOC57228
Unknown/Hypothetical LOC162427
Unknown/Hypothetical SLAC2-B
Unknown/Hypothetical MGC17330
Unknown/Hypothetical MGC3222
Unknown/Hypothetical C14orf116
Unknown/Hypothetical
Unknown/Hypothetical DKFZp434K12
Unknown/Hypothetical
Unknown/Hypothetical KIAA0404
Unknown/Hypothetical SP192
Unknown/Hypothetical KIAA1815
Unknown/Hypothetical C6orf79
Unknown/Hypothetical FLJ10116
Unknown/Hypothetical N4BP1
Unknown/Hypothetical KIAA1102
Unknown/Hypothetical
Unknown/Hypothetical BCR

Figure 6R

KIAA0483 protein
DKFZP586A0522 protein
hypothetical protein FLJ10901
chromosome 14 open reading frame 137
KIAA1536 protein
cysteine-rich hydrophobic domain 2
alpha-2-glycoprotein 1, zinc
WD repeat domain 26

KIAA0930 protein
hypothetical protein LOC149603
hypothetical protein from clone 643
hypothetical protein LOC162427
SLAC2-B
HGFL gene
hypothetical protein MGC3222
chromosome 14 open reading frame 116

hypothetical protein DKFZp434K1210

KIAA0404 protein
hypothetical protein SP192
KIAA1815
chromosome 6 open reading frame 79
hypothetical protein FLJ10116
Nedd4 binding protein 1
KIAA1102 protein

breakpoint cluster region

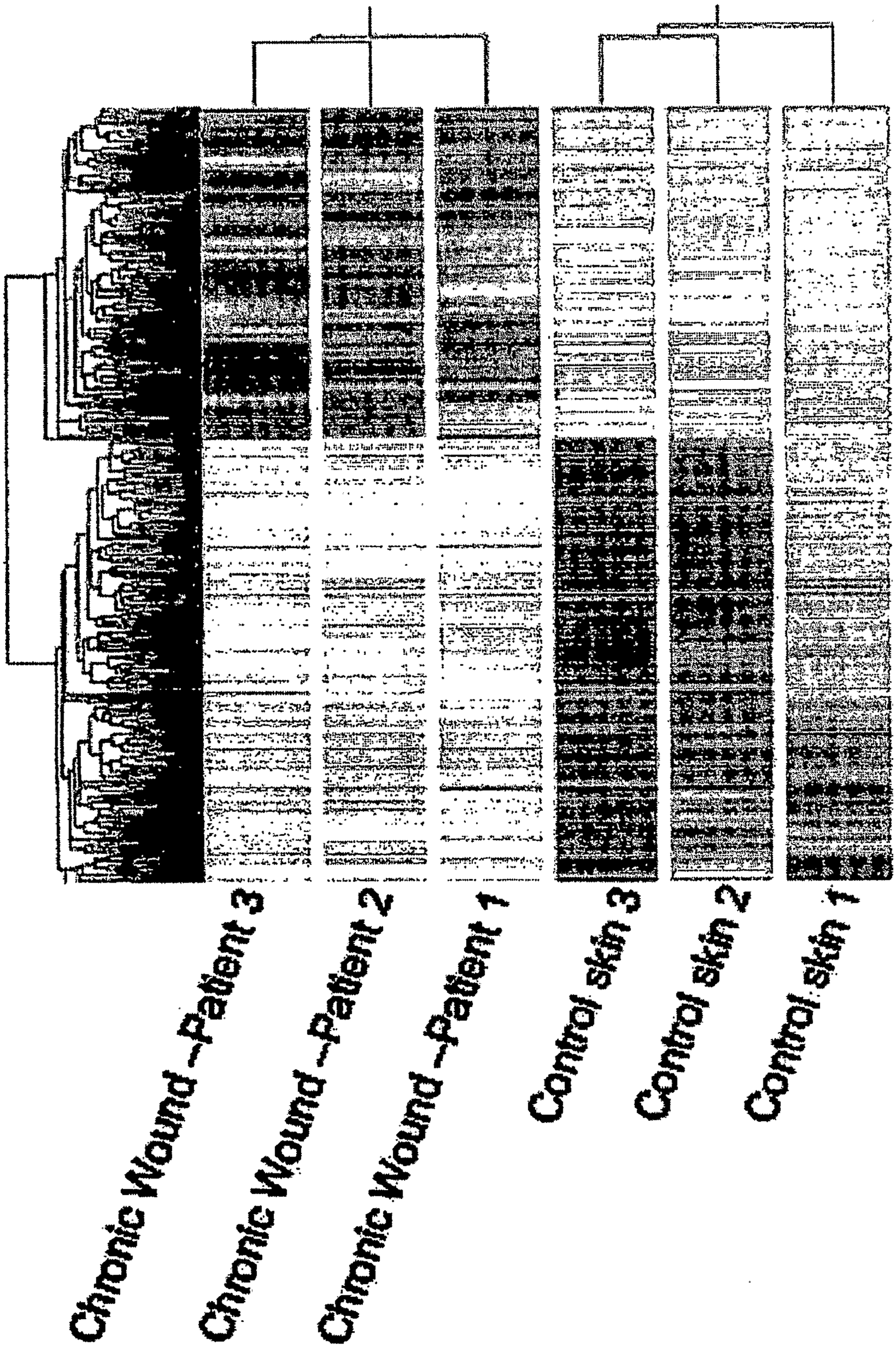

Chronic Wound -Patient 3
Chronic Wound -Patient 2
Chronic Wound -Patient 1
Control skin 3
Control skin 2
Control skin 1

Figure 7

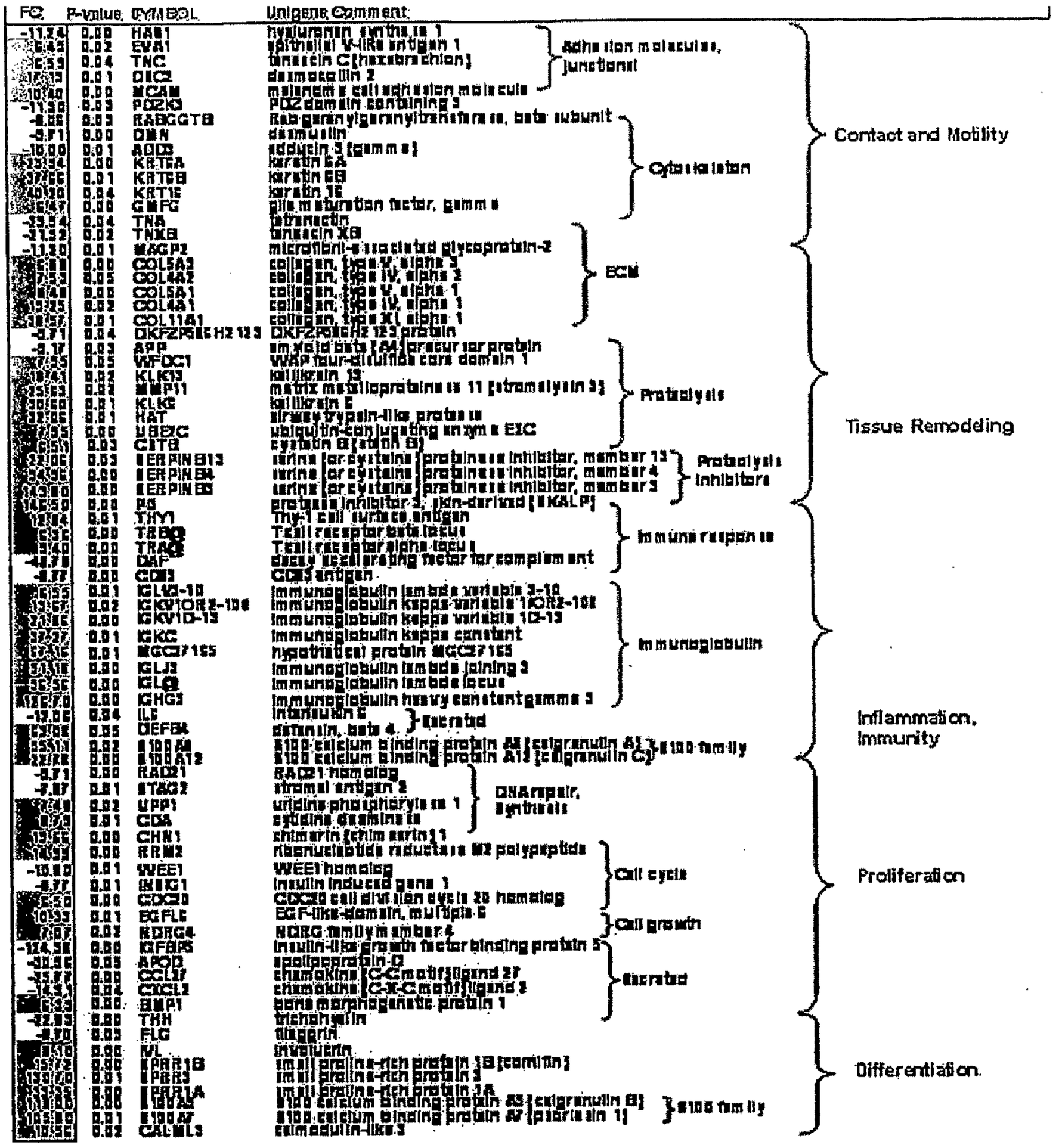

FC
P-value
SYMBOL
Unigene Comment
HAS1
hyaluronan synthase 1
EVA1
epithelial V-like antigen 1
TNC
tenascin C (hexabrachion)
DSC2
desmocollin 2
MCAM
melanoma cell adhesion molecule
Adhesion molecules, junctional
PDZK3
PDZ domain containing 3
RABGGTB
Rab geranylgeranyltransferase, beta subunit
DMN
desmuslin
ADD3
adducin 3 (gamma)
KRT6A
keratin 6A
KRT6B
keratin 6B
KRT16
keratin 16
GMFG
glia maturation factor, gamma
Cytoskeleton
TNA
tetranectin
TNXB
tenascin XB
MAGP2
microfibril-associated glycoprotein-2
COL5A2
collagen, type V, alpha 2
COL4A2
collagen, type IV, alpha 2
COL5A1
collagen, type V, alpha 1
COL4A1
collagen, type IV, alpha 1
COL11A1
collagen, type XI, alpha 1
ECM
DKFZP586H2123
DKFZP586H2123 protein
APP
amyloid beta (A4) precursor protein
WFDC1
WAP four-disulfide core domain 1
KLK13
kallikrein 13
MMP11
matrix metalloproteinase 11 (stromelysin 3)
KLK6
kallikrein 6
HAT
airway trypsin-like protease
UBE2C
ubiquitin-conjugating enzyme E2C
CSTB
cystatin B (stefin B)
Proteolysis
SERPINB13
serine (or cysteine) proteinase inhibitor, member 13
SERPINB4
serine (or cysteine) proteinase inhibitor, member 4
SERPINB3
serine (or cysteine) proteinase inhibitor, member 3
Proteolysis inhibitors
PI3
protease inhibitor 3, skin-derived (SKALP)
THY1
Thy-1 cell surface antigen
TRBC
T cell receptor beta locus
TRAC
T cell receptor alpha locus
DAF
decay accelerating factor for complement
Immune response
CD53
CD53 antigen
IGLV3-10
immunoglobulin lambda variable 3-10
IGKV1OR2-108
immunoglobulin kappa variable 1OR2-108
IGKV1D-13
immunoglobulin kappa variable 1D-13
IGKC
immunoglobulin kappa constant
MGC27165
hypothetical protein MGC27165
IGLJ3
immunoglobulin lambda joining 3
IGL@
immunoglobulin lambda locus
IGHG3
immunoglobulin heavy constant gamma 3
Immunoglobulin
IL6
interleukin 6
Secreted
DEFB4
defensin, beta 4
S100A8
S100 calcium binding protein A8 (calgranulin A)
S100A12
S100 calcium binding protein A12 (calgranulin C)
S100 family
Inflammation, Immunity
RAD21
RAD21 homolog
STAG2
stromal antigen 2
UPP1
uridine phosphorylase 1
CDA
cytidine deaminase
DNA repair, synthesis
CHN1
chimerin (chimaerin) 1
RRM2
ribonucleotide reductase M2 polypeptide
WEE1
WEE1 homolog
Cell cycle
INSIG1
insulin induced gene 1
CDC20
CDC20 cell division cycle 20 homolog
EGFL6
EGF-like-domain, multiple 6
Cell growth
NDRG4
NDRG family member 4
IGFBP5
insulin-like growth factor binding protein 5
APOD
apolipoprotein D
CCL27
chemokine (C-C motif) ligand 27
CXCL2
chemokine (C-X-C motif) ligand 2
BMP1
bone morphogenetic protein 1
Secreted
Proliferation
TRH
trichohyalin
FLG
filaggrin
IVL
involucrin
SPRR1B
small proline-rich protein 1B (cornifin)
SPRR3
small proline-rich protein 3
SPRR1A
small proline-rich protein 1A
S100A9
S100 calcium binding protein A9 (calgranulin B)
S100A7
S100 calcium binding protein A7 (psoriasin 1)
S100 family
CALML3
calmodulin-like 3
Differentiation
Tissue Remodeling
Contact and Motility

Figure 8a

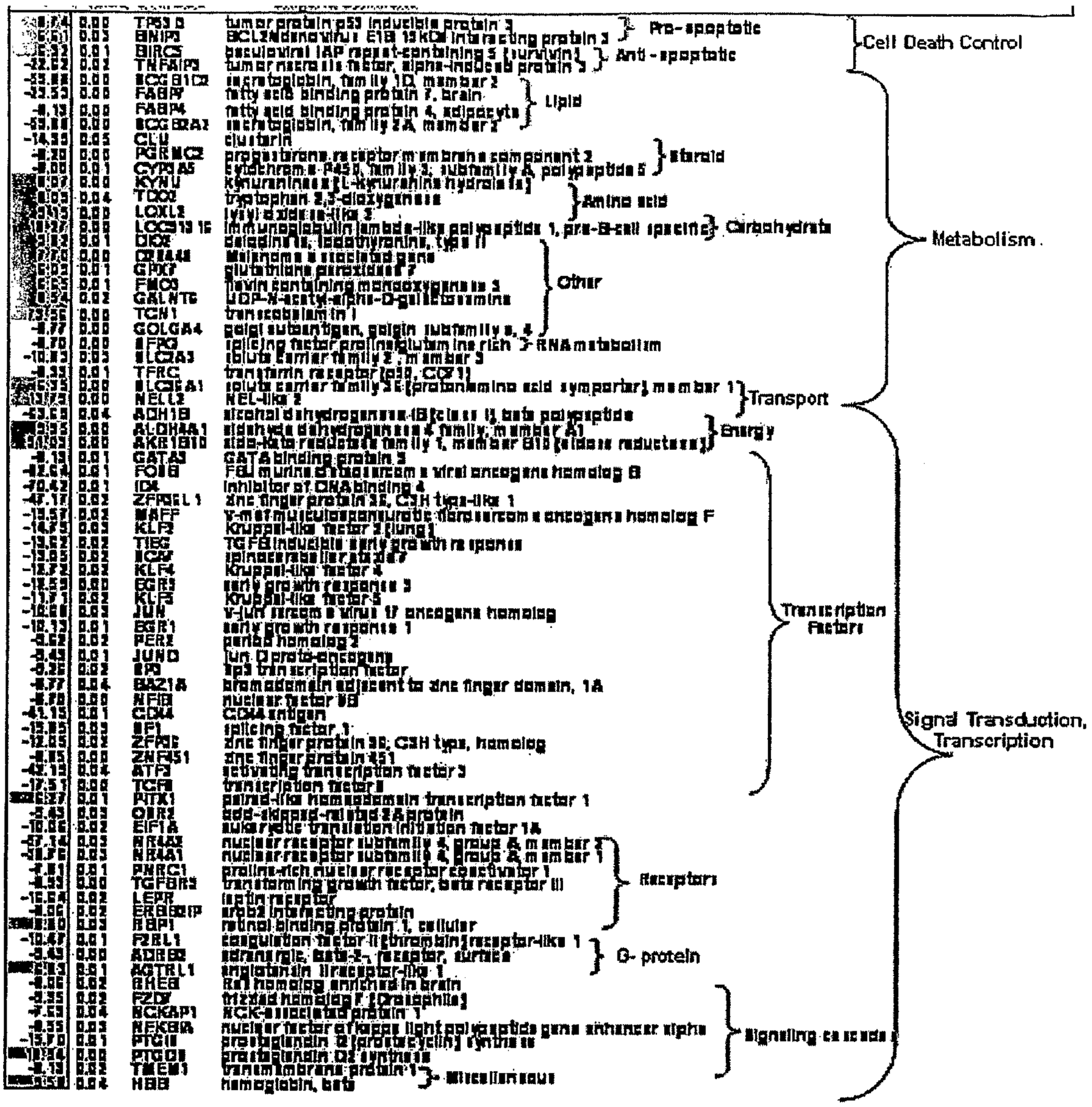

TP53I3 tumor protein p53 inducible protein 3
BNIP3 BCL2/adenovirus E1B 19kDa interacting protein 3
BIRC5 baculoviral IAP repeat-containing 5 (survivin)
TNFAIP3 tumor necrosis factor, alpha-induced protein 3
SCGB1D2 secretoglobin, family 1D, member 2
FABP7 fatty acid binding protein 7, brain
FABP4 fatty acid binding protein 4, adipocyte
SCGB2A2 secretoglobin, family 2A, member 2
CLU clusterin
PGRMC2 progesterone receptor membrane component 2
CYP1A5 cytochrome P450, family 1, subfamily A, polypeptide 5
KYNU kynureninase (L-kynurenine hydrolase)
TDO2 tryptophan 2,3-dioxygenase
LOXL3 lysyl oxidase-like 3
LOC91316 immunoglobulin lambda-like polypeptide 1, pre-B-cell specific
DIO2 deiodinase, iodothyronine, type II
CD44 Melanoma associated gene
GPX7 glutathione peroxidase 7
FMO3 flavin containing monooxygenase 3
GALNT6 UDP-N-acetyl-alpha-D-galactosamine
TCN1 transcobalamin I
GOLGA4 golgi autoantigen, golgin subfamily a, 4
SFRS7 splicing factor proline/glutamine rich
SLC2A3 solute carrier family 2, member 3
TFRC transferrin receptor (p90, CD71)
SLC3A1 solute carrier family 3 (amino acid transporter), member 1
NELL2 NEL-like 2
ADH1B alcohol dehydrogenase 1B (class I), beta polypeptide
ALDH4A1 aldehyde dehydrogenase 4 family, member A1
AKR1B10 aldo-keto reductase family 1, member B10 (aldose reductase)
GATA3 GATA binding protein 3
FOSB FBJ murine osteosarcoma viral oncogene homolog B
ID4 inhibitor of DNA binding 4
ZFP36L1 zinc finger protein 36, C3H type-like 1
MAFF v-maf musculoaponeurotic fibrosarcoma oncogene homolog F
KLF2 Kruppel-like factor 2 (lung)
TIEG TGFB inducible early growth response
ICAM1 intercellular adhesion molecule 1
KLF4 Kruppel-like factor 4
EGR3 early growth response 3
KLF5 Kruppel-like factor 5
JUN v-jun sarcoma virus 17 oncogene homolog
EGR1 early growth response 1
PER2 period homolog 2
JUND jun D proto-oncogene
ELF3 E74-like factor 3
BAZ1A bromodomain adjacent to zinc finger domain, 1A
NFIB nuclear factor IB
CD44 CD44 antigen
SF1 splicing factor 1
ZFP36 zinc finger protein 36, C3H type, homolog
ZNF451 zinc finger protein 451
ATF3 activating transcription factor 3
TCF8 transcription factor 8
PITX1 paired-like homeodomain transcription factor 1
OSR2 odd-skipped related 2 homolog
EIF1A eukaryotic translation initiation factor 1A
NR4A2 nuclear receptor subfamily 4, group A, member 2
NR4A1 nuclear receptor subfamily 4, group A, member 1
PNRC1 proline-rich nuclear receptor coactivator 1
TGFBR3 transforming growth factor, beta receptor III
LEPR leptin receptor
ERBB2IP erbb2 interacting protein
RBP1 retinol binding protein 1, cellular
F2RL1 coagulation factor II (thrombin) receptor-like 1
ADRB2 adrenergic, beta-2-, receptor, surface
AGTRL1 angiotensin II receptor-like 1
RHEB Ras homolog enriched in brain
FZD7 frizzled homolog 7 (Drosophila)
NCKAP1 NCK-associated protein 1
NFKBIA nuclear factor of kappa light polypeptide gene enhancer alpha
PTGIS prostaglandin I2 (prostacyclin) synthase
PTGDS prostaglandin D2 synthase
TMEM1 transmembrane protein 1
HBB hemoglobin, beta
Pro-apoptotic
Anti-apoptotic
Cell Death Control
Lipid
Steroid
Amino acid
Carbohydrate
Other
Metabolism
RNA metabolism
Transport
Energy
Transcription Factors
Receptors
G- protein
Signaling cascade
Miscellaneous
Signal Transduction, Transcription

