

Transcriptomics of dark spots

Aleksei Krasnov

Gardemoen, October 16th, 2014

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?

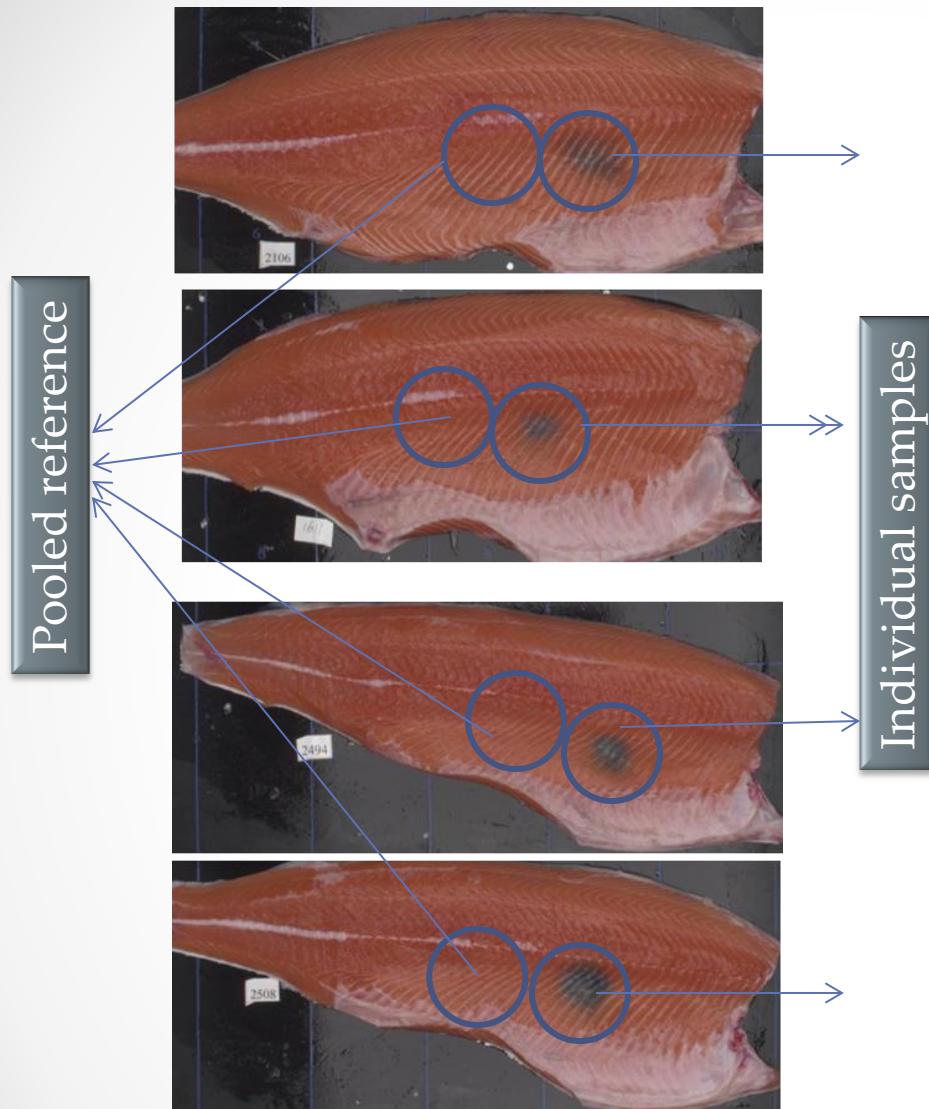
Character of inflammation: acute or chronic? mild or severe? roles of innate and adaptive immunity?

Tissue damage and degeneration?

Composition of new tissue? Healing?

Possible origin of dark spots?

