

# Transcriptomics of dark spots

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Multiple gene expression profiling with oligonucleotide microarray: monitoring of multiple pathways & functional groups in one run



# Questions

Are dark spots same or different? single or multiple disorders?

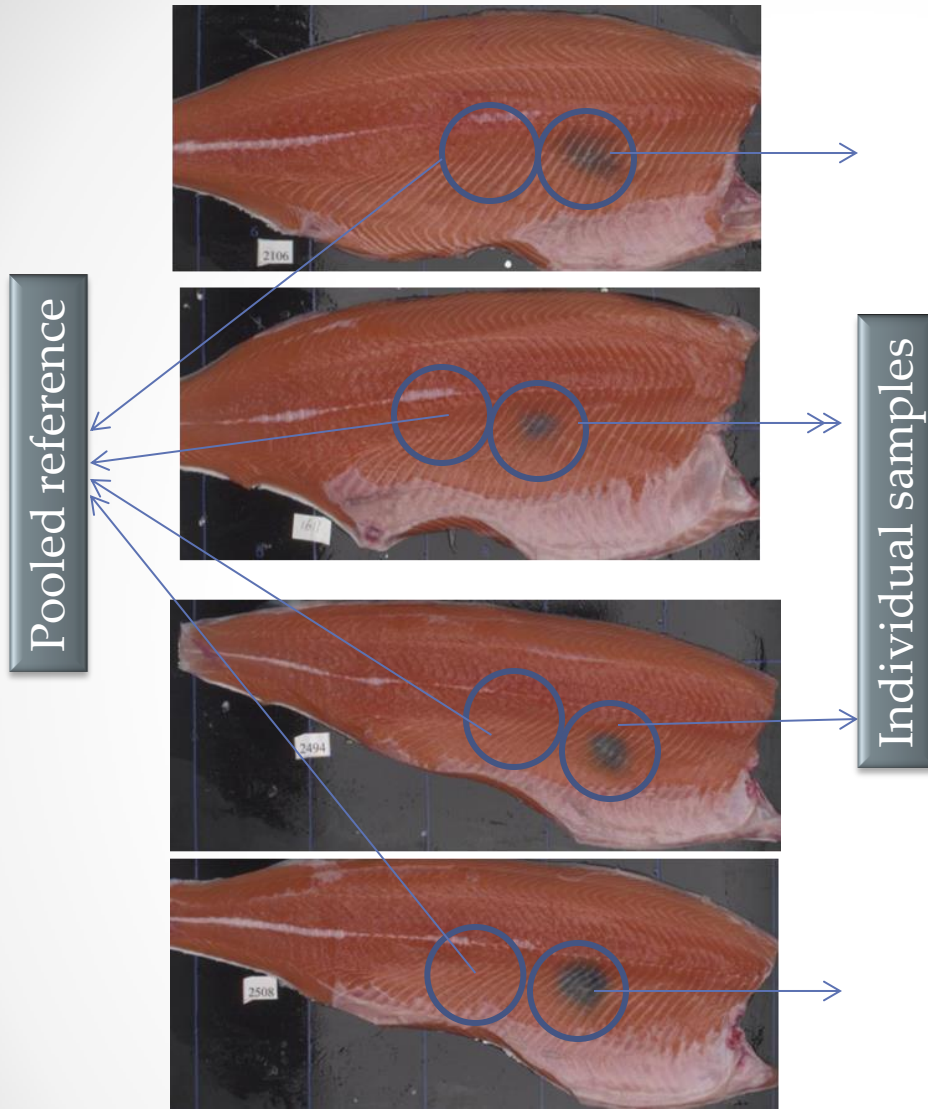
Character of inflammation: acute or chronic? mild or severe? roles of innate and adaptive immunity?

Tissue damage and degeneration?

Composition of new tissue? Healing?