Figure 8b

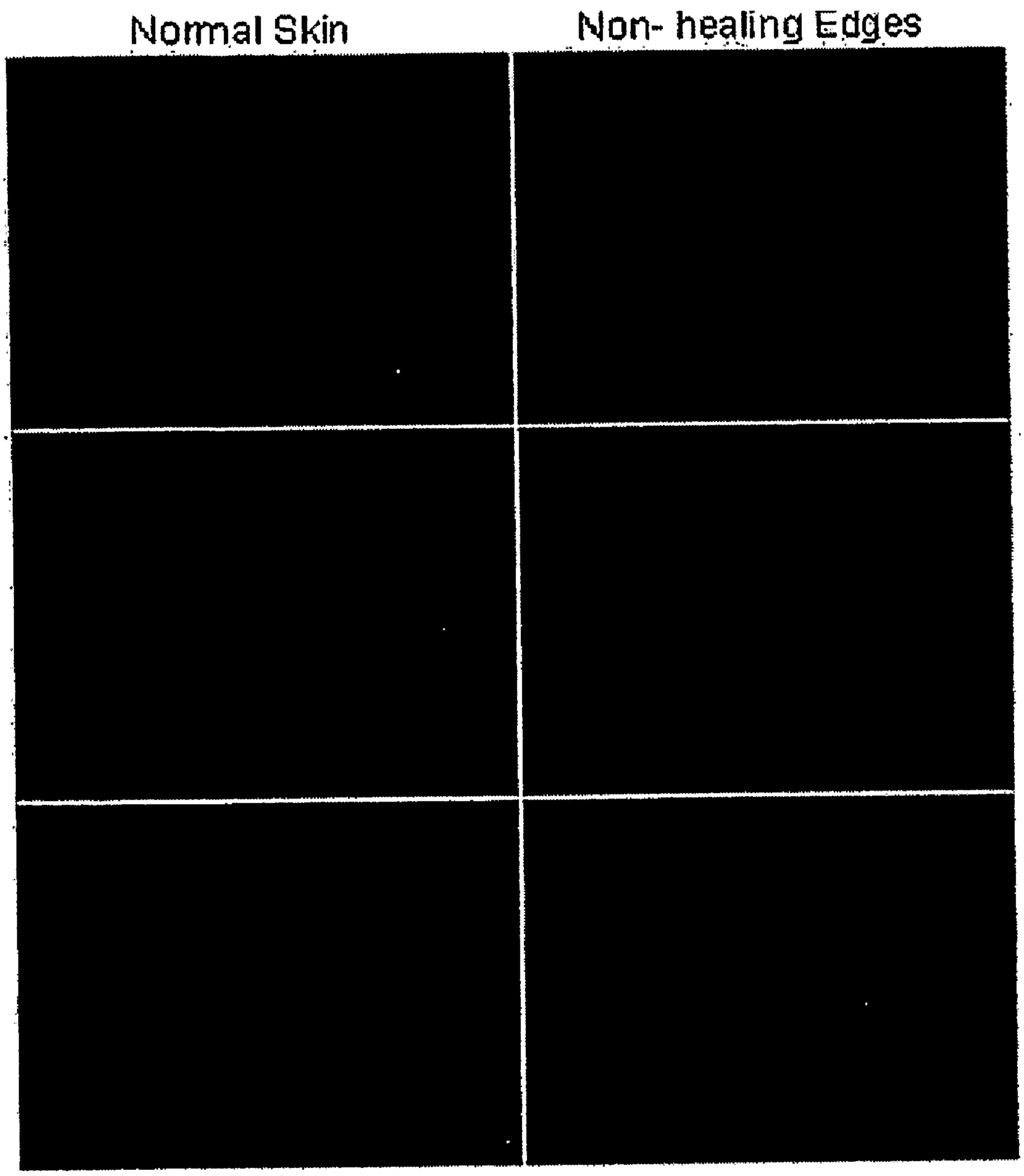


Figure 9

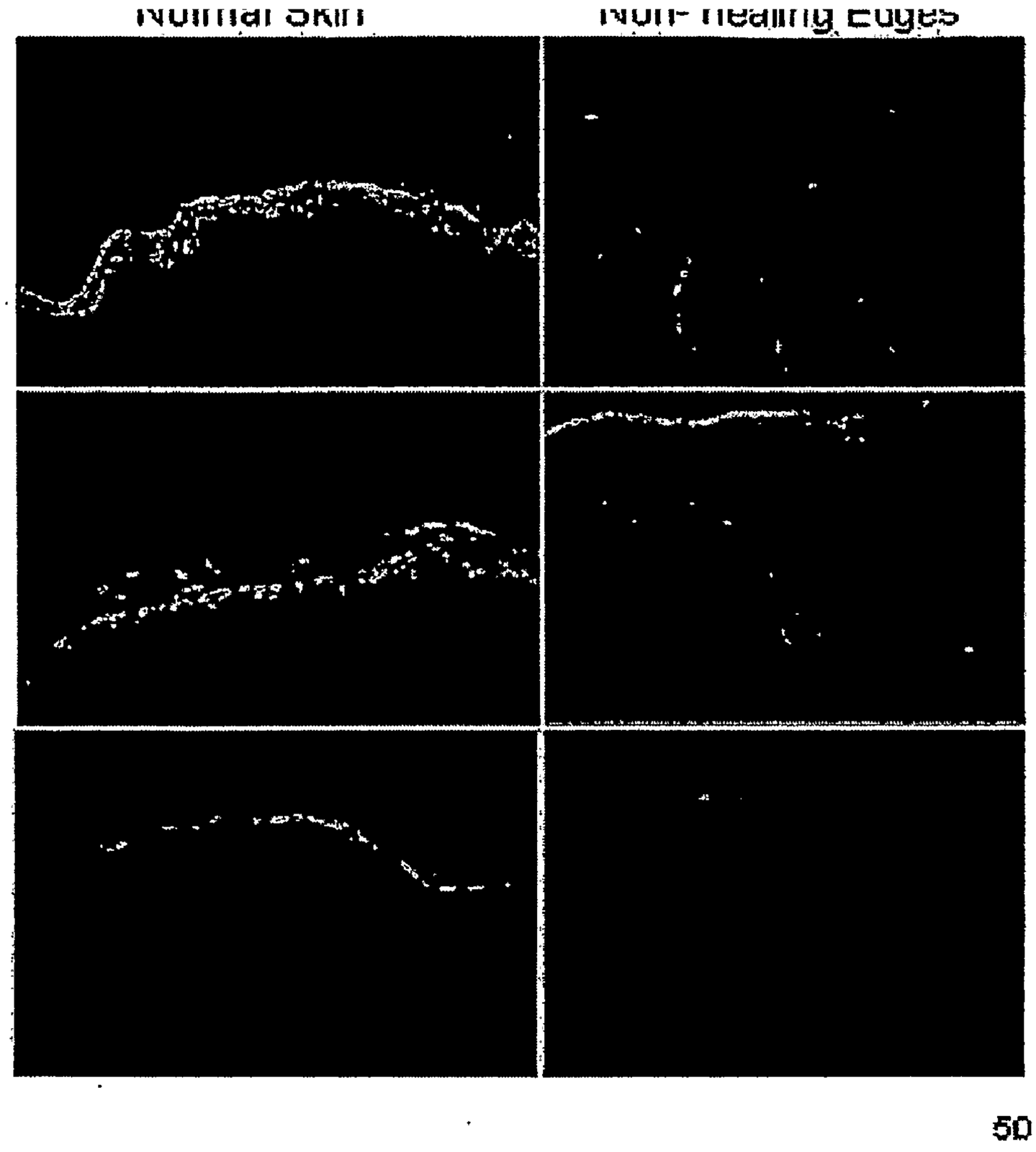
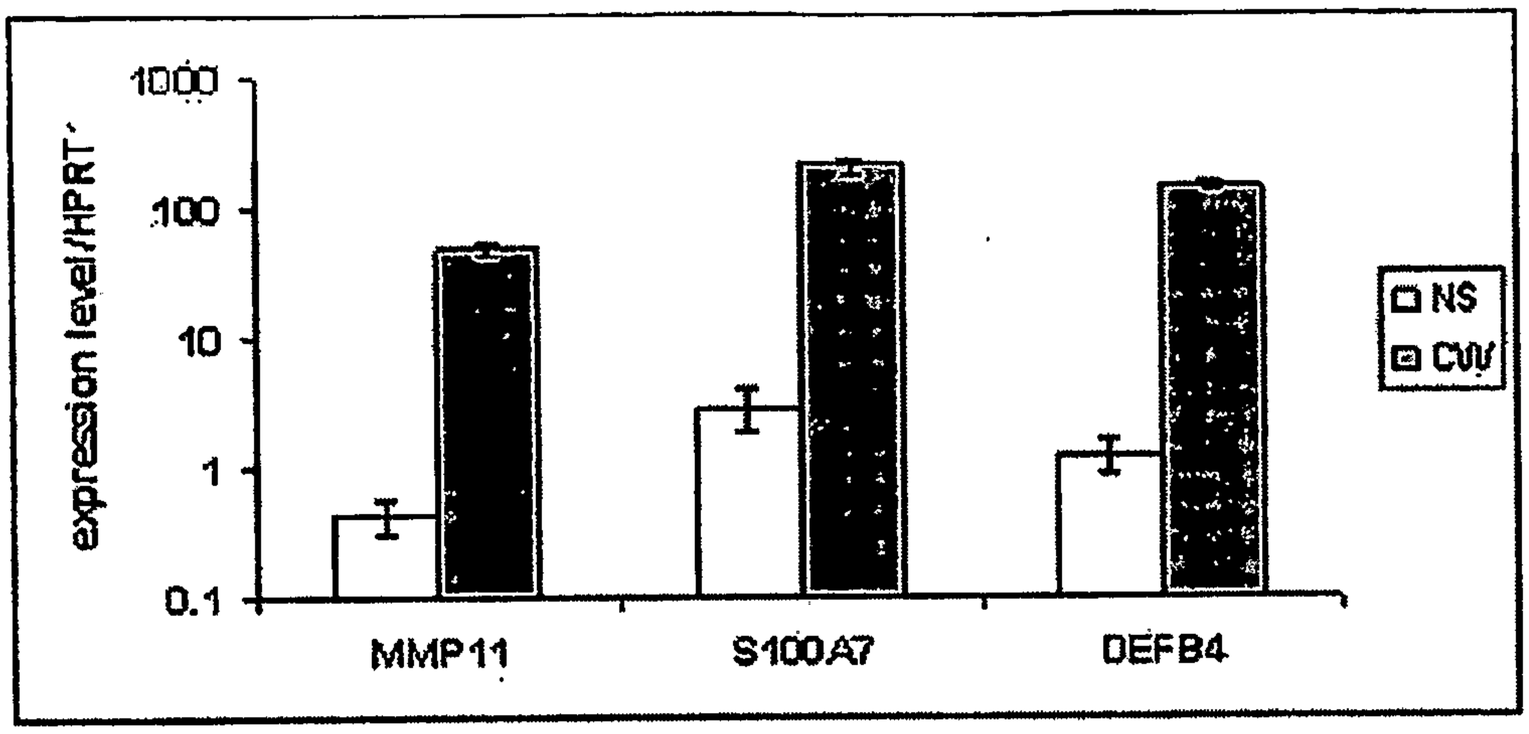
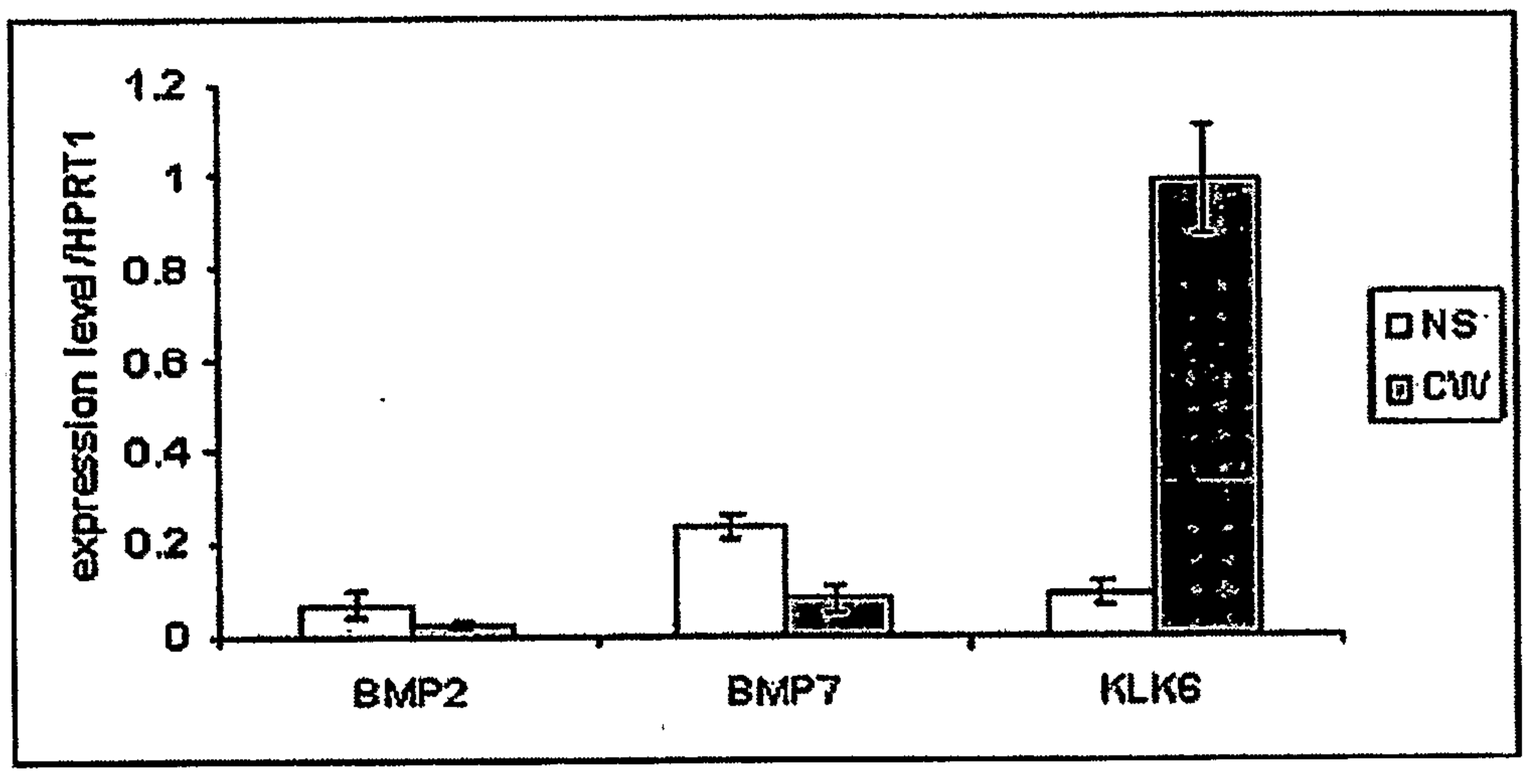


Figure 10

A.



B.



C.

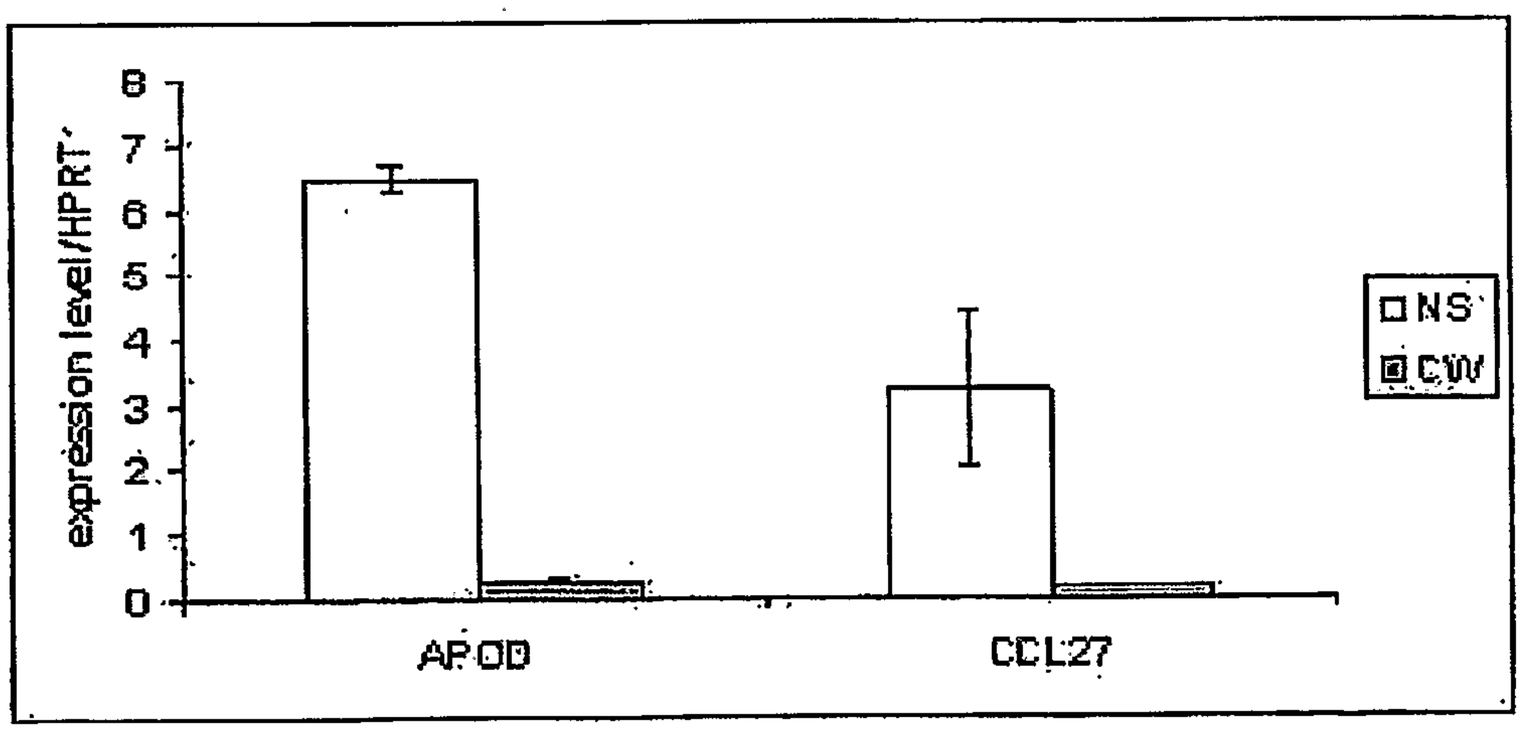


Figure 11

BIOLOGICAL MARKERS OF CHRONIC WOUND TISSUE AND METHODS OF USING FOR CRITERIA IN SURGICAL DEBRIDEMENT

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] The present application is the U.S. National Phase of International Patent Application Serial No. PCT/US07/10577, filed May 1, 2007, which claims the benefit of U.S. Provisional Patent Application Ser. No. 60/796,902, filed May 1, 2006, both of which are hereby incorporated in their entireties.