12 fish



Difference between spots and normal muscle

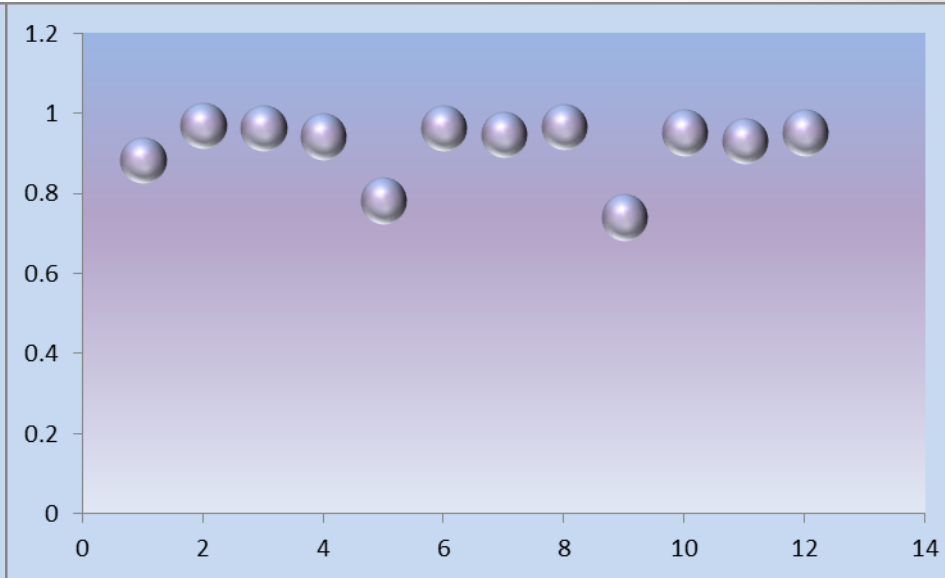
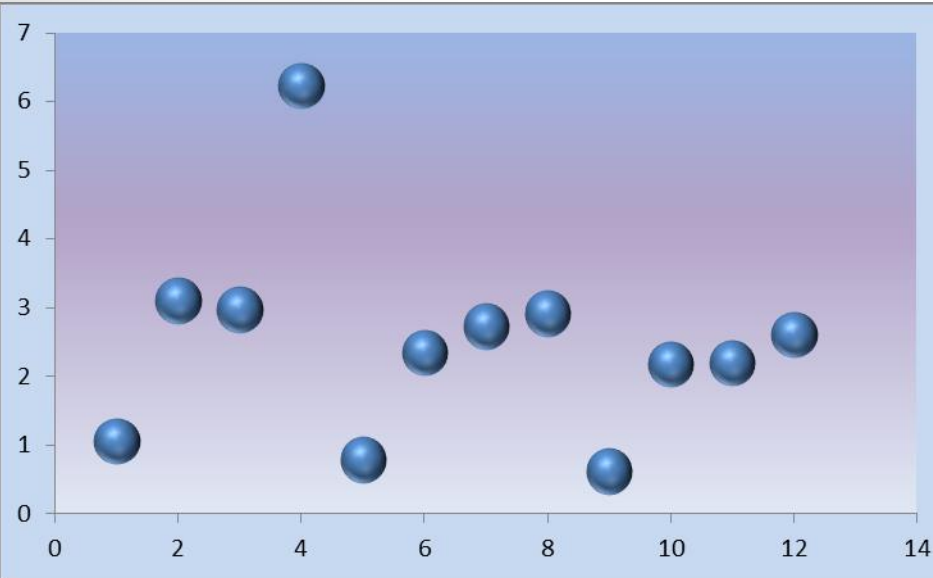
Gene expression data: ratio (fold)

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

946 higher in dark spots

624 lower in dark spots

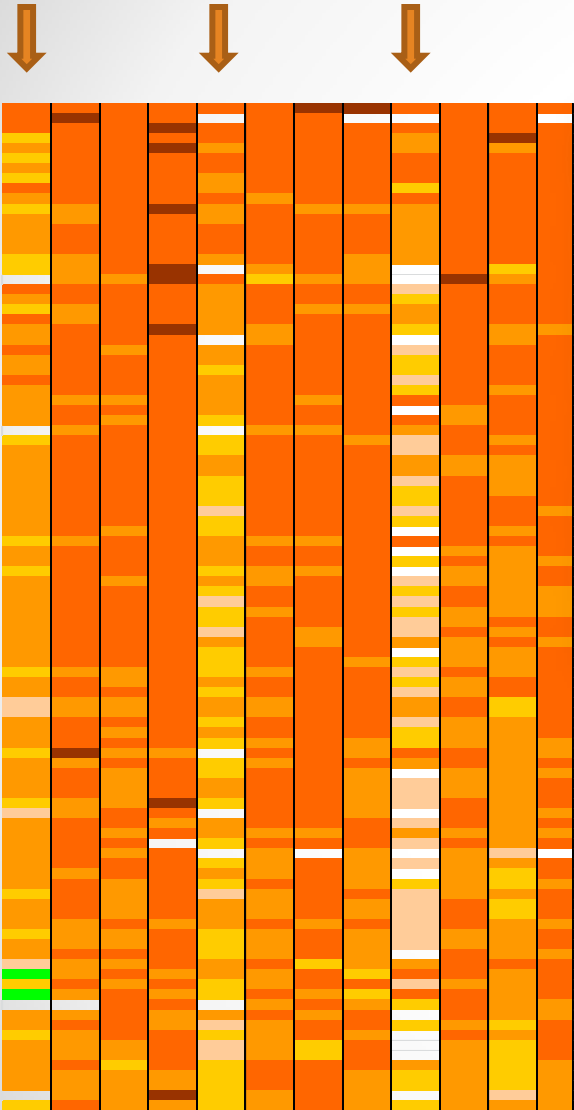
All dark spots are same



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	
Total	370	24

Function	Up	Down
Cell, metabolism		
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
Tissue		
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
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

Different apoptotic pathways

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	
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

Proteolytic damage of tissue?

Lysosomes

Extracellular

Proteases

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 mutiple 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