*Possible origin of dark spots?*

12 fish



Difference between spots and normal muscle

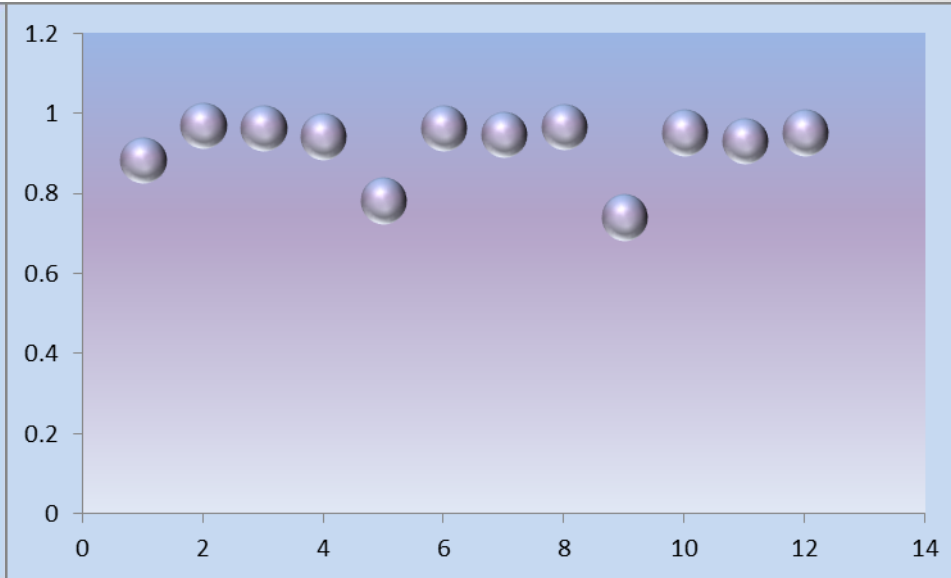
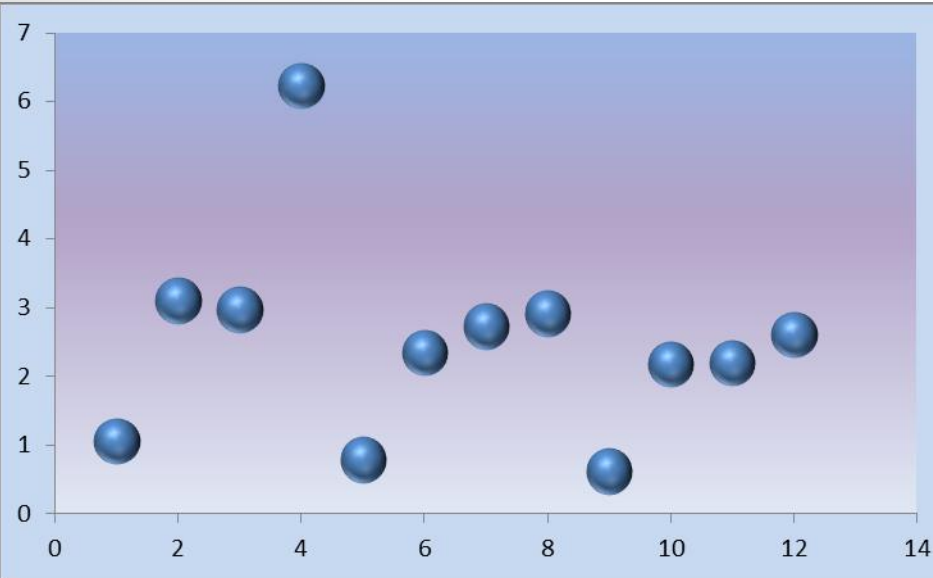
Gene expression data: ratio (fold)