FIELD OF INVENTION

[0002] The present invention relates to biological markers in cells and tissues from sites in and adjacent to chronic wounds. These markers identify whether cells within a site will respond well to surgical debridement and can be used in methods of determining where to debride a chronic wound and/or when a debridement procedure has been successful.

BACKGROUND OF THE INVENTION

[0003] Chronic ulcers, such as venous ulcers, are characterized by physiological impairments, manifested in delays in healing, which results in severe morbidity. These chronic ulcers are reaching epidemic proportions, mostly affecting the elderly and disabled (Brem et al. (2003) *Surg. Tech. Int.* 11:161-167). Not only do these chronic ulcers significantly impair an affected person's life, the cost of caring for such chronic wounds is burdensome. Over twenty-five billion dollars was spent in the United States alone on the treatment of chronic wounds, including the costs of surgical debridement, the mainstay of treatment of chronic wounds (Williams et al. (2005) *Wound Repair Regen.* 13:131-137; Steed et al. (1996) *J. Amer. Coll. Surg.* 77:575-586).

[0004] Accumulation of devitalized tissue, cellular exudates and infection at the outer surface of the wound is characteristic of a chronic wound and prevents adequate cellular response to wound healing stimuli. Wound bed preparation facilitates restoration and regeneration of damaged tissue and provides enhanced function of new therapies (Davies et al. (2005) *Brit. J. Nurs.* 14:393-97). This wound bed preparation is accomplished by debridement, which is a method of removing devitalized tissue from chronic wounds and decreasing bacterial contamination, while stimulating contraction and epithelialization of the wound (Brem et al. (2004) *Amer. J. Surg.* 188:1-8). Proper debridement of a chronic ulcer is important for a good clinical outcome. Typically, patients are debrided weekly and it has been shown that sharp debridement increases the healing rate of venous ulcers when compared to the healing rate of non-debrided wounds. Between weeks 8 and 20 post-debridement, 16% of debrided ulcers versus 4.3% of non-debrided ulcers achieved complete healing (Williams et al. (2005) *Wound Repair Regen.* 13:131-137; Steed et al. (1996) *J. Amer. Coll. Surg.* 77:575-586). Nevertheless, in contrast to tumor excision and other surgical procedures, objective histological, biological and molecular markers have not been developed for debridement and the procedure remains relatively primitive, as new surgeons are taught to "debride until it bleeds." Moreover, about 20% of patients never heal. Thus, there is a need to find an objective determinant as to the borders of surgical debridement.

[0005] Microarray technology has the ability to simultaneously analyze the expression patterns of the entire genome, thus allowing the identification of pathogenic profiles. Such gene expression profiles of various human tumors have led to the identification of transcriptional patterns related to tumor classification, disease outcome, or response to therapy (Grose (2004) *Genome Biol.* 5:228; Golub et al. (1999) *Science* 286:531-37; Risinger et al. (2003) *Cancer Res.* 63:6-11; and Van de Vijver et al. (2002) *New Eng. J. Med.* 347:1999-2009). Microarray technology has also been used to study the mechanism of action of specific therapeutics (Wang (2005) *Opin. Mol. Ther.* 7:246-250) and identify the profile of repair of several tissues, such as cornea, tendons, skin and bone (Cao et al. (2002) *Invest. Opthalmol. Vis. Sci.* 43:2897-2904; Nakazawa et al. (2004) *J. Ortho. Res.* 22:520-525; Cole et al. (2001) *Wound Repair Regen.* 9:360-70). While it has been previously reported that the activation of the β-catenin pathway leads to the induction of c-myc, which contributes to chronic wound development through the inhibition of epithelialization (Stojadinovic et al. (2005) *Am. J. Pathol.* 167:56-59), the identification of a gene expression profile for the pathogenesis of chronic ulcers remains to be elucidated.

[0006] Additionally, therapies other than surgical debridement that stimulate healing of the wound is an essential step in eliminating morbidity caused by the wounds, as well as improving the patients' lives and decreasing healthcare costs. However, there are only two products that are currently approved by the Food and Drug Administration for the treatment of chronic wounds, platelet derived growth factors (Wieman (1998) *Amer. J. Surg.* 176:74 S-79S) and a cellular therapy called Human Skin Equivalent (Sibbald (1998) *J. Cutan. Med. Surg.* 3:S1-24-28; Brem et al. (2000) *Arch. Surg.* 135:627-34). A critical step in development and testing of new therapies is the ability to target responsive cells within the wound that would properly respond to wound healing stimuli.

SUMMARY OF THE INVENTION

[0007] The present invention overcomes the problems in the art by providing markers and methods that identify viable tissue within a wound that has a greater potential to respond to healing stimuli. The present invention also provides methods for determining if a debriding procedure has been successful or if additional debriding treatment is necessary.

[0008] The present invention is based upon the surprising discovery that the gene expression profiles of cells and tissues in sites within and adjacent to chronic wounds directly correlate to particular cellular biology and responses. In particular, it has been found that tissue from the site adjacent to a chronic wound (for convenience, herein referred to as "ACW") contains cells with a morphology similar to that of healthy cells, an increased capacity to migrate, and good response to wound healing stimuli. The tissue from sites within the wound, such as the non-healing edge of the wound (hereinafter referred to as "NHE"), contains cells that exhibit pathological morphology, a decreased ability to migrate, and poor response to wound healing stimuli. More importantly, the tissues from these two sites possess distinct gene expression profiles. Thus, these gene expression profiles provide a convenient marker for determining which tissue is suitable for debriding as well as whether a debriding procedure has been successful.

[0009] Additionally it has been found that certain genes are induced or suppressed in the cells in the tissues in the specific

wound sites. Thus, these genes can be used as markers for further determining the metes and bounds of a debridement procedure.

[0010] One embodiment of the present invention provides for a method for the identification of a margin of debridement within or adjacent to a chronic wound, by (a) obtaining a tissue sample from a site within or adjacent to the chronic wound; (b) determining a gene expression profile of the tissue sample; and (c) comparing the gene expression profile of the tissue sample with a known gene expression profile of tissue from a known site adjacent to the chronic wound (ACW). If the gene expression profile of the tissue sample, such as from the NHE, is the same or similar to the known gene expression profile of the tissue from the known site, such as the ACW, then the site of the tissue sample is within the margin of debridement (i.e., debrided sufficiently).

[0011] A preferred embodiment of this method of the invention is that the tissue from the known site contains cells with healthy, normal morphology that respond well to wound healing stimuli. A further preferred embodiment of this method would be that the tissue from the known site be from the non-ulcerated skin adjacent to the chronic wound.

[0012] It is also preferred that the gene expression profile for both the tissue sample (NHE) and the tissue from the known site be determined by microarray analysis. The known site is preferably from the ACW. The gene expression profile of the tissue from the known site could be determined prior to performing the method of the invention. After this gene expression profile of the tissue of the known site is determined, it can be used for comparison in performing the method of the invention once or several subsequent times.

[0013] It is also contemplated that the gene expression profile of the tissue sample be compared to the known gene expression profile for non-ulcerated skin adjacent to the chronic wound (ACW) as set forth in FIG. **2**. If the gene expression profile of the tissue sample is the same or similar to the known gene expression profile, then the site is within the margin of debridement (i.e., debrided sufficiently).

[0014] In a further embodiment of this method, particular genes, i.e., "marker genes," are either induced or suppressed in the cells in the tissue from the known site, such as the ACW or normal healthy skin away from the wound. These marker genes for the tissue from the known site can also be determined by microarray analysis. A comparison of the expression of genes by cells in the tissue sample, such as from the NHE, to the expression of the marker genes in the cells of the known site can then also be used to determine if the site of the tissue sample is suitable for debriding.

[0015] This method can be used in a clinical setting to determine where in a wound a debridement procedure should commence, as well as determine the margin of debridement. This method can also be used to identify sites in and adjacent to a wound that would respond well to other therapeutic agents that are being used or tested to further treat the chronic wound.

[0016] Another embodiment of the invention provides for a method for determining whether a chronic wound is in further need of debridement, by (a) obtaining a tissue sample from within the chronic wound (NHE); (b) determining a gene expression profile for the tissue sample; (c) comparing the gene expression profile of the tissue sample with a known gene expression profile of tissue from a known site adjacent to the chronic wound. If the gene expression profile of the tissue sample is the same or similar to the known gene expression profile of the tissue from the known site adjacent to the wound (ACW), then the wound is not in need of further debridement. If the gene expression profile of the tissue sample is not the same or similar to the known gene expression profile of the tissue from the known site adjacent to the wound (ACW), then the debriding procedure should continue until the known gene expression profile is obtained.

[0017] Again a preferred embodiment of this method of the invention is that the tissue from the known site contains cells with healthy, normal morphology that respond well to wound healing stimuli.

[0018] It is also preferred that the gene expression profile for both the tissue sample (NHE) and the tissue from the known site, such as ACW, be determined by microarray analysis. The gene expression profile of the tissue from the known site could be determined prior to performing the method of the invention. After the gene expression profile of the tissue of the known site is determined, it can be used for comparison in performing the method of the invention once or several subsequent times.

[0019] It is also contemplated that the gene expression profile of the tissue sample be compared to the known gene expression profile for the non-ulcerated skin adjacent to a chronic wound (ACW) as set forth in FIG. **2**. If the gene expression profile of the tissue sample is the same or similar to the known gene expression profile, then debridement has been successful. It is also preferred but not necessary that the sample tissue come from a site that has been previously debrided.

[0020] In a further embodiment of this method, particular genes, i.e., "marker genes," are either induced or suppressed in the cells in the tissue from the known site, either the ACW or normal healthy skin. These marker genes for the tissue from the known site can also be determined by microarray analysis. A comparison of the expression of genes by cells in the tissue sample, such as from the NHE, to the expression of the marker genes in the cells of the known site can then also be used to determine if debridement has been successful.

[0021] This method can be used in a clinical setting to determine if a wound has been successfully debrided. This method can also be used to identify sites in a chronic wound that because it has been successfully debrided would now respond well to other therapeutic agents that are being used or tested to further treat the chronic wound.

[0022] A further embodiment of the invention is the gene expression profile of the non-ulcerated skin adjacent to a chronic wound (ACW) as set forth in FIG. **2**, the gene expression profile of normal healthy skin as set forth in FIG. **7**, and the gene expression profile of the non-healing edge of a chronic wound (NHE) as set forth in both FIGS. **2** and **7**. Such expression profiles are convenient and useful markers for comparing the gene profile expression of tissue samples in and adjacent to a chronic wound to determine if the tissue is suitable for debridement, if it is within the margin of debridement and/or if debriding has been successful

BRIEF DESCRIPTION OF THE DRAWINGS

[0023] FIG. **1** shows that distinct wound locations have specific histology. FIG. **1**(*a*) depicts a typical venous stasis ulcer. Arrows point to the regions from which tissue biopsies were obtained. Location A is the non-healing edge of the ulcer (NHE) and location B is the adjacent, non-ulcerated skin (ACW). FIG. **1**(*b*) depicts hematoxylin and eosin stained biopsies of epidermis from the non-healing edge (location A),

the adjacent, non-ulcerated skin (location B), and normal skin. FIG. **1**(*c*) depicts hematoxylin and eosin stained biopsies of dermis from the non-healing edge (location A), the adjacent, non-ulcerated skin (location B), and normal skin. FIG. **1**(*d*) depicts the staining of the biopsies from the non-healing edge (location A), the adjacent, non-ulcerated skin (location B), and normal skin with pro-collagen. Circles demarcate the location from which the enlarged images are shown in the insets below. The scale bar is 100 μm.

[0024] FIG. **2** depicts the distinct gene expression patterns for the tissues from the different wound locations, the non-healing edge (NHE) (location A) and the adjacent, non-ulcerated skin (ACW) (location B).

[0025] FIG. **3** shows fibroblast cells grown from the tissue from the non-healing edge (NHE) (location A) and the adjacent, non-ulcerated skin (ACW) (location B).

[0026] FIG. **4** shows the results of an in vitro wound scratch assay. FIG. **4**(*a*) depicts the actual experiment with the full lines indicating the initial wound area and the dotted line demarcating the migrating front of the cells. FIG. **4**(*b*) depicts a graph showing the average coverage of the scratch wound widths in percent (%) relative to baseline wound at 0, 4, 8 and 24 hours for each cell type.

[0027] FIG. **5** shows the gene expression profiles for tissues obtained from three wound locations: location A, the non-healing edge of the wound (NHE); location B, the adjacent, non-ulcerated skin (ACW); and location *, an intermediate location between location A and location B.

[0028] FIG. **6** shows the gene annotation table describing the molecular function and biological categories of the genes present on the Affymetrix Human Genome U133 GeneChip®. The light gray areas depict genes that are up-regulated in the tissue at location B, the non-ulcerated skin adjacent to the chronic wound (ACW), as compared to the tissue at location A, the non-healing edge of the wound (NHE). The dark gray areas depict genes that are down-regulated in tissues from location B as compared to location A. The numbers within the light and dark gray shaded areas depict the fold change. The two different columns depict the comparison of the two locations in two different patients.

[0029] FIG. **7** depicts the distinct gene expression patterns for the tissues from the two different skin samples, chronic non-healing wounds, and normal healthy skin.

[0030] FIG. **8** depicts the 100 most differentially regulated genes between skin from chronic non-healing wounds and normal healthy skin. Fifty (50) of the genes are up-regulated in skin from chronic non-healing wounds as compared to normal skin, and fifty (50) are down-regulated. The genes are grouped by cellular functions and biological processes. Associated fold changes and p-values are also presented.

[0031] FIG. **9** shows the results of immunohistochemistry analysis of normal healthy skin and skin from the non-healing edge of a chronic wound stained with antibodies that recognize desmoglein 2, desmoglein 3, and desmoplakin.

[0032] FIG. **10** shows the results of immunohistochemistry analysis of normal healthy skin and skin from the non-healing edge of a chronic wound stained with antibodies that recognize involucrin, keratin 10, and filaggrin.

[0033] FIG. **11** depicts the results of RT-PCR using tissue from non-healing chronic wounds and normal healthy skin. FIG. **11**(A) shows results for the measurement of expression of genes MMP11, S100A7, and DEFB4. FIG. **11**(B) shows the results for the measurement of the expression of genes BMP2, BMP7, and KLK6. FIG. **11**(C) shows the results for the measurement of the expression of genes APOD and CCL27.

DETAILED DESCRIPTION OF THE INVENTION

[0034] There are presently no objective indicia to serve as a guide in surgical debridement, either as to which portion of a chronic wound should be debrided or as to how a wound is responding to debriding treatment. The present invention sets forth criteria and methods for determining both.

[0035] To assess the pathogenic state of wound tissue before and after wound debridement, biopsies from distinct locations in a chronic wound were analyzed as to their histology, biology and gene expression profile. It was found that biopsies from the non-healing edges of a wound have a specific identifiable and reproducible gene expression profile and primary fibroblasts deriving from this site have impaired migration capacity. In contrast, biopsies from the adjacent non-ulcerated locations of the wound have a different specific gene expression profile and the primary fibroblasts deriving from this location have a similar migration capacity as normal primary fibroblasts. Thus, chronic ulcers contain distinct subpopulations of cells with different capacities to heal and gene expression profiling can be used to identify them.

DEFINITIONS

[0036] The terms used in this specification generally have their ordinary meanings in the art, within the context of this invention and the specific context where each term is used. Certain terms are discussed below, or elsewhere in the specification, to provide additional guidance to the practitioner in describing the methods of the invention and how to use them. Moreover, it will be appreciated that the same thing can be said in more than one way. Consequently, alternative language and synonyms may be used for any one or more of the terms discussed herein, nor is any special significance to be placed upon whether or not a term is elaborated or discussed herein. Synonyms for certain terms are provided. A recital of one or more synonyms does not exclude the use of the other synonyms. The use of examples anywhere in this specification, including examples of any terms discussed herein, is illustrative only, and in no way limits the scope and meaning of the invention or any exemplified term. Likewise, the invention is not limited to its preferred embodiments.

[0037] The term "adjacent" refers to a location near or close to a chronic wound edge that may or may not be in actual contact with the wound.

[0038] The terms "expression profile" or "gene expression profile" are used interchangeably and refer to any description or measurement of one of more of the genes that are expressed by a cell, tissue, or organism under or in response to a particular condition. Expression profiles can identify genes that are up-regulated, down-regulated, or unaffected under particular conditions. Gene expression can be detected at the nucleic acid level or at the protein level. The expression profiling at the nucleic acid level can be accomplished using any available technology to measure gene transcript levels. For example, the method could employ in situ hybridization, Northern hybridization or hybridization to a nucleic acid microarray, such an oligonucleotide microarray, or a cDNA microarray. Alternatively, the method could employ reverse transcriptase-polymerase chain reaction (RT-PCR). The expression profiling at the protein level can be accomplished

by any available technology to measure protein levels, e.g., using peptide-specific capture agent arrays (see, e.g., International PCT Publication No. WO 00/04389).

[0039] The term "same" as used herein as related to gene expression profiles from cells of a tissue means that upon visual examination alone, the gene expression profiles appear identical.

[0040] The term "similar" as used herein as related to gene expression profiles from cells of a tissue means that upon visual examination alone, the gene expression profiles appear nearly but not exactly identical.

[0041] The phrase "identical or similar expression" (and the like) as used herein refers to an expression level of a gene or product thereof (i.e., an mRNA transcript or protein) in a tissue sample that is ±30%, preferably ±20%, and more preferably ±10% of a given numerical value of the expression level of the same gene or gene product from the tissue of a known site as determined by any quantitative assay known in the art.