1570 DE genes (>2-fold,  $p < 0.01$ )

946 higher in dark spots

624 lower in dark spots

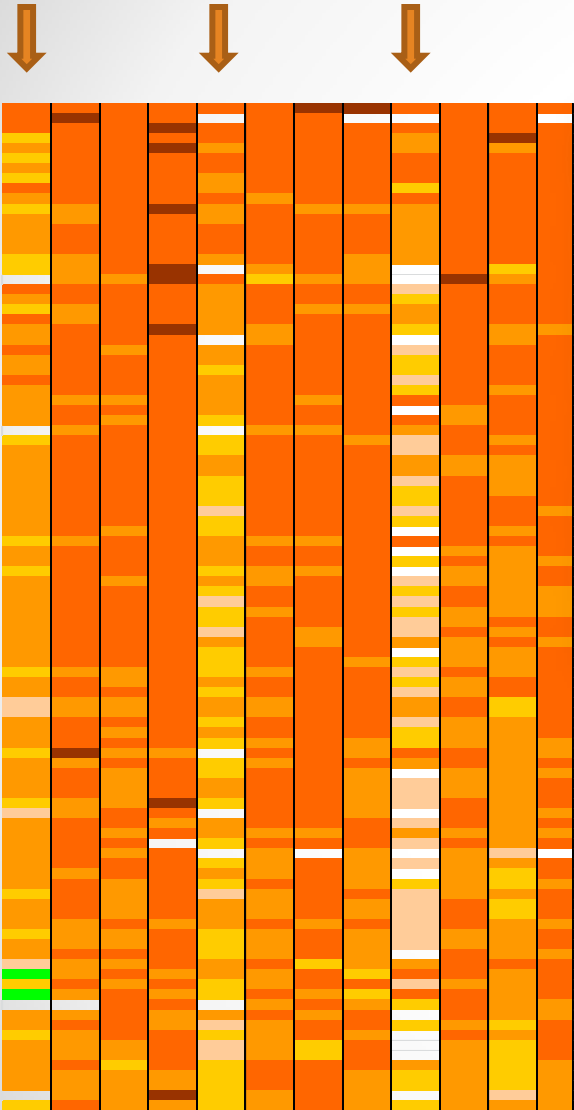
# All dark spots are same



Magnitude:  $\sum(\log-ER)^2/n$

Correlation: Pearson r (mean = 0.917)

# What happens: an overview



Term	Plus	Minus
Acute phase	11	
Antigen presentation	44	1
Chemokines & receptors	14	3
Complement & Ig receptors	20	2
Cytokines & receptors	30	3
Effector	32	3
Eicosanoid	7	
IFN-virus response	69	8
Ig	35	
Lymphocyte	32	3
PRR	7	1
T cell	34	2
TNF	16	4
Transducer	15	
<b>Total</b>	<b>370</b>	<b>24</b>

Function	Up	Down
<b>Cell, metabolism</b>		
Apoptosis	27	2
Cell cycle	23	13
cytoskeleton	69	18
Protein folding	13	11
GTP signaling	33	10
Lysosome	18	3
Myofiber	20	39
Redox	7	9
Reticulum	16	15
Retinoid signaling	1	6
RNA	9	9
Signaling	29	13
Transcription	16	12
Transport	7	8
Ubiquitin	13	22
Xenobiotic metabolism	6	17
Amino acid	16	21
Calcium	5	8
Ion	9	3
Lipid	22	30
mitochondria	10	79
Nucleotide	15	7
Protease	32	5
protease inhibitor	9	3
Steroid, bile	5	6
Sugar	17	30
<b>Tissue</b>		
Adhesion	20	2
Differentiation	30	13
differentiation hox	0	15
Erythrocyte	4	3
Globin	0	19
glycan	21	5
Growth factor	15	11
Secretory	15	4

Strong activation of immunity, complex changes in other functional groups & pathways

# Innflammation: innate responses

Gene		Fold
Serum amyloid P component	Acute phase	6.51
Jeltraxin		6.12
Natterin-like protein		4.85
Lysozyme C II		23.43
Apolipoprotein C-I		6.87
Bactericidal permeability-increasing protein		4.07
Cathelicidin antimicrobial peptide		4.07
Pentraxin		4.92
Serum amyloid A-5 protein		2.67
Complement C1qB -1		Complement
Complement C1qC-1	5.66	
Complement C1qB-2	3.40	
Complement C1qB-3	3.05	
Complement C1qC-2	2.29	
High affinity immunoglobulin gamma Fc receptor 1	Cell	
High affinity immunoglobulin gamma Fc receptor 2		6.71
High affinity immunoglobulin gamma Fc receptor 3		6.61
Neutrophil cytosolic factor 2		4.12
Neutrophil cytosolic factor 1		3.04

**Strong humoral responses  
Classical complement pathways &  
eicosanoid signaling**

15-hydroxyprostaglandin dehydrogenase	Lipid, Eicosanoid	3.95
Epidermis-type lipoxygenase 3		3.66
PLAC8-like		3.64
Arachidonate 5-lipoxygenase		2.68
Arachidonate 5-lipoxygenase		2.02
Prostaglandin E synthase 3	1.87	

Gene	Fold
Mucin 5AC	8.84
IFNg-inducible lysosomal thiol reductase	5.42
Galectin-3-binding protein	4.91
Tyrosine-protein kinase Jak1	4.20
Interferon regulatory factor 8	3.95
Barrier-to-autointegration factor	3.64
Plexin D1	3.64
Interferon-induced protein 44	3.48
Proteasome subunit beta type-8	3.23
TRIM21-like	3.22
CD151, tetraspanin 3	3.15
Macrosialin	3.01
Gig2-6	2.99
Interferon regulatory factor 1	2.80
Gig2-9	2.80
XIAP-associated factor 1	2.77
VHSV-induced protein-10	2.74
GTPase IMAP family member 7	2.73
Fish virus induced TRIM-6	2.67
Very large inducible GTPase 1-3	2.66
Gig2-5	2.65
opioid growth factor receptor	2.60

**Most active VRG are not on the top  
Change of gene expression or cellular  
composition?**

fish virus induced TRIM protein	2.45
Sacsin	2.45
Cholesterol 25-hydroxylase-like protein A	2.44

# Antigen presentation, differentiation of lymphocytes

Gene	Fold
CD83	4.26
C-type lectin domain family 4 member E	3.91
HLA MHCII antigen, DP alpha chain	6.44
HLA MHCII antigen, gamma chain 1	5.95
MHC class II alpha	5.72
HLA MHCII antigen, DP alpha chain	5.32
HLA MHCII antigen, gamma chain 2	5.03
Cathepsin S	5.97
MHC class II	4.85
HLA MHCII antigen, gamma chain 3	4.74
PSMB9b-like protein 1	6.77
Beta-2 microglobulin	6.22
PSMB9b-like protein 2	5.24
PSMB9	4.00
PSMB6a	3.56
PSMB10	3.48

MHCII

MHCI

**Strong MHCII response**

Gene	Fold
Transcription factor PU.1 -1	5.60
Transcription factor PU.1 -2	4.07
Leukocyte surface antigen CD53	7.14
Fermitin family	6.20
Tyrosine-protein kinase SYK	5.57
SH3 protein expressed in lymphocytes	5.48
B-cell linker protein	5.31
SH2 domain-containing protein 1A	5.11
Kelch-like protein 6	5.10
Hematopoietic lineage cell-specific prote	5.01
Src kinase-associated phosphoprotein 2	4.71
Plastin-2	4.35
Lymphocyte cytosolic protein 2	4.28
CD83	4.26
CD97 antigen	4.20
Fermitin family homolog 3	4.16
CD80-like protein	3.95
Transcription factor MafB	3.85
SLAM family member 8	2.20
Dual adapter for phosphotyrosine a	2.06