[0042] The term "margin of debridement" as used herein means an area of skin at the non-healing edge that contains tissue that is biologically responsive to wound healing stimuli and where the debridement procedure should end.

[0043] The term "agent" is used herein to mean a substance capable of producing a chemical reaction or a physical or a biological effect. An agent could be, among other things, a chemical, including a nucleic acid; a drug; a virus; or a bacterium.

[0044] Cells from different regions of a chronic wound exhibit different cell morphology. Cells derived from tissue from the non-healing edge of a wound (NHE) exhibited pathological morphology whereas cells derived from tissue adjacent to the wound (ACW) exhibited normalized pathology.

[0045] Additionally, cells from different specific regions of a chronic wound exhibit unique characteristics, such as cell migration and cellular response to wounding, that would influence the success of debridement treatment, since the aim of debridement of a wound is not only to clean the necrotic tissue but to reach out to the cells within the wound that are biologically capable of responding to wound healing stimuli. Cells grown from tissue obtained from the non-healing edge of a chronic wound (NHE) show a diminished capacity to migrate and respond to wounding, whereas cells derived from tissue from the adjacent, non-ulcerated area of the chronic wound (ACW) show an increased capacity to migrate and respond well to wound healing stimuli. Typically, this area adjacent to the ulcer is the margin where debridement ends. However, based upon the ability of the cells in this area to migrate and heal, this area should be included in the debridement treatment since the time of healing could be reduced if more permissive cells were exposed to wound healing signals. Moreover, these cells with the greater ability to respond to wound healing stimuli would also be a preferred target for other therapeutic treatment for a chronic wound, such a pharmaceutical or biological agent.

[0046] Perhaps, more surprising is that these cells from different regions of the chronic wound are not only characterized by unique biological properties, but are also characterized by a unique gene expression profile. Gene expression profiles resemble a bar code and allow overall visualization of an entire expression pattern rather than specific gene regulation. Since there is a direct correlation between biological properties that may be useful determining criteria for debridement and a unique gene expression profile in cells from different regions of a chronic wound, gene expression profiling can serve as a guide for surgical debridement in the treatment of chronic ulcers. The differences in the gene expression maps of the particular wound locations are definitive and can be grouped as specific patterns that can be used as a diagnostic tool.

[0047] As shown in FIG. **2**, the gene expression profiles or patterns from tissues in the non-healing edge of a wound (NHE) are the same or similar to each other but markedly different from the gene profiles of the tissues in the non-ulcerated skin adjacent to the wound (ACW). These profiles resemble bar codes with the dark gray lines representing up-regulated genes, the lighter gray lines representing down-regulated genes, and the lightest gray lines representing the expressed genes. By referring to the gene expression profiles set forth FIG. **2**, it can be seen that the gene expression profiles of the tissue from the non-ulcerated skin adjacent to the wound comprises mostly lightest gray lines in its pattern whereas the gene expression profiles of the tissues from the non-healing edge of the wound are mostly dark gray on top and lighter gray on the bottom. Thus, the cells in the tissue in the non-healing edge of the chronic wound (NHE) either up- or down-regulate many genes that are expressed in the cells of the non-ulcerated skin adjacent to the wound (ACW).

[0048] As shown in FIG. **7**, the gene expression profiles or patterns from tissues in the non-healing edge of a wound (NHE) are similar to each other and the profiles for chronic wounds in FIG. **2**. The gene expression profiles or patterns for the healthy control skin away from the chronic wound is also markedly different from the gene profiles of the skin from the chronic wounds. Referring to FIG. **7**, it can be seen that the gene expression profiles of the tissue from the chronic wounds comprise dark gray and lighter gray lines at opposite areas in the pattern as compared to the profiles for the healthy skin. Thus, the cells in the tissues of the chronic non-healing wounds differentially regulate genes as compared to healthy skin.

[0049] The similarity of the patterns of the gene expression profiles from tissue derived from the same location (either the NHE or the ACW or healthy skin), and the differences in the patterns of the gene expression profiles of the different types of tissue are easily visually discernable by the naked eye. Thus, by generating a gene expression profile of the specific wound region, one could quickly identify, by visual examination only, from which region a tissue biopsy originates and determine if it contains cells which would respond well to debridement as well as determine how well the wound has been debrided.

[0050] It is also possible to quantify the data in the gene expression profiles and determine which genes in particular are being up-regulated, i.e., induced, or down-regulated, i.e., suppressed, in the tissues from the different locations. Table 1 lists genes that are up-regulated in the non-ulcerated skin adjacent to the wound (ACW) (in alphabetical order as to function) relative to the genes in the non-healing edge of the wound (NHE) and Table 2 lists the genes that are down-regulated in the non-ulcerated skin adjacent to the wound (ACW) (in alphabetical order as to function) relative to the genes in the non-healing edge of the wound (NHE). Thus, the specific regulation of any one gene or combination of genes in a tissue sample or biopsy can be determined and compared to the regulation of genes in the non-ulcerated skin adjacent to the wound. This comparison of the regulated genes in the tissue sample to the regulation of any of the marker genes in the non-ulcerated skin adjacent to the wound can assist in further determining if the tissue sample contains cells which will respond well to debridement and/or how well a wound has been debrided.

TABLE 1

Genes which are up-regulated or induced in the non-ulcerated skin adjacent to a wound as compared to the non-healing edge of a wound

Function	Gene
Adhesion	tenascin C (hexabrachion)
Adhesion	desmocollin 2
Adhesion	CD47 antigen (Rh-related antigen, integrin-associated signal transducer)
Adhesion	melanoma cell adhesion molecule
Adhesion	carcinoembryonic antigen-related cell adhesion molecule 6
Adhesion	caldesmon 1
Anti-oxidant	glutathione S-transferase omega 1
Apoptosis	tumor necrosis factor receptor superfamily, member 21
Apoptosis inhibitor	immediate early response 3
Ca binding	EGF-like domain, multiple 6
Ca binding	reticulocalbin 3, EF-hand calcium binding domain
Ca binding	calumenin
Cell cycle	CDC20 cell division cycle 20 homolog (*S. cerevisiae*)
Cell cycle	CDC28 protein kinase regulatory subunit 2
Cell cycle	ZW10 interactor
Cell cycle	regulator of G-protein signaling 2, 24 kDa
Cell cycle	cell division cycle 25 B
Cell cycle inhibitor	quiescin Q6
Cell growth proliferation	cysteine-rich, angiogenic inducer 61
Cytoskeletal	thymosin, beta 10
Cytoskeletal	transgelin
Cytoskeletal, actin	tropomyosin 2 (beta)
Cytoskeletal, actin	actin related protein 2/3 complex, subunit 1B, 41 kDa
Cytoskeletal, actin	actinin, alpha 1
Cytoskeletal, actin	erythrocyte membrane protein band 4.1-like 3
Cytoskeletal, actin	actin, alpha 2, smooth muscle, aorta
Cytoskeletal, actin	actin, beta
Cytoskeletal, keratin	keratin 17
Cytoskeletal, keratin	keratin 16
Cytoskeletal, keratin	cytokeratin type II
Cytoskeletal, keratin	keratin 6A
Cytoskeletal, myosin	myosin, heavy polypeptide 10, non-muscle
Cytoskeletal, tubulin	tubulin, beta 4
Cytoskeletal, tubulin	tubulin, alpha, ubiquitous
Cytoskeletal, tubulin	tubulin, beta MGC4083
Cytoskeletal, tubulin	tubulin, alpha 6
Cytoskeletal, tubulin	tubulin, beta 5
Cytoskeletal, tubulin	tubulin, alpha 3
Cytoskeletal, tubulin	tubulin beta 2
DNA binding, histone	H2A histone family, member X
DNA binding, histone	H2A histone family, member Z
DNA repair, synthesis	ribonucleotide reductase M2 polypeptide
DNA repair, synthesis	uridine phosphorylase 1
DNA repair, synthesis	cytidine deaminase
ECM	fibronectin 1
ECM	spondin 2, extracellular matrix protein
ECM	collagen, type XI, alpha 1
ECM	collagen, type V, alpha 3
ECM	thrombospondin 1
ECM	syndecan 2
ECM	collagen, type IV, alpha 2
ECM	biglycan
ECM	fibronectin 1
Energy	lactate dehydrogenase B
Energy	aldo-keto reductase family 1, member B1
Enzyme	transketolase (Wernicke-Korsakoff syndrome)
Epidermal differentiation	S100 calcium binding protein A2
Epidermal differentiation	S100 calcium binding protein A6 (calcyclin)
Epidermal differentiation	small proline-rich protein 2B
Epidermal differentiation	small proline-rich protein 1A
Epidermal differentiation	S100 calcium binding protein A11 (calgizzarin)
Epidermal differentiation	S100 calcium binding protein A10 (annexin II ligand, calpactin I, light polypeptide (p11)
Epidermal differentiation	S100 calcium binding protein A4 (calcium protein, calvasculin, metastasin, murine placental homolog)
Golgi apparatus	coatomer protein complex, subunit zeta 2
Hemoglobin	hemoglobin, gamma G

TABLE 1-continued

Genes which are up-regulated or induced in the non-ulcerated skin adjacent to a wound as compared to the non-healing edge of a wound

Function	Gene
Immunoglobulin	immunoglobulin kappa variable ID-13
Interferon-regulated	interferon, alpha-inducible protein
Membrane protein	tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, theta polypeptide
Membrane protein	Thy-1 cell surface antigen
Membrane protein	transmembrane 4 superfamily member 1
Membrane protein	CD151 antigen
Metabolism, amino acid	lysyl oxidase-like 2
Metabolism, amino acid	lysyl oxidase-like 1
Metabolism	ornithine decarboxylase 1
Metabolism, steroid	aldo-keto reductase family 1, member C1
Metabolism, steroid	aldo-keto reductase family 1, member C2
Mitochondrial	cytochrome c oxidase subunit VIIa polypeptide 1 (muscle)
Nuclear receptor	nucleophosmin
Nucleoskeletal	karyopherin alpha 2
Oncogenesis	four and half LIM domains 2
Phosphatase	endoglin (Osler-Rendu-Weber Syndrome 1)
Proteolysis	calpain 2, (m/II) large subunt
Proteolysis	protease, serine, 23
Proteolysis	WAP four-disulfide core domain 1
Proteolysis	cathepsin L
Proteolysis inhibitor	serine (or cysteine) proteinase inhibitor, clade B, member 13
Proteolysis inhibitor	cystatin B
Proteolysis inhibitor	secretory leukocyte protease inhibitor
Proteolysis inhibitor	protease inhibitor 3, skin-derived
Proteolysis inhibitor	serine (or cysteine) proteinase inhibitor, clade B, member 1
Proteolysis inhibitor	serine (or cysteine) proteinase inhibitor, clade H, member 1
Proteolysis inhibitor	tissue inhibitor of metalloproteinase 1
Proteolysis, extracellular	kallikrein 12
Proteolysis, ubiquitin	ubiquitin-conjugating enzyme E2C
Proteolysis, ubiquitin	ubiquitin-conjugating enzyme E2S
Receptor	low density lipoprotein receptor
Receptor	angiotensin II receptor-like 1
Regulator	annexin A1
Regulator	guanylate cyclase 1, soluble, alpha 3
Regulator	annexin A11
Regulator	CAP 1
Regulator	annexin A6
Regulator	annexin A5
Regulator	SH3 domain binding glutamic acid-rich protein-like 3
Regulator	RAB 31
Secreted	lectin, galactoside-binding, soluble 1
Secreted	latent transforming growth factor beta binding protein 1
Secreted	insulin-like growth factor binding protein 2
Secreted	insulin-like growth factor binding protein 6
Secreted	chemokine (C-C motif) ligand 18
Secreted	transforming growth factor, beta induced, 68 kDa
Secreted	endothelial cell growth factor 1 (platelet-derived)
Secreted	angiopoietin-like 2
Trafficking, vesicles	KDEL endoplasmic reticulum protein retention receptor 3
Trafficking, vesicles	plasmalemma vesicle associated protein
Transcription	pituitary tumor-transforming 1
Transcription	polymerase (RNA) II polypeptide L, 7.6 kDa
Transcription factor	cysteine-rich protein 1
Transcription repressor	inhibitor of DNA binding 3, dominant negative helix-loop-helix protein
Transcription repressor	eukaryotic translation initiation factor 4E binding protein 1
Translation	ribosomal protein S26
Translation	FXYD domain containing ion transport regulator 5
Transporter, channel	NEL-like 2

TABLE 1-continued

Genes which are up-regulated or induced in the non-ulcerated skin adjacent to a wound as compared to the non-healing edge of a wound

Function	Gene
Transporter	chloride intracellular channel 3
Tumor suppressor	serologically defined colon cancer antigen 33
Tumor antigen	melanoma associated gene
Unknown	Raft-linking protein
Unknown	Calcium regulated heat stable protein 1, 24 kDa
Unknown	DKFZP586L151 protein
Unknown	Hematological and neurological expressed 1
Unknown	Ring finger protein 141
Unknown	Proteoglycan 1, secretory granule
Unknown/hypothetical	hypothetical protein PRO1855
Unknown/hypothetical	hypothetical protein FLJ23221

TABLE 2

Genes which are down-regulated or suppressed in the non-ulcerated skin adjacent to a wound as compared to the non-healing edge of a wound

Function	Gene
Adhesion	calsytenin 1
Adhesion	discs, large homolog (*Drosophila*)
Adhesion	protocadherin 21
Adhesion	FAT tumor suppressor homolog 2 (*Drosophila*)
Adhesion	catenin, delta 1
Adhesion	cadherin, EGF LAG seven-ass G-type receptor 2
Adhesion	desmocollin 1
Adhesion	bullous pemphigold antigen 1, 230/240 kDa
Adhesion	gap junction protein, beta 3, 31 kDa
Antioxidant	glutathione S-transferase A4
Antioxidant	selenoprotein P, plasma, 1
Antioxidant	microsomal glutathione S-tranferase 2
Antioxidant	glutaredoxin (thioltransferase)
Antioxidant	catalase
Apoptosis	p8 protein
Apoptosis	programmed cell death 4
Apoptosis	PRKC, apoptosis, WT1 regulator
Apoptosis inhibitor	secreted frizzle-related protein
Apoptosis inhibitor	sema domain, immunoglobulin domain (Ig), transmembrane domain and short cytoplasmic domain
Ca binding	signal peptide, CUB domain, EGF-like 2
Cell cycle	cullin 3
Cell cycle	transforming, acidic coil containing protein 2
Cell cycle inhibitor	sestrin 1
Cell cycle inhibitor	B-cell translocation gene 1, anti-proliferative
Cell cycle inhibitor	BTG family, member 2
Cell cycle inhibitor	growth arrest-specific 7
Cell growth proliferation	four and a half LIM domains 1
Cytoskeletal	supervillin
Cytoskeletal, actin	spectrin, beta, non-erythrocytic 5
Cytoskeletal, actin	GABA(A) receptor associated protein-like 2
Cytoskeletal, actin	Huntington interacting protein-1 related
Cytoskeletal, keratin	keratin 15
Cytoskeletal, keratin	keratin 2A
Cytoskeletal, keratin	keratin 23
Cytoskeletal, keratin	keratin 9
Cytoskeletal, keratin	keratin 10
Cytoskeletal, keratin	keratin 1
Cytoskeletal, membrane	uroplakin 1A
Cytoskeletal, motility	dynein, cytoplasmic, light polypeptide 2A
Cytoskeletal, myosin	myosin X
Cytoskeletal, Rho, CDC42	PTPL1-associated RhoGAP 1
Cytoskeletal, Rho, CDC42	CDC42 effector protein
Cytoskeletal, Rho, CDC42	T-cell lymphoma invasion and metastasis 1
Cytoskeletal, Rho, CDC42	Rho guanine nucleotide exchange factor (GEF) 5
Cytoskeletal, tubulin	micro-tubule-associated protein 1 light chain 3 beta
Detoxification	paraoxonase 2
Detoxification	monoamine oxidase A
Detoxification	flavin containing monooxygenase 2

TABLE 2-continued

Genes which are down-regulated or suppressed in the non-ulcerated skin adjacent to a wound as compared to the non-healing edge of a wound

Function	Gene
DNA repair, synthesis	deoxyribonuclease I-like 2
DNA repair, synthesis	cell death-inducing DFFA effector
DNA repair, synthesis	adenylate kinase 3
DNA repair, synthesis	DNA-damage-inducible transcript 4
ECM	tuftelin 1
ECM	microfibrillar-associated protein 4
ECM	chitinase 3-like 2
ECM	cartilage oligomeric matrix protein
ECM	chondroitin sulfate proteoglycan 2
ECM	fibulin 2
ECM	dermatopontin
Energy	aldolase C, fructose-biphosphate
Energy	thioredoxin interacting protein
Energy	aldehyde dehydrogenase 3 family, member A1
Energy	aldehyde dehydrogenase 4 family, member A1
Energy	aldehyde dehydrogenase 3 family, member B2
Enzyme	P450 (cytochrome) oxidoreductase
Epidermal differentiation	small proline-rich protein 3
Epidermal differentiation	S100 calcium binding protein A12
Epidermal differentiation	S100 calcium binding protein A13
Epidermal differentiation	calmodulin-like 5
Epidermal differentiation	ARS component B
Epidermal differentiation	small proline rich-like (epidermal differentiation complex) 1B
Epidermal differentiation	psoriasis susceptibility 1 candidate 2
Epidermal differentiation	annexin A9
Epidermal differentiation	loricrin
Epidermal differentiation	filaggrin
Epidermal differentiation	transglutaminase 3
Epidermal differentiation	sciellin
Golgi apparatus	bicaudal D homolog 2 (*drosophila*)
Golgi apparatus	golgi auto antigen, golgin subfamily a, 7
Golgi apparatus	DNA segment on chromosome 4, 234 expressed sequence
G-regulated protein	ADP-ribosylation factor-like 4
G-regulated protein	ADP-ribosylation factor-like 5
G-regulated protein	ADP-ribosylation factor-like 10C
G-regulated protein	ral guanine nucleotide dissociation stimulator
Heat shock, chaperone	heat shock 70 kDa protein 2
Heat shock, chaperone	heat shock 70 kDa protein 1A
Immune response	D component of complement
Immune response	major histocompatibility complex, class I, F
Immune response	major histocompatibility complex, class I, A
Immune response	major histocompatibility complex, class I, C
Immune response	major histocompatibility complex, class II, DR beta 4
Immunoglobulin	Fc fragment of IgG binding protein
Immunoglobulin	immunoglobulin superfamily, member 3
Immunoglobulin	lymphocyte antigen 6 complex, locus G6C
Interferon regulated	guanylate binding protein 2, interferon inducible
Melanogenesis	tyrosinase-related protein I
Melanogenesis	tyrosinase (oculocutaneous albinism 1A)
Melonogenesis	dopochrome tautomerase
Membrane protein	epithelial membrane protein 2
Membrane protein	melan-A
Membrane protein	perixisomal membrane protein 4, 24 kDa
Membrane protein	glycoprotein (transmembrane) NMB
Membrane protein	transmembrane 7 superfamily member 2
Membrane protein	adipose differentiation-related protein
Membrane protein	KIAA0247
Membrane protein	sema domain, immunoglobulin domain transmembrane domain, short cytoplasmic domain (semaphorin) 4C
Membrane protein	membrane interacting protein of RGS16
Metabolism, amino acid	histidine ammonia-lyase
Metabolism, amino acid	arginase, liver
Metabolism, amino acid	autism susceptibility candidate 2
Metabolism, amino acid	ornthine aminotransferase (gyrate atrophy)
Metabolism, amino acid	phosphoglycerate dehydrogenase
Metabolism, carbohydrate	sorbitol dehydrogenase
Metabolism, lipid	degenerative spermatocyte homolog, lipid desaturase (*Drosophila*)
Metabolism, lipid	acyl-CoA synthetase long-chain family member 1