**Lymphocyte differentiate at the sites**



# Inflammation: adaptive responses, B & T cells

Gene	Fold
Ig light chain	13.06
Ig kappa chain V-IV region JI precursor	11.42
Ig mu chain C region membrane-bound fo	9.41
Ig kappa chain V-IV region B17 precursor	9.32
Ig kappa chain V-IV region JI precursor	8.94
Ig kappa chain	8.63
Ig kappa chain V-III region MOPC 63 precu	8.14
Ig kappa chain V region 3368	7.75
Immunoglobulin heavy chain AF141606_1	7.53
Ig kappa chain V-III region CLL precursor	7.51
Ig light chain Immunoglobulin light chain	6.87
Immunoglobulin lambda-like polypeptide	6.40
Ig kappa chain V-IV region B17 precursor	6.35
Ig heavy chain	5.73
immunoglobulin mu heavy chain [Salmo t	4.88
Ig kappa chain V region Mem5	4.42
immunoglobulin heavy chain [Oncorhynch	4.37
immunoglobulin tau-2 heavy chain consta	4.34
Ig kappa chain V-IV region JI precursor	4.31
Ig kappa chain V-IV region STH	3.88
Ig kappa chain V region 3368	3.67
Ig kappa chain V-IV region B17 precursor	3.64
AF273019_1 immunoglobulin light chain p	3.22
Ig kappa chain V-III region CLL precursor	3.12
Ig heavy chain	3.11
Ig kappa chain V-III region MOPC 63 precu	2.90

**Strong B cells response**

Gene	Fold
CD4 -1	9.47
CD4-2	7.91
CD8 alpha	3.31
CD8 beta	1.92
CD3 gammadelta-A	3.78
CD3 epsilon	2.75
CD3 zeta	2.03
CD28 T-cell-specific surface glycoprotein	2.78
CD45	3.34
T-cell receptor beta chain T17T-22	6.51
T-cell antigen receptor	3.63
T-cell receptor beta chain	2.49
T cell receptor alpha chain	3.10
Granzyme K	3.27
Tyrosine-protein kinase BTK	4.90
Hmha1 protein	4.44
T-cell activation Rho GTPase-activating pr	3.93
lymphocyte cytosolic protein 1 precursor	3.67
SRC-like-adaptor	3.66
T-cell activation Rho GTPase-activating pr	3.46
SH2 domain-containing protein 2A	3.13
Proto-oncogene tyrosine-protein kinase L	3.04
Differentially expressed in FDCP 6	2.84
Novel NACHT domainin containing protein	2.79
Transcription factor SOX-4	2.67
Stat4	2.39
Tyrosine-protein kinase SYK	2.29
Differentially expressed in FDCP 6 homolo	2.19

**Strong CD4 response**

# Tissue damage & degeneration

Gene	Fold
CD274	5.10
Nck-associated protein 1	4.96
Pleckstrin homology-like domain family A	3.97
DNA-damage-inducible transcript 4-like p	3.61
Serine/threonine-protein kinase 17A	3.54
Dnase2 protein	3.32
Apoptosis-associated speck-like protein c	3.27
Apoptosis-associated speck-like protein c	2.85
EF-hand domain-containing protein D2	2.84
Pleckstrin homology domain-containing fa	2.81
Damage-regulated autophagy modulator	2.68
Caspase	2.50
PTB domain-containing engulfment adapt	2.45
Death-associated protein-like 1-A	2.42
Programmed cell death protein 6	2.05
Cellular tumor antigen p53	1.95
Caspase recruitment domain-containing p	1.95
Engulfment and cell motility protein 1	1.93
programmed cell death 6	1.92
Caspase-1	1.92
Apoptosis regulator Bcl-X	1.88
<b>Different apoptotic pathways</b>	
Novel protein with zinc finger _csmc4 typ	1.88
Programmed cell death 1 ligand 1	1.83
Programmed cell death protein 6	1.74
Tax1-binding protein 1	-1.78
Caspase-1	-1.92
Apoptosis regulatory protein Siva	-2.41

Gene	Fold
Lipase/cholesteryl ester hydrolase	12.45
Proactivator polypeptide	7.46
Cathepsin K	6.16
Cathepsin S	5.97
Lysosomal thioesterase PPT2-A	5.42
Cathepsin K	4.35
Cathepsin K	4.26
Cathepsin B	4.08
Cathepsin Z	3.96
Lysosomal protective protein	3.78
Cathepsin B	2.63
Cathepsin D	2.37
Cathepsin O	2.23
Lysosomal protective protein	2.16
Cathepsin H	2.11
Vacuolar proton pump subunit E	1.92
Matrix metalloproteinase 13	15.47
72 kDa type IV collagenase	3.90
Matrix metalloproteinase-9	3.70
Matrix metalloproteinase-25	3.18
MMP 13 or Collagenase 3	2.80
Se	
Pro	
<b>Proteolytic damage of tissue?</b>	
Dipeptidyl-peptidase 1	4.37
Metalloproteinase inhibitor 2	2.97
Serine protease HTRA1	2.94
Enpep protein - Ident 36	2.91
Kunitz-type protease inhibitor 2	6.67

Lysosomes

Extracellular

teases

# Muscle, blood

Gene	Fold
Nebulin-like	11.86
Myosin heavy chain	6.63
Fast myotomal muscle troponin-T-2	6.24
Troponin C, slow muscles	4.28
Cardiac calsequestrin-like protein	4.12
Cardiac tropomyosin [Salmo trutta]	3.87
Fast myotomal muscle actin 2	3.67
Myomesin 2	-3.15
Myosin heavy chain	-2.91
Actin	-2.65
Cardiomyopathy associated 5 like	-2.65
Myosin binding protein H-like	-2.58
Titin	-2.22
Myozenin 1-like	-2.19
Myosin heavy chain	-2.11
Sarcoendoplasmic calcium ATPase	-2.06
Myf5 protein	2.35
Myocyte-specific enhancer factor 2A	1.81
Myocyte enhancer factor 2D MEF2D	-1.81
Myocyte enhancer factor 2cb	-1.90
Myogenic factor 6	-1.99

Gene	Fold
Heme oxygenase	4.15
Heme oxygenase	3.19
Hepcidin-1	2.72
Ferritin-H subunit	1.93
Ferritin, middle subunit	1.84
Ferritin, heavy subunit	1.83
Ferritin, heavy subunit	-4.24
Kruppel-like factor 11	-1.84
5-aminolevulinate synthase, nonspecific	-2.52
5-aminolevulinate synthase, erythroid	-3.13
Carbonic anhydrase, CAH	-2.34
Hemoglobin subunit alpha	-3.62
Hemoglobin subunit beta	-2.86
Hemoglobin subunit alpha	-2.79
Hemoglobin subunit beta	-2.68
Hemoglobin subunit alpha-4	-2.64
Hemoglobin subunit beta-1	-2.57
alpha-globin IV	-2.33
Hemoglobin subunit alpha	-2.29
Hemoglobin subunit alpha	-2.29
Hemoglobin subunit alpha-4	-2.28
Hemoglobin subunit beta	-2.21
Hemoglobin subunit alpha-4	-2.20
Hemoglobin subunit alpha-4	-2.17
Hemoglobin subunit alpha-4	-2.12

Muscle: rather change of composition  
than degeneration

Possible haemorrhage, reduced flux of RBC

# Melanogenesis, mitochondria

Gene	Fold
Tyrosinase-related protein 1	4.12
Melanocyte protein Pmel 17	3.05
MCSF receptor	2.53
Kinase C eta type	2.36
Protein Wnt-5b	2.17
Transcription factor EC 2	2.04
Catenin beta-1	2.04
Guanine nucleotide binding protein G(o)	1.93
Androgen-induced proliferation inhibitor	1.82
Adenylate cyclase type 8	1.81
cAMP-dependent transcription factor ATF-1	-1.85
Segment polarity protein dishevelled DVL-3	-1.90

Gene	Fold
Mitochondrial uncoupling protein 2	3.85
Cytochrome b-c1 complex subunit 6	3.56
ADP/ATP translocase 2	2.91
Electron transfer flavoprotein subunit alpha	-3.74
Pyruvate dehydrogenase kinase isozyme 2	-3.58
ES1 protein homolog	-3.54
Calcium-binding carrier protein Aralar1	-3.25
Dihydrolipoyl dehydrogenase	-3.15
NAD-dependent deacetylase sirtuin-3	-2.77
Cytochrome b-c1 complex subunit Rieske	-2.77
Succinate dehydrogenase [ubiquinone]	-2.72
Mitochondrial Rho GTPase 1-A [MIRO-1-A]	-2.69
Ubiquinol-cytochrome c reductase core I protein	-2.62
Cytochrome c	-2.58
Succinate-CoA ligase_ ADP-forming_ beta subunit	-2.52
ATP synthase gamma chain	-2.50
Aspartate aminotransferase	-2.43
3-ketoacyl-CoA thiolase	-2.43
Pyruvate kinase	-2.42
Malate dehydrogenase	-2.39
ATP synthase, H+ transporting FO complex, subunit b	-2.38
ATP synthase H+ transporting mitochondrial F1 complex	-2.35
NADH dehydrogenase 1 alpha subcomplex subunit 5	-2.33
ATP synthase	-2.32
Mitochondrial	-2.32
ATP11 precursor	-2.29
NADP transhydrogenase	-2.28