TABLE 2-continued

Genes which are down-regulated or suppressed in the non-ulcerated skin adjacent to a wound as compared to the non-healing edge of a wound

Function	Gene
Metabolism, lipid	phosphatidic acid phosphatase type 2B
Metabolism, lipid	phospholipid transfer protein
Metabolism, lipid	phospholipase A2, group IVB (cytosolic)
Metabolism, other	transcobalamin I
Metabolism, other	arylsulfatase F
Metabolism, other	arylacetamide deacetylase (esterase)
Metabolism, other	lactotransferrin
Metabolism, other	carbonic anhydrase XII
Metabolism, other	anhydrolase domain containing 9
Metabolism, other	spermine oxidase
Metabolism, other	glycine amidinotransferase
Metabolism, steroid	24-dehydrocholesterol reductase
Metabolism, steroid	START domain containing 5
Metabolism, steroid	oxysterol binding protein-like 8
Mitochondrial	PET112-like yeast
Nuclear receptor/RA	RAR-related orphan receptor A
Nuclear receptor/RA	retinoid X receptor, alpha
Phosphatase	acid phosphatase, prostate
Phosphatase	protein phosphatase 3, catalytic subunit, alpha isoform
Phosphatase	dual specificity phosphatase 1
Phosphatase	protein phosphatase 2, regulatory subunit B, alpha
Protein binding	KIAA0795 protein
Protein kinase	casein kinase 2, alpha prime polypeptide
Protein kinase	SFRS protein kinase 1
Protein kinase	casein kinase 2, beta polypeptide
Protein kinase	serum/glucocortoid regulated kinase
Protein kinase	MAP kinase-interacting serine/threonine kinase 2
Protein kinase	protein kinase C and casein kinase substrate in neurons 2
Protein kinase inhibitor	protein kinase, lysine deficient 1
Protein modification	phosphatidylinositol glycan, class C
Proteolysis	insulin-degrading enzyme
Proteolysis	cathepsin L2
Proteolysis	bleomycin hydrolase
Proteolysis	calpain 3
Proteolysis	cathepsin H
Proteolysis	carboxypeptidase A4
Proteolysis	cathepsin D
Proteolysis	protein x 0001
Proteolysis inhibitor	cystatin E/M
Proteolysis inhibitor	serine (or cysteine) proteinase inhibitor, clade B, member 7
Proteolysis inhibitor	serine (or cysteine) proteinase inhibitor, clade B, member 8
Proteolysis, extracellular	protease, serine, 8
Proteolysis, extracellular	serine protease inhibitor, Kunitz type 1
Proteolysis, ubiquitin	F-box and WD-40 domain protein 7
Receptor	Coxsackie virus and adenovirus receptor
Receptor	CD36 antigen
Receptor	discoidin domain receptor family, member 1
Receptor	insulin receptor substrate 2
Receptor	putative chemokine receptor
Receptor	EphB6
Receptor	G protein-coupled receptor 87
Receptor	fibroblast growth factor receptor 2
Receptor	fibroblast growth factor receptor 3
Receptor	activin A receptor, type IB
Receptor	v-erb-b2 erythroblastic leukemia viral oncogene homolog 2, neuron/glioblastoma derived oncogene homolog (avian)
Receptor	epidermal growth factor receptor (erythroblastic leukemia viral (v-erb-b) oncogene homolog (avian)
Receptor	protein tyrosine phosphatase, receptor type F
Regulator	annexin A4
Regulator	SH3 domain containing Ysc84-like 1 (*s. cerevisiae*)
Regulator	SH3 domain binding glutamic acid rich protein like
Regulator	vav 3 oncogene
Regulator	glucosidase, beta: acid

TABLE 2-continued

Genes which are down-regulated or suppressed in the non-ulcerated skin adjacent to a wound as compared to the non-healing edge of a wound

Function	Gene
Regulator	sphingomyelin phosphodiesterase acid-like 3A
Regulator	sphingomyelin phosphodiesterase 1 acid lysosomal
Regulator	inositol(myo)-1(or 4)-monophosphatase 2
Regulator	inositol hexaphosphate kinase 2
Regulator	phosphoinositide-3-kinase, regulatory subunit, polypeptide 1 (p85 alpha)
Regulator	phosphotidylinositol transfer protein
Regulator	inositol 1,4,5-triphosphate 3-kinase B
Regulator	protein associated with myc
Regulator	v-myc myelocytomatosis viral oncogene (avian)
Regulator	hydroxyprostaglandin dehydrogenase 15-(NAD)
Regulator	prostaglandin-endoperoxide synthetase 1
Regulator	arachidonate lipoxygenase 3
Regulator	prostaglandin D2 synthase 21 kDa (brains)
Regulator	ras-related GTP binding D
Regulator	retinoblastoma-associated factor 600
Secreted	lectin, galactoside-binding, soluble 3
Secreted	chemokine-like factor superfamily 6
Secreted	chemokine (C—X—C) motif ligand 12
Secreted	angiopoietin-like 4
Secreted	ephrin-A1
Secreted	apolipoprotein E
Secreted	putative secreted protein ZSIG11
Signal transduction	link guanine nucleotide exchange factor ii
Signal transduction	SPRY domain-containing SOCS box protein SSB-3
Trafficking, vesicles	reticulon 3
Trafficking, vesicles	chromosome 12 open reading frame 8
Trafficking, vesicles	vesicle amine transport protein 1 homolog
Trafficking, vesicles	adaptor-related protein complex 1, gamma 1 subunit
Transcription	GATA binding protein 3
Transcription	SRY (sex determining region Y)-box 9
Transcription	polymerase (RNA) II (DNA directed) polypeptide J
Transcription factor	catenin, beta interacting protein 1
Transcription factor	nuclear factor I/B
Transcription factor	v-kit Hardy-Zukerman 4 feline sarcoma viral oncogene homolog
Transcription factor	Kruppel-like factor 4 (gut)
Transcription factor	v-ets erythroblastosis virus E26 oncogene homolog 2 (avian)
Transcription factor	MAX interacting protein 1
Transcription factor	zinc finger protein 36, C3H type-like 2
Transcription factor	forkhead box O3A
Transcription factor	v-fos FBJ murine osteosarcoma viral oncogene homolog
Transcription factor	proline-rich nuclear receptor coactivator 2
Transcription factor	OGT(O-Glc-NAc-transferase)-interacting protein, 106 kDa
Transcription factor	myogenic factor 3
Transcription factor	delta sleep inducing peptide, immunoreactor
Transcription factor	HMG-box transcription factor 1
Transcription factor	v-maf musculoaponeurotic fibrosarcoma oncogene homolog (avian)
Transcription factor	MAX protein
Transcription factor	pre-B-cell leukemia transcription factor interacting protein 1
Transcription factor	homeodomain-only protein
Transcription factor	B-cell CLL/lymphoma 6 (zinc finger protein 51)
Transcription factor	B-cell CLL/lymphoma 11A (zinc finger protein)
Transcription factor	MYST histone acetyltransferase (monocytic leukemia) 3
Transcription repressor	cellular repressor of E1A-stimulated genes
Transcription repressor	transcription factor 8 (represses interleukin 2 expression)
Translation	membrane protein expressed in epithelial-like lung adenocarcinoma
Translation	ribosomal protein L15

TABLE 2-continued

Genes which are down-regulated or suppressed in the non-ulcerated skin adjacent to a wound as compared to the non-healing edge of a wound

Function	Gene
Translation	eukaryotic translation initiation factor 4A, isoform 2
Translation	eukaryotic translation initiation factor 4B
Translation	ribosomal protein L3
Translation	glutaminyl-tRNA synthetase
Transporter	solute carrier family 31, member 2
Transporter	aldehyde dehydrogenase 3, family member A2
Transporter	ATPase, class V, type 10B
Transporter	hypothetical protein FLJ20296
Transporter	solute carrier family 39, member 6
Transporter	solute carrier family 25, member 6
Transporter	solute carrier family 25, member 11
Transporter	solute carrier family 30, member 1
Transporter	ATPase Ca++ transporting, plasma membrane
Transporter	solute carrier family 39, member 2
Transporter	solute carrier family 1, member 4
Transporter	aquaporin 9
Transporter	kelch domain containing 2
Transporter	sodium channel, non-voltage-gated 1, beta (Liddle syndrome)
Transporter	ATPase H+ transporting, lysosomal 50/57 kDa, V1 subunit H
Tumor antigen	silver homolog (mouse)
Tumor antigen	hepatocellular carcinoma antigen gene 520
Tumor suppressor	phosphatidic acid phosphatase 2A
Tumor suppressor	FGF receptor activating protein 1
Unknown	chromosome 6 open reading frame 48
Unknown	chromosome 7 open reading frame 24
Unknown	alpha-2-glycoprotein 1, zinc
Unknown	premature ovarian failure 1B
Unknown	KIAA0483 protein
Unknown	DKFZP586A0522 protein
Unknown	chromosome 14 open reading frame 137
Unknown	KIAA 1536 protein
Unknown	cysteine-rich hydrophobic domain 2
Unknown	alpha-2-glycoprotein
Unknown	WD repeat domain 26
Unknown	KIAA0930 protein
Unknown	SLAc2-B
Unknown	HGFL gene
Unknown	KIAA0404 protein
Unknown	KIAA1815
Unknown	chromosome 6 open reading frame 79
Unknown	Nedd4 binding protein 1
Unknown	KIAA1102 protein
Unknown	breakpoint cluster region
Unknown/hypothetical	hypothetical protein MGC10940
Unknown/hypothetical	hypothetical protein FLJ22679
Unknown/hypothetical	hypothetical protein MGC11308
Unknown/hypothetical	hypothetical protein FLJ10134
Unknown/hypothetical	hypothetical protein FLJ10901
Unknown/hypothetical	hypothetical protein LOC149603
Unknown/hypothetical	hypothetical protein from clone 643MGC10940
Unknown/hypothetical	hypothetical protein LOC149427
Unknown/hypothetical	hypothetical protein MGC3222
Unknown/hypothetical	hypothetical protein DKFZp43K1210
Unknown/hypothetical	hypothetical protein SP192
Unknown/hypothetical	hypothetical protein FLJ10116

[0051] Moreover, there are 100 genes that are the most differentially regulated between the skin of chronic non-healing wounds (NHE) and normal healthy skin. FIG. **8** shows these genes, 50 of which are the most up-regulated in chronic non-healing wound skin as compared to normal skin, and 50 of which are the most down-regulated.

[0052] Normal basal epidermal keratinocytes are proliferating and as they exit the basal cell compartment, they commit to a differentiation program. Keratinocyte differentiation requires DNA degradation, nuclear destruction, and substantial proteolytic activity that leads to cell death and the formation of the cornified layer. Most of the 100 differentially regulated genes fall into one of the three main biological processes of keratinocytes: proliferation; differentiation; and apoptosis; thus, showing that these processes are aberrantly regulated in the cells of tissue from chronic non-healing wounds.

[0053] As shown in Examples 1 and 7, chronic wound tissue exhibits a specific morphology. Chronic wound tissue

exhibits thick hyperproliferative epidermis with hyperkeratotic (hypertrophy of the cornified layer of skin) and parakeratotic (presence of nuclei in the cornified layer) epidermis (FIG. **1**(*b*)). This morphology indicates aberrant proliferation and improper keratinocyte differentiation (Stojadinovic et al. (2005) *Am. J. Pathol.* 167:59-69). Results from the microarray analysis confirm that keratinocytes in chronic wound epidermis do not execute either of these processes in a proper manner.

[0054] Studies from transgenic mice suggest that differential expression of desmosomal proteins within the epidermis participate in the regulation of the tissue proliferation and differentiation (Brennan et al. (2007) *J. Cell Science* 120:758-771; Hardman et al. (2005) *Mol Cell. Biol.* 25:969-978; Smith et al. (2004) *Biochem. J.* 380:757-765; Merritt et al. (2002) *Mol. Cell. Biol.* 22:5846-5858; Garrod et al. (1996) *Curr. Opin. Cell Biol.* 8:670-678). In agreement with these findings, the microarray analysis showed desmosomal molecules are differentially regulated in chronic wounds as compared to normal skin. Specifically, desmosomal cadherin desmocollin 2 (Dsc2) was up-regulated in chronic wounds, while desmocollin 3 (Dsc3) was down-regulated. Desmoglein 3 (Dsg3) was up-regulated and desmoglein 2 (Dsg2) down-regulated. When human Dsg3 was over-expressed under control of keratin1 promoter in suprabasal epidermis of transgenic mice, histological analysis of the skin revealed hyperproliferative epidermis with hyper- and para-keratosis along with abnormal epidermal differentiation (Merritt (2002)). This suggests that in chronic wounds Dsg3 is up-regulated and expressed through the hyperproliferative epidermis, and that the atypical expression of the desmosomal molecules plays a role in epidermal morphogenesis and altered keratinocyte differentiation. Desmoplakin (DP) and plakophilin 2 (PKP2), additional desmosomal molecules, were down-regulated.

[0055] Moreover, keratinocyte differentiation markers, keratin 1 (K1) and keratin 10 (K10), were also shown to be down-regulated in chronic non-healing wound tissue by microarray analysis, the down-regulation of the latter protein being confirmed by immunohistochemistry analysis. Additional differentiation markers, filaggrin (FLG) and trichohyalin (THH) that associate with the keratin cytoskeleton during terminal differentiation were also down-regulated, the down-regulation of the former protein being confirmed by immunohistochemistry analysis.

[0056] Involucrin (IVL), a major early cross-linked component of the cornified envelope, and small proline rich proteins (SPRR1A, SPRR1B, SPRR2B, AND SPRR3) were up-regulated, the increased expression of the former protein in chronic wound tissue being confirmed by immunohistochemistry analysis. Transglutaminase 1 (TGM1), one of the enzymes responsible for crosslinking the SPRR proteins and involucrin in to the cornified envelope found in proliferating keratinocytes, but more abundantly expressed in differentiating keratinocytes, was up-regulated. These data suggest improper cornified envelope assembly in chronic wound epidermis (Steinert et al. (1997) *J. Biol. Chem.* 272:2021-2030).

[0057] S100A7, a gene which is part of the human epidermal differentiation complex (EDC) and the S100 family, and S100A8 and S110A9 were also among the 50 most up-regulated genes in the skin of chronic non-healing wounds as found by microarray analysis, the increased expression of the former being confirmed by RT-PCR. These genes are induced in normal primary keratinocytes by high levels of calcium, and found to be highly expressed in inflammatory and hyperproliferative skin diseases (Martinsson et al. (2005) *Exp. Dermatol.* 14:161-168; Eckert et al. (2004) *J. Invest. Dermatol.* 123:341-355; Marenholz et al. (2001) *Genome Res.* 11:341-355).

[0058] Kuppel-like factor (KLF4) was down-regulated in the chronic non-healing wound tissue. KLF4 is a transcription factor expressed in the differentiated layers of epidermis important in the establishment of skin barrier function and expression and cross-linking of cornified envelope proteins (Segre et al. (2003) *Curr. Opin. Cell Biol.* 15:776-782; Bazzoni et al. (2002) *J. Cell. Biol.* 156:947-949). Manic Fringe protein (MFNG), a protein whose expression is normally restricted to the proliferative basal layer during embryonic epidermal stratification (Thelu et al. (1998) *J. Invest. Dermatol.* 111:903-906), was up-regulated. This finding, along with the presence of mitoticaly active cells in the suprabasal layer, suggests its role in the induction of keratinocyte proliferation.

[0059] NOTCH-2 was downregulated. This protein is involved in the Notch signaling pathway that has been shown to play a role in defining different steps of keratinocyte differentiation (Rangarajan et al. (2001) *Embo J.* 20:3427-3436; Thelu (1998)).

[0060] Phospholipase D (PLD) has been implicated in late keratinocyte differentiation (Jung et al. (1999) *Carcinogenesis* 20:569-576). PLD1 was found to be down-regulated in chronic wound tissue and PLD2 up-regulated. Moreover, PLD1 mRNA levels are increased during differentiation (Nakashima et al. (1999) *Chem. Phys. Lipids* 98:153-164), and the highest level of PLD1 expression is found in the more differentiated layers of epidermis (Griner et al. (1999) *J. Biol. Chem.* 274:4663-460). The finding by microarray analysis of the down-regulation of PLD1 in chronic wound tissue suggests that there are less differentiated keratinocytes in chronic wounds.

[0061] Kalikrein 6 (KLK6), implicated in keratinocyte proliferation and differentiation and the pathogenesis of psoriasis (Kishibe et al. (2007) *J. Biol. Chem.* 282:5834-5841), was found to be up-regulated by microarray analysis, and confirmed by RT-PCR.

[0062] Among newly identified potential markers of keratinocyte terminal differentiation (Radoja et al. (2006) *Physiol. Genomics* 27:65-78), protease inhibitor 3, skin-derived (SKALP, PI3), oxysterol binding protein-like 8 (OSBPL8), adducing 3 (ADD3), early growth response 3 (EGR3), inhibitor of DNA binding 4 (ID4), occluding (OCLN) and decay accelerating factor for complement (DAF) were found to be down-regulated in chronic non-healing wound tissue, whereas septin (SEPT_8), serine/threonine kinase 10 (STK 10), and serine/cysteine proteinase inhibitor, clade B, member 3 (SERPINB3) were up-regulated. These data indicate that aberrant cornified envelope assembly and incomplete terminal differentiation play a role in the pathogenesis of chronic non-healing wounds.

[0063] One of the key goals of keratinocyte terminal differentiation is to form a physical barrier that acts as a permeability barrier against water loss, foreign microbes, and toxins. The two important components of the barrier function of the skin is cornified cell envelope and recently introduced tight junctions (TJs) (Bazzoni (2002)). Tight junctions in the skin are complex structures localized in the granular layer and are composed of transmembrane (claudins 1-20, ocludin) and plaque (Symplekin and ZO 1-3) proteins (Denning (2007) *J. Invest. Dermatol.* 127:742-744; Brandner et al. (2006) *Skin Pharmacol. Physiol.* 19:71-77). It was found by microarray

analysis that many of the different structural proteins of TJ are down-regulated in chronic wound skin as compared to normal skin. This suggests loss of permeability function in the epidermis of chronic wounds. Studies using knock-out mice for different claudins found that while there was TJ formation in the KO mice, the TJ function was completely altered (Furuse et al. (2002) *J. Cell. Biol.* 156:1099-1111; Pummi et al. (2001) *J. Invest. Dermatol* 117:1050-1058). These down-regulated TJ proteins include: tight junction protein 3 (TJP3); tight junction proteian, zona ocludens 3 (ZO3); spectrin 1 (SPTBN1); multiple PDZ domain protein (MUPP1); InaD-like protein (INADL); occluding (OCLN); claudin 5 (CLDN5); and claudin 8 (CLDN 8). Only symplekin (SYMPK) was up-regulated.