**Reduced energy metabolism?**

# Tissue composition

Gene	Fold
Angiopoietin-like 7	8.68
Transcription factor MafB	3.85
Pigment epithelium-derived factor	3.35
CCAAT/enhancer-binding protein beta 1	3.32
CCAAT/enhancer-binding protein beta 2	3.28
CCAAT/enhancer binding protein beta2	3.22
Transcription factor 21, TCF21	3.05
Connective tissue growth factor	3.02
DNA-binding protein inhibitor ID-2	2.93
Syntaxin-11	2.79
Transcription factor SOX-11	2.68
Transcription factor SOX-4	2.67
Filamin A-interacting protein 1-like	2.55
Pigment epithelium-derived factor	2.49
Neuropilin 1a	2.44
Class B basic helix-loop-helix protein 3	2.36
Osteoclast-stimulating factor 1	2.32
Protein Wnt-5b	2.17
Forkhead box P3	2.08
Periphilin-1	2.08
Signal peptide_ CUB and EGF-like domain-cont	2.02
Secreted frizzled-related protein 1	1.90

Gene	Fold
Homeobox protein HoxC10ba	-4.59
Homeobox protein HoxA11ab	-4.47
Homeobox protein HoxD11aa	-4.45
Homeobox protein HoxA9ab	-4.01
Pancreatic progenitor cell differentiation	-3.61
Homeobox protein HoxB6ab	-2.46
sine oculis Homeobox like 1	-2.30
SET and MYND domain containing 1b	-2.15
Homeobox protein six2.1	-2.12
Homeobox protein HoxD12aa	-2.08
Homeobox protein HoxB6aa	-2.07
Pbx1a homeodomain protein	-2.02
Homeobox protein Hox-B7a	-1.95
Homeobox protein HoxA5aa	-1.94
Homeobox protein HoxD9aa	-1.86
Pbx1a homeodomain protein	-1.82
Homeobox protein HoxB7ab	-1.80

Unclear but impressive

Differentiation of multiple cell lineages



# Cell contacts

Gene	Fold
Tetraspanin-8	8.54
Gap junction beta-6 protein	7.91
Integrin beta-2	5.32
Cell adhesion molecule n-cam	5.29
Poliovirus receptor-related protein 1	4.60
Sialic-acid binding protein-4	4.01
Alpha M integrin	3.92
Ependymin	3.54
Leupaxin	2.58
Integrin alpha 11 subunit	2.56
CD166 antigen homolog	2.53
Epithelial cadherin	2.48
Epithelial cadherin	2.46
CD209 antigen-like protein D	2.23
Gap junction alpha-1 protein	2.19
Epithelial cadherin	2.17
T-box transcription factor TBX1	2.07
Epithelial cadherin	1.92
Epithelial cadherin	1.92
Zinc finger protein SLUG	1.86
Integrin alpha 11 subunit	1.81
Coiled-coil domain-containing protein 80	1.76
Gap junction beta-6 protein	-2.18
Basal cell adhesion molecule	-2.66