[0064] Formation of TJs in epidermis, as part of differentiation, is a precisely spatiotemporally regulated process. Important components of this regulation include polarity complex Par3, Par6, atypical PKC-iota, and CDC42 (Schneeberger et al. (2004) *J. Physiol. Cell. Physiol.* 286:C1213-1228). Recent findings suggest that the activity of this complex in the granular layer of the epidermis is necessary for TJs formation and keratinocyte differentation (Helfrich et al. (2007) *J. Invest. Dermatol.* 127:782-791). Furthermore, during calcium induced differentiation of keratinocytes, atypical PKC-iota was found necessary for the establishment of barrier formation. This complex has characteristic redistribution during wound healing and may also be an endogenous regulator of asymetric cell division of basal keratinoctyes (Denning (2007); Lechler et al. (2005) *Nature* 437:275-280). Asymmetric skin division promotes stratification and wound healing in the skin by keeping balance between basal proliferation and differentiation. PKC-iota and CDC42 were found to be down-regulated in chronic wound tissue as compared to normal skin, indicating a loss of cell polarity, further indicating a loss of balance between basal proliferation and differentiation, resulting in deregulation of TJ formation.

[0065] In mammalian cells, a crucial checkpoint control for proliferation is provided by pocket proteins of the retinoblastoma (Rb) family (Scherr (1996) *Science* 274:1672-1677; Weinberg (1996) *Cell* 81:323-330). All three pocket proteins of the Rb family, Rb, p107, and p130 were found to be down-regulated in chronic wound tissue by microarray analysis. Cyclin B1, cyclin D2, cyclin A2, cyclin F, and cyclin M4 were upregulated, as was CDC2, suggesting an increase of CDC2/cyclin B1 and CDC2/cyclin A2 complexes and the promotion of both cell cycle G1/S and G2/M transitions. The microarray data also suggests that there is a loss of cell cycle checkpoint regulation in the epidermis of chronic non-healing wounds. Checkpoint suppressor (CHES1) and WEE1 were down-regulated in chronic wound tissue. WEE1 catalyzes the inhibitory tyrosine phosphorylation of CDC2/cyclinB kinase, and appears to coordinate the transition between DNA replication and mitosis by protecting the nucleus from cytoplasmically activated CDC2 kinase. Without being bound by any theory, the up-regulation of CDC and cyclin B coupled with the loss of inhibitory phosphorylation, may contribute to the hyperproliferative phenotype of chronic wound tissue.

[0066] Cyclin D1 was down-regulated in chronic non-healing wound tissue. Over-expression of this gene is frequently observed in a variety of tumors, and may contribute to tumorgenesis. Moreover, EIF4E, which promotes the nuclear export of cyclin D1 is also down-regulated. EIF4E, a translation initiation factor, is a critical modulator of cellular growth, and levels are often elevated in tumors (Culjkovic et al. (2005) *J. Cell. Biol.* 169:245-256).

[0067] Two of the cyclin-dependent kinase inhibitors, CDKNB and CDKN3, were up-regulated. Keratins K6 and K16 were up-regulated, indicating keratinocyte activation.

[0068] Among secreted molecules, insulin-like growth factor binding protein (IGFBP5) was among the 50 most down-regulated genes in chronic wounds. Bone morphogenetic proteins (BMP) were differently regulated. BMP2 and BMP7 were down-regulated in chronic wound tissue as shown by both microarray analysis and RT-PCR. In normal human keratinocytes, BMP2 inhibits cell proliferation and promotes terminal differentiation (Gosselet et al. (2007) *Cell Signal* 19:731-739). The down-regulation of BMP2 in chronic wounds may contribute to the keratinocyte hyperproliferation and have an inhibitory effect on terminal differentiation. The expression of BMP1 was up-regulated.

[0069] Leptin enhances wound re-epithelialization (Frank et al. (2000) *J. Clin. Invest.* 106:510-509). The leptin receptor was found to be down-regulated.

[0070] Microarray analysis showed angiogenesis factors, vascular endothelial growth factors (VEGF), epiregulin (EREG) and angiopoetin-like 6 (ANGPTL6) were all down-regulated. ANGPTL6 promotes epidermal proliferation, remodeling, and regeneration (Oike et al. (2003) *PNAS* 100: 9494-9499). Other pro-angiogenic growth factors and receptors were found to be up-regulated in chronic wound tissue such as platelet-derived endothelial cell growth factor (ECGF1), receptor neuropilin (NRP1), and stromal cell-derived factors 1-alpha (CXCL12, SDF-1α). SDF-1α has an important role in homing endothelial progenitor cells.

[0071] The microarray analysis showed the strong down-regulation of apolipoprotein D (APOD) (associated with suprabasal differentiated keratinocytes (Radoja (2006)) and the strong up-regulation of defensin B4 (DEFB4) (associated with benign hyperplasia in skin (Haider et al. (2006) *J. Invest. Dermatol.* 126:869-881) in chronic wound tissue. These data were confirmed by RT-PCR.

[0072] Chemokines that mediate T cell chemotaxis were down-regulated, as was the expression of cutaneous T-cell attracting chemokine (CCL27) and IL-7, essential for memory T-cell generation. The expression of the IL-7 receptor was up-regulated, as was the expression of platelet-derived growth factors, PDGFB and PDGFA. The expression of TGFB2, TGFBR3, FGF13, and IL-6 was down-regulated in chronic wound skin. IL deficient mice display significantly delayed cutaneous wound closure (Gallucci et al. (2006) *J. Invest. Dermatol.* 126:561-568).

[0073] The stromelysin-3 gene (MMP-11) was up-regulated as found by microarray analysis and confirmed by RT-PCR. It has been suggested that MMP-11 expression may be under the control of factors produced by inflammatory cells during wound healing and by cancer cells during carcinoma progression (Basset et al. (1993) *Breast Cancer Res. Treat.* 24:185-193).

[0074] Lastly, some of the Fas-mediated apoptosis genes were up-regulated in chronic wound tissue (FASTH, FAF1, PACAP, FASTK) while some were down-regulated (PHLDA2, PCDN6, PTPN13, APAF1). Bcl-2 associated protein, BAX, involved in p53 mediated apoptosis was up-regulated as well as p53 inducible protein 3 (TP53I3). Some inhibitors of apoptosis were down-regulated (BAG4, SER-

PINB2) while some were up-regulated (NOL3, AVEN, BIRC5). Inhibitor of TNFα mediated apoptosis (TNFAIP3) was down-regulated.

[0075] Using the direct correlation between cell biology and gene expression profiles, one can determine a tissue site that is suitable for debriding, i.e., a site with cells which would respond well to debriding. This particular method can be used to determine where in a chronic wound to start debridement as well as to determine the debridement margin. It can also be used to identify tissues with cells that would respond well to other chronic wound treatment. This is an important tool in both further treatment of a chronic wound by pharmaceutical and/or biological agents as well as for testing potential therapeutic agents for chronic wound therapy. If it is known prior to testing such agents that tissues and cells are being targeted that respond well to wound healing stimuli, the outcome of the clinical tests of the agents can be better evaluated. In other words, it would be known that the success or failure of the agent being tested was not related to the cells being targeted and due to some other variable.

[0076] To perform this method, one or more tissue samples or biopsies are taken from within or adjacent to a chronic wound. A gene expression profile is then determined for the cells in the site or sites of the tissue biopsies. This gene expression profile is compared to a known gene expression profile from cells that derive from tissue in a site adjacent to the wound (ACW) that is known to respond well to debriding. This known second gene expression profile can be from the non-ulcerated skin adjacent to the wound (ACW) shown in FIG. **2**, or from another site adjacent to the wound or away from the wound that has been found to contain cells that respond well to wound healing stimuli. Additional sites can be found by testing the cells in the site for response to wound healing stimuli and determining a gene expression profile from cells with good responses.

[0077] Using the correlation between cell biology and gene expression profiles, it can be also determined if a debridement treatment has been successful or if such treatment needs to continue.

If the gene expression profile of a sample tissue biopsy is the same or similar to the cells in the non-healing edge of the wound (NHE), further debridement is required to reach the appropriate cells. If the gene expression profile of the tissue sample is the same or similar to the cells in the non-adjacent non-ulcerative area (ACW), then the debridement was sufficient. Again this information is also useful in both a clinical setting in determining treatment for particular patients, as well as for testing potential therapeutic agents for chronic wound treatment. If it is known prior to testing a therapeutic agent that a wound has been successfully and fully debrided, the outcome of the testing can be better evaluated.

[0078] In performing this method, one or more biopsies or tissue samples from in or adjacent to the chronic wound may be taken. It is preferable, but not necessary, that the sample be from any area of the chronic wound where debridement has already been performed. A gene expression profile is then determined for the cells in the site or sites of the tissue biopsies. Once the gene expression profiles for the biopsied tissue are determined, they can be compared to the known gene expression profile of the cells from the adjacent non-ulcerated skin (ACW) found in FIG. **2**. However, comparison can also be made to the gene expression profiles of tissue adjacent to the chronic wound that have been shown to have cells with a healthy morphology and/or a good response to wound healing stimuli, or other healthy skin. If the gene expression profile of the biopsied tissue is the same or similar to the gene expression profile of the tissue containing cells with healthy morphology and/or good response to wound healing stimuli, then the debriding has been sufficient and can be terminated.

[0079] Any methods known in the art can be used to test for the various biological characteristics of the cells. A preferred method for testing the response to wound healing stimuli is an in vitro wound scratch assay performed on fibroblasts grown from the tissue samples. This method requires growing fibroblasts from the biopsied tissue and once the culture is established, scratching the cells with a sterile pipet or other instrument. The capacity of the cells to respond to the wound healing stimuli is measured by the distance the cells migrate to cover the initial scratch. The further the cells migrate, the better their response to the scratch, i.e., wound healing stimuli. Cells with further migration would be predicted to grow better and heal after surgical debridement.

[0080] The preferred method for determining the morphology of the cells is staining by hematoxylin, eosin and/or an antibody such as one for pro-collagen.

[0081] The current preferred technology that would be used to determine the gene expression profiles or "bar codes" of the tissue is microarrays. Processing the tissue samples from obtaining a biopsy to obtaining a gene expression "bar code" takes approximately three days. However, under current treatment protocols, this information is still clinically useful as there is often waiting periods in debridement procedures.

[0082] The terms "array" or "microarray" are used interchangeably and refer generally to any ordered arrangement (e.g., on a surface or substrate) of different molecules, referred to herein as "probes." Each different probe of any array is capable of specifically recognizing and/or binding to a particular molecule, which is referred to herein as its "target" in the context of arrays. Examples of typical target molecules that can be detected using microarrays include mRNA transcripts, cRNA molecules, and proteins.

[0083] Microarrays are useful for simultaneously detecting the presence, absence and quantity of a plurality of different target molecules in a sample (such as an mRNA preparation isolated from a relevant cell, tissue or organism, or a corresponding cDNA or cRNA preparation). The presence and quantity, or absence, of a probe's target molecule in a sample may be readily determined by analyzing whether (and how much of) a target has bound to a probe at a particular location on the surface or substrate.

[0084] In a preferred embodiment, arrays used in the present invention are "addressable arrays" where each different probe is associated with a particular "address."

[0085] The arrays utilized in the present invention are preferably nucleic acid arrays that comprise a plurality of nucleic acid probes immobilized on a surface or substrate. The different nucleic acid probes are complementary to, and therefore can hybridize to, different target nucleic acid molecules in a sample. Thus, such probes can be used to simultaneously detect the presence and quantity of a plurality of different nucleic acid molecules in a sample, to determine the expression of a plurality of different genes, e.g., the presence and abundance of different mRNA molecules, or of nucleic acid molecules derived therefrom (for example, cDNA or cRNA).

[0086] There are two major types of microarray technology: spotted cDNA arrays and manufactured oligonucleotide

arrays. The Example section below describes the use of a high density oligonucleotide Affymetrix GeneChip® human genome array.

[0087] The arrays are preferably reproducible, allowing multiple copies of a given array to be produced and the results from each easily compared to one another. Preferably microarrays are small, usually smaller than 5 cm^2, and are made from materials that are stable under binding (e.g., nucleic acid hybridization) conditions. A given binding site or unique set of binding sites in the microarray will specifically bind the target (e.g., the mRNA of a single gene in the cell). Although there may be more than one physical binding site (hereinafter "site") per specific target, for the sake of clarity the discussion below will assume that there is a single site. It will be appreciated that when cDNA complementary to the RNA of a cell is made and hybridized to a microarray under suitable hybridization conditions, the level or degree of hybridization to the site in the array corresponding to any particular gene will reflect the prevalence in the cell of mRNA transcribed from that gene. For example, when detectably labeled (e.g., with a fluorophore) cDNA complementary to the total cellular mRNA is hybridized to a microarray, the site on the array corresponding to a gene (i.e., capable of specifically binding a nucleic acid product of the gene) that is not transcribed in the cell will have little or no signal, while gene for which the encoded mRNA is highly prevalent will have a relatively strong signal.

[0088] By way of example, GeneChip® expression analysis (Affymetrix, Santa Clara, Calif.) generates data for the assessment of gene expression profiles and other biological assays. Oligonucleotide expression arrays simultaneously and quantitatively "interrogate" thousands of mRNA transcripts (genes or ESTs), simplifying large genomic studies. Each transcript can be represented on a probe array by multiple probe pairs to differentiate among closely related members of gene families. Each probe set contains millions of copies of a specific oligonucleotide probe, permitting the accurate and sensitive detection of even low-intensity mRNA hybridization patterns. After hybridization intensity data is captured, e.g., using optical detection systems (e.g., a scanner), software can be used to automatically calculate intensity values for each probe cell. Probe cell intensities can be used to calculate an average intensity for each gene, which correlates with mRNA abundance levels. Expression data can be quickly sorted based on any analysis parameter and displayed in a variety of graphical formats for any selected subset of genes. Gene expression detection technologies include, among others, the research products manufactured and sold by Hewlett-Packard, Perkin-Elmer and Gene Logic.

[0089] It is contemplated that technological developments will allow more rapid processing of the RNA from tissue to chips, such as a desktop machine that has been recently reported that allows doctors to access a patient's DNA from a drop of blood in just an hour (Cyranoski (2005) *Nature* 437: 796).

[0090] As shown, certain genes are up-regulated or induced in the cells from tissue from chronic non-healing wounds as compared to healthy skin, and certain genes are down-regulated or suppressed. This differential regulation of certain genes can also be used to identify a suitable site for debridement as well as determine if the debridement needs to be continued on a wound.

[0091] To perform a method for identifying a suitable site for debridement, one of more tissue samples are taken from within or adjacent to a chronic wound. The expression of a gene or genes known to be differentially regulated in chronic non-healing wound tissue (NHE) as compared to normal skin is determined. If the expression of the gene or genes is identical or similar in the sample, i.e., up-regulated or down-regulated, to the known expression of the gene or genes in the cells from the tissue of chronic non-healing wounds (NHE), then the site is suitable for debridement. If the expression of the gene or genes is identical or similar in the sample, i.e., up-regulated or down-regulated, to the known expression of the gene or genes in the cells of healthy skin, then the site is not suitable for debridement.

[0092] To perform a method for determining if a debridement treatment has been successful or if such treatment needs to continue, one of more tissue samples are taken from within or adjacent to a chronic wound. It is preferable, but not necessary, that the sample be from an area of the wound where debridement has already been performed. The expression of a gene or genes known to be differentially regulated in chronic non-healing wound tissue (NHE) as compared to normal skin is determined. If the expression of the gene or genes is identical or similar in the sample, i.e., up-regulated or down-regulated, to the known expression of the gene or genes in the cells from tissue of chronic non-healing wounds (NHE), then further debridement is necessary. If the expression of the gene or genes is identical or similar in the sample, i.e., up-regulated or down-regulated, to the known expression of the gene or genes in the cells of healthy skin, then the debridement has been successful.

[0093] Any method known in the art can be used to determine the expression of the gene or genes in the sample. Such methods include, but are not limited to, microarray analysis, RT-PCR, quantitative RT-PCR, immunohistochemistry, Southern, Northern and Western blots.

[0094] Genes that are known to be up-regulated or induced in the cells of tissue from chronic non-healing wounds (NHE) as compared to the cells in normal healthy skin, include, but are not limited to desmocollin 2 (Dsc2), desmoglein 3 (Dsg3), involucrin (IVL), small proline rich protein 1A (SPRR1A), small proline rich protein 1B (SPRR1B), small proline rich protein 2B (SPRR2B), small proline rich protein 3 (SPRR3), transglutaminase 1 (TGM1), S100 calcium binding protein A7 (S100A7), S100 calcium binding protein A8 (S100A8), S100 calcium binding protein A9 (S100A9), manic fringe protein (MFNG), phospholipase D 2 (PLD2), kalikrein 6, (KLK6), septin (SEPT_8), serine/threonine kinase 10 (STK10), serine/cysteine proteinase inhibitor, clade B, member 3 (SERPINB3), symplekin (SYMPK), cyclin B1, cyclin D2, cyclin A2, cyclin F, cyclin M4, cell division cycle 2 homolog (CDC2), cyclin dependent kinase inhibitor NB (CDKNB), cyclin dependent kinase inhibitor N3 (CDKN3), keratin 6 (K6), keratin 16 (K16), bone morphogenetic protein 1 (BMP-1), platelet derived endothelial growth factor (ECGF1), receptor neuropilin (NRP1), stromal cell derived factor 1-alpha (SDF-1α), defensin B4 (DEFB4), IL-7 receptor (IL-7R), platelet derived growth factor B (PDGFB), platelet derived growth factor A (PDGFA), Fas-activated serine/theorine kinase (FASTK), Fas (TNFRSF6) associated factor (FAF1), proapoptotic caspase adaptor protein (PCAP), bcl-2 associated protein (BAX), p53 inducible protein (TP5313), nucleolar protein 3(NOL3), apoptosis, caspase activation inhibitor (AVEN), and baculoviral IAP repeat-containing 5 (surivin) (BIRC5).

[0095] Genes that are known to be down-regulated or suppressed in the cells of tissue from chronic non-healing wounds (NHE) as compared to cells in normal, healthy skin, include, but are not limited to desmocollin 3 (Dsc3), desmoglein 2 (Dsg2), desmoplakin (DP), plakophilin 2 (PKP2), filaggrin (FLG), thrichohyalin (THH), kuppel-like factor (KLF4), NOTCH, drosophila, homolog OF, 2 (NOTH2), phospholipase D 1 (PLD1), protease inhibitor 3, skin-derived (SKALP, PI3), oxysterol binding protein-like 8 (OSBPL8), adducing 3 (ADD3), early growth response 3 (EGR3), inhibitor of DNA binding 4 (ID4), occluding (OCLN), decay accelerating factor for complement (DAF), tight junction protein, zona occludens 3 (ZO3), tight junction protein 3 (TJP3), spectrin 1 (SPTBN1), multiple PDZ domain protein (MUPP1), InaD-like protein (INADL), claudin 5 (CLDN5), claudin 8 (CLDN8), protein kinase C-iota (PKC-iota), cell division cycle homolog 42 (CDC42), retinoblastoma protein (Rb), retinoblastoma protein (p107), retinoblastoma protein (p103), checkpoint suppressor (CHES1), WEE1 homolog (WEE1), translation initiation factor (EIF4E), insulin-like growth factor binding protein (IGFBP5), bone morphogenetic protein 2 (BMP2), bone morphogenetic protein 7 (BMP7), leptin receptor (LEPR), vascular endothelial growth factor (VEGF), epiregulin (EREG), angiopoetin-like 6 (ANGPTL6), apolipoprotein D (APOD), cutaneous T cell attracting chemokine 27 (CCL27), IL-7, transforming growth factor, beta 2 (TGFB2), transforming growth factor, beta 3, (TGFBR3), fibroblast growth factor 13 (FGF13), interleukin 6 (IL-6), pleckstrin homology-like domain, family A, member 2 (PHLDA2), programmed cell death (PDCD6), protein tyrosine phosphatase, non-receptor type 13 (APO-1/CD95 (Fas)-associated phophatase (PTPN13), apoptotic peptidase activating factor 1 (APAF1), and TNFα mediated apoptosis inhibitor (TNFAIP3).

EXAMPLES

Example 1

Skin Specimens and Histology

Materials and Methods

[0096] A total of eight skin sample biopsies were obtained from three consented patients with venous reflux ulcers as discarded tissue after debridement procedures. The biopsies were obtained in a blinded fashion, i.e., the wound location was under code. As shown in FIG. **1**A, the biopsies were obtained from two distinct locations in the wounds: the non-healing edge (NHE) (location A) and the adjacent non-ulcerated skin (ACW) (location B).

[0097] A small portion of the specimens were fixed in formalin and processed for paraffin embedding. The paraffin embedded tissues were sectioned and 5 μm thick sections were stained with hematoxylin and eosin. The sections were also stained with pro-collagen type I antibody M-38 (Developmental Studies Hybridoma Bank at University of Iowa, described in McDonald et al. (1986) *J. Clin. Invest.* 78:1237-1244) following the published protocol of Stojadinovic et al. (2005) *Am. J. Pathol.* 167:59-69. The sections were analyzed using a Carl Zeiss microscope (Carl Ziess, Thornwood, N.Y.) and digital images collected using an Adobe TWAIN_32 program.

Results

[0098] The results of the histological staining showed that the locations of non-healing wounds differed in their morphology. FIG. **1**(*b*) shows the results of stained tissue from the epidermis layer. The hematoxylin and eosin stained biopsy obtained from the non-healing edge (NHE) (location A as shown in FIG. **1**(*a*)) showed thick, hyperproliferative epidermis with hyperkeratotic (hypertrophy of the cornified layer of the skin) and parakeratotic (presence of nuclei in the cornified layer) epidermis (FIG. **1**(*b*)). Following the debridement margin towards healthy skin, the morphology of the skin biopsies transformed. Epidermis from adjacent, non-ulcerated skin (ACW) (location B as shown in FIG. **1**(*a*)) was normalized and exhibited a well-defined cornified layer and significantly less hyperproliferation as compared to the non-healing edge. However, it was still more hyperproliferative than epidermis of normal skin that is not part of the wound (FIG. **1**(*b*)).

[0099] FIG. **1**(*c*) shows stained tissue from the dermis layer. Epidermal ridges (projections of the epidermis into the dermis) were also present in the adjacent, non-ulcerated skin, although they extended deeper in the dermis than in normal skin. Evidence of fibrosis was also found in both the dermis in the non-healing edge and the non-ulcerated skin adjacent to the wound, although to a lesser extent in the non-ulcerated skin. The dermis of the skin from the non-healing edge exhibited increased cellularity when compared to adjacent, non-ulcerated or normal skin (FIG. **1**(*c*)).

[0100] Finally, intracellular pro-collagen was most pronounced in the dermis from the non-healing edge when compared with skin from the adjacent, non-ulcerated area or normal skin (FIG. **1**(*d*)).

[0101] In summary, the stained biopsies from non-healing edge of the wound (NHE) (location A) exhibited severe pathogenesis as compared to the adjacent, non-ulcerated skin (ACW) (location B). It was concluded that the biology of the skin within the wound edge differs from healthy skin.

Example 2

Total RNA Isolation and Microarray Analysis

Materials and Methods

[0102] Samples from Example 1 were stored in an RNAlater (Ambion) for subsequent RNA isolation. Total RNA from the samples of Example 1 was then isolated using RNeasy (QIAGEN, Valencia, Calif.) following the commercial protocol. Northern Blot analysis was performed to assess the quality of the isolated mRNA. Using RNeasy protocol, 5 μg of total RNA was reversed-transcribed, amplified and labeled. Labeled cRNA was hybridized to GeneChip®) Human Genome U133 arrays (Affymetrix, Santa Clara, Calif.) following commercial protocol. The arrays were washed and stained with anti-biotin streptavidin-phycoerythin labeled antibody using Affymetrix fluidics station and then scanned using the Agilent GeneArray Scanner system (Hewlett-Packard, Palo Alto, Calif.).

[0103] Microarray Suite 5.0 (Affymetix) was used for data extraction. Data Mining Tool 3.0 (Affymetrix) was used for further analysis. GeneSpring™ software 5.1 (Silicon Genetics, Santa Clara, Calif.) was used for normalization, fold change calculations, and clustering.

[0104] Differential expressions of transcripts were determined by calculating the fold change. Genes were considered regulated if the expression levels differed by more than 2-fold

to healing edges. Clustering was performed based upon similarity of the expression pattern in all samples using GeneSpring™.

Results

[0105] Using the Affymetrix HU133 chips and Gene Spring™ software as described above, hybridizations of the eight samples were performed, four from the non-healing edges (NHE) (location A) and four from the non-ulcerated skin (ACW) (location B). The various samples were compared and a specific transcriptional, i.e., gene expression, profile was obtained. Gene expression was visualized by generating gene trees, a graphic representation in which sample are grouped based on the similarity of their gene expression profiles. The dark gray lines represent up-regulated genes, the lighter gray lines represent down-regulated genes and the lightest gray lines represent expressed genes, but ones that are not significantly regulated. This method allows the overall visualization of the entire gene expression pattern, rather than specific gene regulation. Using this method, it is shown that the expression patterns from the samples from the non-healing edge (location A) are similar, while the expression patterns from the samples taken from the adjacent, non-ulcerated skin (location B) are similar to each other but quite different than the pattern from the samples from the non-healing edge (FIG. **2**).

[0106] These gene expression pattern profiles coupled with the tissue morphology studies in Example 1 show that the cells from the two different wound locations exhibit different biological features.

Example 3

Primary Fibroblast Cell Culture

Materials and Methods

[0107] The 5 mm biopsies obtained from three patients during debridement procedure were used to establish fibroblast cultures. The biopsies were obtained from two different locations: non-healing wound edge (NHE) and adjacent non-ulcerated skin (ACW). The underlying fat beneath the skin was removed, and the tissue washed six times in phosphate buffered saline (PBS), and minced into pieces approximately 1 mm^2 in size. The tissue pieces were placed in 75 cm^2 tissue culture flasks containing Dulbecco's modified Eagle medium (DMEM) supplemented with 10% serum, and a penecillin/streptamycin/gentamycin mixture. After several days in culture, fibroblasts were observed sprouting from the tissue explants. The mono layer was trypsinized to separate the tissue explants from the cells. Dermal fibroblasts were then seeded in DMEM with 10% serum and the penecillin/streptamycin/gentamycin mixture. The fibroblasts were propagated by trypsinization until the fourth passage.

Results

[0108] The fibroblasts grown from the tissue at the non-healing edge of the chronic wounds (NHE) exhibited pathogenic phenotypes, whereas the fibroblasts grown from the adjacent non-ulcerated area (ACW) (location B) had a phenotype similar to primary fibroblasts obtained from healthy skin (control) (FIG. **3**). The fibroblasts from the non-healing edge of the chronic wound were misshaped, inflated with large nuclei, and clumped together as compared to normal cells (FIG. **3**).

Example 4

Wound Scratch Assay

Materials and Methods

[0109] The primary human dermal fibroblasts described in Example 3 were grown to 80% confluency. Cells were transferred to basal medium containing DMEM with 5% stripped serum (Radoja et al. (2000) *Mol. Cell. Biol.* 20:4328-4339) 24 hours prior to the experiment. On day 0, the cells were treated with 8 μg/ml of Mitomycin C (ICN) for one hour and washed with 1×PBS prior to scratch.

[0110] Scratches were performed using sterile yellow pipet tips and photographed using a Carl Zeiss microscope and a Sony digital camera. Cells were further incubated for 4, 8 and 24 hours and re-photographed in the same fields as initially done on day 0. Cell migration was quantified using a Sigma Scan Program. Measurements were taken for each experimental condition and expressed as a percentage of distance covered by the cells moving into the scratch wound area for each time point after wounding. Three images are analyzed per condition and time point, and averages and standard deviations were calculated.

Results

[0111] The fibroblasts grown from the non-healing edge tissue (NHE) (location A) have the slowest migration rate, covering only 33% of the initial scratch in 24 hours. Fibroblasts grown from the adjacent, non-ulcerated tissue (ACW) (location B) covered 75%, only slightly less than the control which closed 89% of the scratched area (FIG. **4**).

[0112] The results from Examples 1-4 indicate a direct correlation between specific location within the wound, cellular biology, cellular response to wounding, and gene expression profile.

Example 5

[0113] Using the microarray analysis described in Example 2, gene expression patterns were obtained for samples from the non-healing edge (NHE) (location A), the adjacent non-ulcerated tissue (ACW) (location B), and an additional sample from an intermediate location between locations A and B (location *). The gene expression patterns for each sample are found in FIG. **5**. As can be seen from the Figure, the gene expression pattern of the intermediate sample (indicated by an "*") was more similar to the gene expression pattern of non-healing edge sample, indicating that debridement procedure needed to proceed further, until a healing pattern, similar to that of location B, is detected. This data suggest that gene expression pattern changes may serve as an indication of the pathogenic progress within the wound, which can further guide the extent of the debridement.

Example 6

Further Analysis of Expression of Specific Genes

Materials and Methods

[0114] Further analysis of the actual genes being up-regulated and down-regulated in the gene expression profiles obtained in Example 2 were done using Microarray Suite 5.0

(Affymetix) for data extraction, Data Mining Tool 3.0 (Affymetrix) for further analysis and GeneSpring™ software 5.1 (Silicon Genetics) for normalization, fold change calculations, and clustering.

[0115] Differential expressions of transcripts were determined by calculating the fold change. To compare data from multiple arrays, the signal of each probe array was scaled to the same target intensity value. Genes were considered regulated if the expression levels differed by more than 2-fold to healing edges at any time point. Fold changes obtained from the first and second experiments were averaged and determined regulated if the fold changes were more than 2 or less than 2. Clustering was performed based upon similarity of the expression pattern in all samples using GeneSpring™.

[0116] An extensive gene annotation table was produced describing the molecular function and biological category of the genes present on the Affymetrix Human Genome chip based upon data from J. M. Ruillard and the Gene Ontology Consortium Data available on the World Wide Web at cgap.nci.nih.gov/Genes/GOBrowser and ot.ped.med.umich.edu:2000/ourimage/pub/shared/JMR_pub_affyannot.html.

Results

[0117] The results, found in FIG. **6**, show the gene annotation table describing the molecular function and biological categories of the genes present on the Affymetrix Human Genome U133 GeneChip®. The light gray areas depict genes that are up-regulated in the tissue at location B, the non-ulcerated skin adjacent to the chronic wound (ACW) as compared to the tissue at location A, the non-healing edge of the wound (NHE). The dark gray areas depict genes that are down-regulated in tissues from location B as compared to location A. The numbers within the light gray and dark gray shaded areas depict the fold change. The two different columns depict the comparison of the two locations in two different patients. As seen by the Figure, over 400 genes are differentially regulated in the cells of the tissue in non-ulcerated skin adjacent to a chronic wound as compared to the cells of the tissue in the non-healing edge.

Example 7

Additional Skin Specimens and Histology

[0118] Additional skin sample biopsies were obtained from both the non-healing edge of chronic wounds (NHE) and normal healthy skin specimens. Skin biopsies from the non-healing edge of chronic wounds were obtained after surgical debridement procedures from three consenting patients with venous reflux ulcers. Three normal skin specimens were obtained as discarded tissue from voluntary corrective surgery.

[0119] A small portion of skin biopsies were embedded in OCT compound (Tissue Tek, Torrance, Calif.) and frozen in liquid nitrogen. The majority of the samples were stored in RNAlater (Ambion, Foster City, Calif.) for subsequent RNA isolation.

[0120] Hematoxylin and eosin staining were performed on tissue from the samples as described in Example 1. Similar to the results in Exhibit 1, all of the samples from the chronic wounds (NHE) showed hyperproliferative, hyper and parakeratotic epidermis typical for non-healing edges of chronic ulcers.

Example 8

Total RNA Isolation and Microarray Analysis of Additional Skin Samples

Materials and Methods

[0121] Samples from Example 7 (three from the patients with the chronic wounds and three from normal skin), stored in RNAlater were used for RNA isolation and gene array data analysis, using the procedure described in Example 2.

Results

[0122] Using the Affymetric HU133 chips and Gene Spring™ software previously described (Example 2), a gene tree utilizing all genes present on the chip, and a visualized expression profile of each sample were generated. This method allows overall visualization of the entire gene expression pattern, rather than specific gene regulation. As shown in FIG. **7** and previously described in Example 2, it is shown that the expression patterns of the skin samples from the chronic wound biopsies are similar, while the expression patterns of the samples taken from the normal skin samples are similar to each other but quite different from the pattern of the samples from the chronic wound.

Example 9

Further Analysis of Expression of Specific Genes of the Additional Skin Samples

Materials and Methods

[0123] Using the samples from Example 7, further analysis of the actual genes being up-regulated and down-regulated in the gene expression profile obtained in Example 8 were done using the methods described previously in Example 6.

Results

[0124] Of approximately 22,000 genes presented on the chip, 1557 genes were found to be differentially regulated between non-healing edges of the chronic wounds and normal healthy skin. Out of the 1557 genes, 55% of the genes were down-regulated and 45% were up-regulated in normal skin as compared to skin from the non-healing edges of a chronic wound. The regulated genes sorted by biological function and regulation are shown in Table 3.

TABLE 3

Percent of up-regulated and down-regulated genes in normal skin as compared to skin from the non-healing edge of a chronic wound

PERCENT OF DOWN-REGULATED GENES	BIOLOGICAL FUNCTION OF THE GENES	PERCENT OF UP-REGULATED GENES
52	Adhesion	48
60	Antioxidants	40
53	Apoptosis	47
20	Ca Binding	80
47	Cell cycle	53
83	Cell growth, proliferation	17
75	Cytochrome	25
59	Cytoskeletal	41
29	Detoxification	71
80	Development	20
80	DNA Binding	20

TABLE 3-continued

Percent of up-regulated and down-regulated genes in normal skin as compared to skin from the non-healing edge of a chronic wound

PERCENT OF DOWN-REGULATED GENES	BIOLOGICAL FUNCTION OF THE GENES	PERCENT OF UP-REGULATED GENES
45	DNA Repair, Synthesis	55
45	ECM	55
23	Energy	77
42	Enzyme	58
13	Epidermal Differentiation	87
47	Golgi Apparatus	53
54	G-regulated Protein	46
67	Heat Shock	33
47	Immune Response Related	53
8	Immunoglobulin	92
67	INF-Regulated	33
44	Membrane Protein	56
38	Membrane, cell-surface	62
38	Metabolism	62
27	Mitochondrial	73
86	Nuclear Receptors	14
54	Nucleoskeletal	46
67	Oncogenesis	33
46	Proteolysis	54
64	Phosphatase	36
80	Protein Binding	20
53	Protein Kinase	67
33	Protein Inhibitor	67
18	Protein Modification	82
58	Receptors	42
58	Regulators	42
85	RNA Metabolism	15
63	Secreted	37
63	Signal Transduction	37
58	Trafficking	42
70	Transcription	30
79	Transcription Factor	21
83	Transcription Repressor	17
70	Translation	30
47	Transporter	53
73	Tumor Antigen	27
57	Tumor Suppressor	43

[0125] The 100 most regulated genes, 50 being the most up-regulated and 50 being the most down-regulated, along with associated fold-changes and p-values, grouped by cellular functins and biological processes, are shown in FIG. **8**. The most regulated genes fall into the following categories for biological processes: 1) contact and motility; 2) tissue remodeling; 3) inflammation; 4) proliferation; 5) differentiation; 6) cell death control; 7) metabolism; and 8) signal transduction and transcription.

Example 10

Immunohistochemistry

Materials and Methods

[0126] In order to confirm the microarray data obtained in Example 9, the normal healthy skin samples and the skin samples from the chronic wounds (Example 7) were stained with antibodies recognizing various proteins that were differentially regulated in the chronic wound tissue.

[0127] Frozen skin specimens from both normal skin biopsies and biopsies from chronic wounds were cut with a cryostat (Jung Frigocut 28006, Leica, Germany) and stored at −80° C. Slides containing the frozen 5 micrometer skin sections were fixed in cold acetone for 1 minute. Sections stained with desmoglein 2 (1:2, AbCam, Cambridge, Mass.), desmoglein 3 (1:100, Santa Cruz Biotech, Santa Cruz, Calif.), and desmoplakin (1:200, a gift from Dr. Jim Wahl, University of Toledo) as a primary antibody were blocked with 0.1% Triton-X in 1% BSA for 60 minutes and incubated overnight at 4° C.

[0128] Sections stained with a monoclonal antibody against filaggrin (1:1000 as described in Dale et al. (1985) *J. Cell. Biol.* 101: 1257-1269), keratin 10 (1:500, a gift from Dr. Tung-Tien Sun, New York University School of Medicine), and involucrin (1:500, NeoMarkers, Waltham, Mass.) as a primary antibody were blocked with 5% bovine serum albumin (BSA) and incubated with a primary antibody diluted in 5% BSA in 1× phosphate buffered saline (PBS).

[0129] Signals were visualized using Alexa-Fluor 488 or Alexa-Fluor 594 (Molecular Probes, Carlsbad, Calif.) as a secondary antibody. Slides were mounted with mounting media containing Dapi (Vector Labs, Burlingame, Calif.).

[0130] All negative controls were prepared by substituting the primary antibody with PBS. Staining was analyzed using a Nikon Eclipse E800 microscope and digital images were collected using SPOT-Camera Advanced Program.

Results

[0131] Desmoglein 2 (Dsg2), desmoglein 3 (Dsg3), and desmoplakin (DP) are adhesion junction molecules. Some adhesions junction molecules, including these three, were found to be differentially regulated in chronic wounds in the microarray analysis performed in Example 9. Specifically, the microarray analysis showed that Dsg3 was up-regulated in chronic non-healing wounds, and Dsg2 and DP were down-regulated. As shown in FIG. **9**, staining with Dsg3 showed an increased signal throughout the epidermis of the chronic wounds as compared to normal skin, while the staining signal of the Dsg2 and DP was decreased in the epidermis of the chronic wound. These data confirm that there is deregulation of major desmosomal proteins in the epidermis of chronic non-healing wounds.