Gene	Fold
Alpha-N-acetylgalactosaminidase	6.90
Myelin-associated glycoprotein	6.61
Beta-1_4-galactosyltransferase 1	3.54
Alpha-1,3-fucosyltransferase	3.41
UDP-Gal:betaGlcNAc beta 1_3-galactosyltransferase	3.01
Hyaluronan and proteoglycan link protein 4	2.98
Lactosylceramide 1_3-N-acetyl-beta-D-glucosaminyltransferase	2.68
N-acetylglucosamine-6-sulfatase	2.41
Syndecan-2-A	2.38
Beta-hexosaminidase beta chain	2.30
Carbohydrate sulfotransferase 10	2.21
Galactosamine (N-acetyl)-6-sulfate sulfatase	2.19
Beta-1_4-galactosyltransferase 1 - Ident 99	2.06
N-acetylneuraminase lyase	2.00
Hyaluronidase-2	1.94
Alpha-N-acetylgalactosaminidase alpha-2,6-sialyltransferase 6	1.89
Lactosylceramide 1_3-N-acetyl-beta-D-glucosaminyltransferase	1.89
Carbohydrate (Chondroitin) synthase 1 - Ident 31	1.76
Alpha-1,3-mannosyl-glycoprotein 2-beta-N-acetylglucosaminidase	1.76
Alpha-(1_6)-fucosyltransferase	1.74
Guanidinoacetate N-methyltransferase	-1.78
Mannose-6-phosphate receptor-binding protein 1	-1.85
UTP--glucose-1-phosphate uridylyltransferase	-1.93

Compact, firm tissue with high contents of glycans

# Extracellular matrix, collagens

Gene	Fold
FAM180A	8.94
Cartilage intermediate layer protein	8.74
FAM180A	7.03
Fibronectin	6.94
Ependymin	4.80
Ependymin	4.50
Galectin-3	4.32
Lumican	3.23
Lumican	2.98
Decorin	2.89
Microfibrillar-associated protein 2	2.72
Tartrate-resistant acid phosphatase type 5	5.74
Protein-lysine 6-oxidase	2.61
Periostin	2.53
Extracellular matrix protein 1	2.47
Acid phosphatase-like protein 2	2.46
Transcription factor Smad4	2.15
Extracellular matrix protein 1	2.14
Procollagen C-endopeptidase enhancer 1	2.03
Transgelin	2.03
Fn1 protein	1.88
Periostin	1.86
Angiopoietin like 2	1.86
Periostin	1.86
Syndecan-2-A	-1.79
Spondin 1a	-3.60

Gene	Fold
Collagen triple helix repeat-containing 1	4.19
Collagen a3(I)	3.45
Collagen I alpha 2 chain	3.08
Collagen V alpha 3	2.96
Collagen a3(I)	2.74
Collagen a3(I)	2.74
Col14a1 protein	2.48
Translocation-associated membrane 2	2.37
Collagen type XI alpha1 short isoform	2.36
Collagen a3(I)	2.36
type I Collagen alpha 2 chain	2.30
type I Collagen alpha 2 chain	2.28
type I Collagen alpha 2 chain	2.26
Col6a2 protein	2.26
type I Collagen alpha 2 chain	2.22
Col6a2 protein	2.06
Col6a2 protein	1.99
Protein-lysine 6-oxidase	1.93
Collagen a3(I)	1.93
Collagen a1(I)	1.89
Collagen a1(I)	1.81
Collagen a1(I)	1.78
ProCollagen type I alpha 2 chain	-2.08
Collagen XV alpha 1 chain	-2.99
Collagen XV alpha 1 chain	-3.45

Healing, substitution of muscle with connective tissue

# Summary: immune

All 12 cases are similar – common origin?

Inflammation: rather chronic than acute – slow development?

- *Combination of innate and adaptive components*
- *Immune responses go in parallel with healing*

Inflammation is skewed to B cells – extracellular antigen?

- *MHCII*
- *classical complement pathway*
- *Ig*
- *CD4 > CD8*

Causative agent?

- *Haemorrhage, tissue damage – degradation of Hb*
- *Virus? – innate antiviral responses*
- *Vaccine?*



# Summary: tissue

Damage? – apoptosis, lysosomal proteases

No vascularization & blood circulation

No degeneration of muscle, change of cellular composition

Highly compact tissue: cell adhesion & ECM

Melanogenesis