[0132] Microarray analysis also showed that keratinocyte differentiation markers were differentially regulated in the epidermis of chronic non-healing wounds. Keratin 10 (K10) was shown to be down-regulated in the epidermis of chronic non-healing wounds. Additional differentiation markers, such as filaggrin (FLG) were also down-regulated, while involucrin (IVL) was up-regulated. The results of the immunohistochemistry analysis confirm the microarray data. As shown in FIG. **10**, there is an increased involucrin expression in the epidermis of the chronic non-healing wounds, whereas the K10 and filaggrin staining was barely detected in the chronic non-healing wound samples.

[0133] In conclusion, the results from the immunohistochemistry analysis confirm and are in agreement with the results from the microarray analysis.

Example 11

Quantitative Real-Time PCR Analysis

Materials and Methods

[0134] 0.5 μg of total RNA from normal skin samples and samples from the chronic wounds were reverse transcribed using Omniscript Reverse Transcription Kit (QIAGEN). The real-time PCR was performed in triplicate using the iCycler

iQ thermal cycler and detection system and an iQ SYBR Supermix (BioRad, Hercules, Calif.). Relative expression was normalized for levels of hypoxanthin-guanine phosphoribosyltransferase (HPRT1). The primer sequences used were as follows:

HPRT1, forward - (5'-AAAGGACCCCACGAAGTGTT-3') (SEQ ID NO 1)

HPRT1, reverse - (5'-TCAAGGGCATATCCTACAACAA-3') (SEQ ID NO 2)

Human β defensin 4 (HBD4), forward - (5'-GGTGGTATAGGCGATCCTGTT-3') (SEQ ID NO 3)

HBD4, reverse - (5'-AGGGCAAAAGACTGGATGACA-3') (SEQ ID NO 4)

Kalikrein 6 (KLK6), forward - (5'CATGGCGGACCCCTGCGACAAGAC-3') (SEQ ID NO 5)

KLK6, reverse - (5'-TGGATCACAGCCCGGACAACAGAA-3') (SEQ ID NO 6)

MMP11, forward - (5'-AGATCTACTTCTTCCGAGGC-3') (SEQ ID NO 7)

MMP11, reverse - (5'-TTCCAGAGCCTTCACCTTCA-3') (SEQ ID NO 8)

CCL27-2, forward - (5'-TCCTGAGCCCAGACCCTAC-3') (SEQ ID NO 19)

CCL27-2, reverse - (5'-CAGTTCCACCTGGATGACCTT-3') (SEQ ID NO 10)

APOD, forward - (5'-AATCAAATCGAAGGTGAAGCCA-3') (SEQ ID NO 11)

APOD, reverse - (5'-ACGAGGGCATAGTTCTCATAGT-3') (SEQ ID NO 12)

S100A7, forward - (5'-GGAGGAACTTCCCCAACTTCC-3') (SEQ ID NO 13)

S100A7, reverse - (5'-ACATCGGCGAGGTAATTTGT-3') (SEQ ID NO 14)

BMP2, forward - (5'-TCAAGCCAAACACAAACAGC-3') (SEQ ID NO 15)

BMP2, reverse - (5'-GTGGCAGTAAAAGGCGTGAT-3') (SEQ ID NO 16)

BMP7, forward - (5'-AGGCCTGTAAGAAGCACGAG-3') (SEQ ID NO 17)

BMP7, reverse - (5'-GGTGGCGTTCATGTAGGAGT-3') (SEQ ID NO 18)

[0135] Statistical comparisons of expression levels from the chronic wounds versus the normal skin were performed using the Student's t-test.

Results

[0136] The results of the PCR analysis are shown in FIG. **11**. S1007A, a gene which is part of the human epidermal differentiation complex (EDC) and belongs to the S100 family, was among the most 50 up-regulated genes in chronic wound epidermis as found by microarray analysis. As shown in FIG. **11**(A), S1007A was expressed almost 100 fold in the chronic wound tissue. Additionally, as shown in FIG. **11**(A), DEFB4, associated with benign hyperplasia in skin, was also expressed almost 100 fold more in the chronic wound epidermis as compared to the normal epidermis. This is consistent with the microarray analysis. Also, the expression of MMP-11 was greatly increased in the chronic wound tissue as compared to the normal skin as shown in FIG. **11**(A). Again, this is consistent with the microarray analysis.

[0137] As shown in FIG. **11**(B), bone morphogenetic proteins, BMP2 and BMP7, had much lower expression levels in the chronic wound skin. This is consistent with the microarray analysis which showed these genes to be among the 50 most down-regulated genes in chronic wound epidermis. Also shown in FIG. **11**(B), RT-PCR analysis showed the expression levels of KLK6 is greatly increased in chronic wound epidermis. This protein has been implicated in keratinocyte proliferation and differentiation and in the pathogenesis of psoriasis.

[0138] FIG. **11**(C) shows that the expression of both APOD and CCL27, cutaneous T cell attracting chemokin, are highly suppressed in the chronic non-healing wounds.

[0139] In conclusion, the RT-PCR analysis confirmed the results of the microarray analysis.

[0140] The present invention is not limited in scope by specific embodiments described herein. Indeed, various modifications of the invention in addition to those described herein will become apparent to those skilled in the art from the foregoing description and the accompanying figures. Such modifications are intended to fall within the scope of the appended claims.

[0141] It is further to be understood that all values are approximate, and are provided for description.

[0142] Patents, patent applications, publications, product descriptions, and protocols are cited throughout this application, the disclosures of which are incorporated herein by reference in their entireties for all purposes.

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1. A method for the identification of a margin of debridement within or adjacent to a chronic wound suitable for debriding, comprising:

(a) obtaining a tissue sample from a site within or adjacent to the chronic wound;

(b) determining a gene expression profile of the tissue sample; and

(c) comparing the gene expression profile of the tissue sample with a known gene expression profile of tissue from a known site of non-ulcerated skin adjacent to the chronic wound,

wherein if the gene expression profile of the tissue sample is the same or similar to the known gene expression profile of the tissue from the known site, then the site of the tissue sample has reached the margin of debridement.

2.-**5**. (canceled)

6. The method of claim **1**, wherein the gene expression profiles of the tissue sample and the tissue from the known site are determined by microarray analysis.

7. The method of claim **1**, wherein the known gene expression profile for skin adjacent to the chronic wound is found in FIG. **2**.

8. A method for determining whether a chronic wound is in further need of debriding, comprising:

(d) obtaining a tissue sample from within or adjacent to the chronic wound;

(e) determining a gene expression profile for the tissue sample;

(f) comparing the gene expression profile of the tissue sample with a known gene expression profile of tissue from a known site adjacent to the chronic wound,

wherein if the gene expression profile of the tissue sample is the same or similar to the known gene expression profile of the tissue from the known site, then the wound is not in need of further debriding.

9.-**12**. (canceled)

13. The method of claim **8**, wherein the gene expression profiles of the tissue sample and the tissue from the known site are determined by microarray analysis.

14. The method of claim **8**, wherein the known gene expression profile for skin adjacent to the chronic wound is found in FIG. **2**.

15. The method of claim **8**, wherein the tissue sample derives from tissue that has been previously debrided.

16. A method for the identification of a site within or adjacent to a chronic wound suitable for debriding, comprising:

(g) obtaining a tissue sample from a site within or adjacent to the chronic wound;

(h) determining the expression of a gene or genes known to be up-regulated or induced in tissue from chronic wounds; and

(i) comparing the expression of the gene or genes of the tissue sample with the known expression of up-regulated gene or genes from the chronic wound tissue;

wherein if the expression of the gene or genes of the tissue sample is identical or similar to the known expression of the gene or genes from the chronic wound tissue, then the site of the tissue sample is suitable for debriding, and if the expression of the gene or genes of the tissue sample is different from the known expression of the gene or genes from the chronic wound tissue, then the site of the tissue sample is not suitable for debriding.

17. The method of claim **16**, wherein the genes that up-regulated or induced in the tissue from chronic wounds are desmocollin 2 (Dsc2), desmoglein 3 (Dsg3), involucrin (IVL), small proline rich protein 1A (SPRR1A), small proline rich protein 1B (SPRR1B), small proline rich protein 2B (SPRR2B), small proline rich protein 3 (SPRR3), transglutaminase 1 (TGM1), S100 calcium binding protein A7 (S100A7), S100 calcium binding protein A8 (S100A8), S100 calcium binding protein A9 (S100A9), manic fringe protein (MFNG), phospholipase D 2 (PLD2), kalikrein 6, (KLK6), septin (SEPT_8), serine/threonine kinase 10 (STK10), serine/cysteine proteinase inhibitor, clade B, member 3 (SERPINB3), symplekin (SYMPK), cyclin B1, cyclin D2, cyclin A2, cyclin F, cyclin M4, cell division cycle 2 homolog (CDC2), cyclin dependent kinase inhibitor NB (CDKNB), cyclin dependent kinase inhibitor N3 (CDKN3), keratin 6 (K6), keratin 16 (K16), bone morphogenetic protein 1 (BMP-1), platelet derived endothelial growth factor (ECGF1), receptor neuropilin (NRP1), stromal cell derived factor 1-alpha (SDF-1α), defensin B4 (DEFB4), IL-7 receptor (IL-7R), platelet derived growth factor B (PDGFB), platelet derived growth factor A (PDGFA), Fas-activated serine/theorine kinase (FASTK), Fas (TNFRSF6) associated factor (FAF1), proapoptotic caspase adaptor protein (PCAP), bcl-2 associated protein (BAX), p53 inducible protein (TP5313), nucleolar protein 3(NOL3), apoptosis, caspase activation inhibitor (AVEN), and baculoviral IAP repeat-containing 5 (surivin) (BIRC5).

18. A method for the identification of a site within or adjacent to a chronic wound suitable for debriding, comprising:

j) obtaining a tissue sample from a site within or adjacent to the chronic wound;

(k) determining the expression of a gene or genes known to be down-regulated or suppressed in tissue from chronic wounds; and

(l) comparing the expression of the gene or genes of the tissue sample with the known expression of down-regulated gene or genes from the chronic wound tissue;

wherein if the expression of the gene or genes of the tissue sample is identical or similar to the known expression of the gene or genes from the chronic wound tissue, then the site of the tissue sample is suitable for debriding, and if the expression of the gene or genes of the tissue sample is different from the known expression of the gene or genes from the chronic wound tissue, then the site of the tissue sample is not suitable for debriding.

19. The method of claim **18**, wherein the genes that down-regulated or suppressed in the tissue from chronic wounds are desmocollin 3 (Dsc3), desmoglein 2 (Dsg2), desmoplakin (DP), plakophilin 2 (PKP2), filaggrin (FLG), thrichohyalin (THH), kuppel-like factor (KLF4), NOTCH, drosophila, homolog OF, 2 (NOTH2), phospholipase D 1 (PLD1), protease inhibitor 3, skin-derived (SKALP, PI3), oxysterol binding protein-like 8 (OSBPL8), adducing 3 (ADD3), early growth response 3 (EGR3), inhibitor of DNA binding 4 (ID4), occluding (OCLN), decay accelerating factor for complement (DAF), tight junction protein, zona ocludens 3 (ZO3), tight junction protein 3 (TJP3), spectrin 1 (SPTBN1), multiple PDZ domain protein (MUPP1), InaD-like protein (INADL), claudin 5 (CLDN5), claudin 8 (CLDN8), protein kinase C-iota (PKC-iota), cell division cycle homolog 42 (CDC42), retinoblastoma protein (Rb), retinoblastoma protein (p107), retinoblastoma protein (p103), checkpoint suppressor (CHES1), WEE1 homolog (WEE1), translation initiation factor (EIF4E), insulin-like growth factor binding protein (IGFBP5), bone morphogenetic protein 2 (BMP-2), bone morphogenetic protein 7 (BMP-7), leptin receptor (LEPR), vascular endothelial growth factor (VEGF), epiregulin (EREG), angiopoetin-like 6 (ANGPTL6), apolipoprotein D (APOD), cutaneous T cell attracting chemokine 27 (CCL27), IL-7, transforming growth factor, beta 2 (TGFB2), transforming growth factor, beta 3, (TGFBR3), fibroblast growth factor 13 (FGF13), interleukin 6 (IL-6), pleckstrin homology-like domain, family A, member 2 (PHLDA2), programmed cell death (PDCD6), protein tyrosine phosphatase, non-receptor type 13 (APO-1/CD95 (Fas)-associated phophatase (PTPN13), apoptotic peptidase activating factor 1 (APAF1), and TNFα mediated apoptosis inhibitor (TNFAIP3).

20. A method for determining whether a chronic wound is in further need of debriding, comprising:

(m) obtaining a tissue sample from a site within or adjacent to the chronic wound;

(n) determining the expression of a gene or genes known to be up-regulated or induced in tissue from chronic wounds; and

(o) comparing the expression of the gene or genes of the tissue sample with the known expression of gene or genes from the chronic wound tissue;

wherein if the expression of the gene or genes of the tissue sample is identical or similar to the known expression of the gene or genes from the chronic wound tissue, then the site of the tissue sample is in need of further debriding, and if the expression of the gene or genes of the tissue sample is differ-

ent from the known expression of the gene or genes from the chronic wound tissue, then the site of the tissue sample is not in need of further debriding.

21. The method of claim **20**, wherein the genes that up-regulated or induced in the tissue from chronic wounds are desmocollin 2 (Dsc2), desmoglein 3 (Dsg3), involucrin (IVL), small proline rich protein 1A (SPRR1A), small proline rich protein 1B (SPRR1B), small proline rich protein 2B (SPRR2B), small proline rich protein 3 (SPRR3), transglutaminase 1 (TGM1), S100 calcium binding protein A7 (S100A7), S100 calcium binding protein A8 (S100A8), S100 calcium binding protein A9 (S100A9), manic fringe protein (MFNG), phospholipase D 2 (PLD2), kalikrein 6, (KLK6), septin (SEPT_8), serine/threonine kinase 10 (STK10), serine/cysteine proteinase inhibitor, clade B, member 3 (SERPINB3), symplekin (SYMPK), cyclin B1, cyclin D2, cyclin A2, cyclin F, cyclin M4, cell division cycle 2 homolog (CDC2), cyclin dependent kinase inhibitor NB (CDKNB), cyclin dependent kinase inhibitor N3 (CDKN3), keratin 6 (K6), keratin 16 (K16), bone morphogenetic protein 1 (BMP-1), platelet derived endothelial growth factor (ECGF1), receptor neuropilin (NRP1), stromal cell derived factor 1-alpha (SDF-1α), defensin B4 (DEFB4), IL-7 receptor (IL-7R), platelet derived growth factor B (PDGFB), platelet derived growth factor A (PDGFA), Fas-activated serine/theorine kinase (FASTK), Fas (TNFRSF6) associated factor (FAF1), proapoptotic caspase adaptor protein (PCAP), bcl-2 associated protein (BAX), p53 inducible protein (TP5313), nucleolar protein 3(NOL3), apoptosis, caspase activation inhibitor (AVEN), and baculoviral IAP repeat-containing 5 (surivin) (BIRC5).

22. (canceled)

23. A method for determining whether a chronic wound is in further need of debriding, comprising:

(p) obtaining a tissue sample from a site within or adjacent to the chronic wound;

(q) determining the expression of a gene or genes known to be down-regulated or suppressed in tissue from chronic wounds; and

(r) comparing the expression of the gene or genes of the tissue sample with the known expression of gene or genes from the chronic wound tissue;

wherein if the expression of the gene or genes of the tissue sample is identical or similar to the known expression of the gene or genes from the chronic wound tissue, then the site of the tissue sample is in further need of debriding, and if the expression of the gene or genes of the tissue sample is different from the known expression of the gene or genes from the chronic wound tissue, then the site of the tissue sample is not in need of further debriding.

24. The method of claim **23**, wherein the genes that down-regulated or suppressed in the tissue from chronic wounds are desmocollin 3 (Dsc3), desmoglein 2 (Dsg2), desmoplakin (DP), plakophilin 2 (PKP2), filaggrin (FLG), thrichohyalin (THH), kuppel-like factor (KLF4), NOTCH, drosophila, homolog OF, 2 (NOTH2), phospholipase D 1 (PLD1), protease inhibitor 3, skin-derived (SKALP, PI3), oxysterol binding protein-like 8 (OSBPL8), adducing 3 (ADD3), early growth response 3 (EGR3), inhibitor of DNA binding 4 (ID4), occluding (OCLN), decay accelerating factor for complement (DAF), tight junction protein, zona ocludens 3 (ZO3), tight junction protein 3 (TJP3), spectrin 1 (SPTBN1), multiple PDZ domain protein (MUPP1), InaD-like protein (INADL), claudin 5 (CLDN5), claudin 8 (CLDN8), protein kinase C-iota (PKC-iota), cell division cycle homolog 42 (CDC42), retinoblastoma protein (Rb), retinoblastoma protein (p107), retinoblastoma protein (p103), checkpoint suppressor (CHES1), WEE1 homolog (WEE1), translation initiation factor (EIF4E), insulin-like growth factor binding protein (IGFBP5), bone morphogenetic protein 2 (BMP-2), bone morphogenetic protein 7 (BMP-7), leptin receptor (LEPR), vascular endothelial growth factor (VEGF), epiregulin (EREG), angiopoetin-like 6 (ANGPTL6), apolipoprotein D (APOD), cutaneous T cell attracting chemokine 27 (CCL27), IL-7, transforming growth factor, beta 2 (TGFB2), transforming growth factor, beta 3, (TGFBR3), fibroblast growth factor 13 (FGF13), interleukin 6 (IL-6), pleckstrin homology-like domain, family A, member 2 (PHLDA2), programmed cell death (PDCD6), protein tyrosine phosphatase, non-receptor type 13 (APO-1/CD95 (Fas)-associated phophatase (PTPN13), apoptotic peptidase activating factor 1 (APAF1), and TNFα mediated apoptosis inhibitor (TNFAIP3).

25. (canceled)

26. A method for the identification of a site within or adjacent to a chronic wound suitable for testing wound-healing therapeutic agents, comprising:

(s) obtaining a tissue sample from a site within or adjacent to the chronic wound;

(t) determining a gene expression profile of the tissue sample; and

(u) comparing the gene expression profile of the tissue sample with a known gene expression profile of tissue from a known site of non-ulcerated skin adjacent to the chronic wound;

wherein if the gene expression profile of the tissue sample is the same or similar to the known gene expression profile of the tissue from the known site, then the site of the tissue sample is suitable for testing wound-healing therapeutic agents.

27. The method of claim **26**, wherein the tissue from the known site contains cells with healthy, normal morphology.

28. (canceled)

29. The method of claim **26**, wherein the tissue from the known site contains cells that respond well to wound healing stimuli.

30. (canceled)

31. The method of claim **26**, wherein the gene expression profiles of the tissue sample and the tissue from the known site are determined by microarray analysis.

32. The method of claim **26** wherein the known gene expression profile for skin adjacent to the chronic wound is found in FIG. **2**.

33. The gene expression profile for skin adjacent to the chronic wound found in FIG. **2**.

34. The gene expression profile for normal healthy skin found in FIG. **7**.

35. The gene expression profile for skin from the non-healing edge of a chronic wound found in FIGS. **2** and **7**.

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