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Gene Expression Profiling in Familial Adenomatous Polyposis Adenomas and Desmoid Disease

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Abstract

Gene expression profiling is a powerful method by which alterations in gene expression can be interrogated in a single experiment. The disease familial adenomatous polyposis (FAP) is associated with germline mutations in the APC gene, which result in aberrant β-catenin control. The molecular mechanisms underlying colorectal cancer development in FAP are being characterised but limited information is available about other symptoms that occur in this disorder. Although extremely rare in the general population, desmoid tumours in approximately 10% of FAP patients. The aim of this study was to determine the similarities and differences in gene expression profiles in adenomas and compare them to those observed in desmoid tumours. Illumina whole genome gene expression BeadChips were used to measure gene expression in FAP adenomas and desmoid tumours. Similarities between gene expression profiles and mechanisms important in regulating formation of FAP adenomas and desmoid tumours were identified. This study furthers our understanding of the mechanisms underlying FAP and desmoid tumour formation.

Introduction

Familial adenomatous polyposis (FAP) is a rare form of colorectal cancer caused by germline mutations in the adenomatous polyposis coli (APC) gene. Approximately 70-90% of FAP patients have identifiable germline mutations in *APC* [1, 2]. FAP is clinically characterized by the formation of hundreds to thousands of adenomas that carpet the entire colon and rectum [3]. Although initially benign the risk of malignant transformation increases with age such that, if left untreated, colorectal carcinoma usually develops before the age of 40 years [4].

Loss of APC results in dysregulation of the Wnt signalling pathway that leads to the constitutional activation of the transcription factor Tcf-4, which has been associated with adenoma formation [5]. Alterations in Wnt signalling cause stem cells to retain their ability to divide in the upper intestinal crypt, thereby forming monocryptal adenomas [6]. Eventually the adenomas may acquire metastatic potential, resulting in carcinoma development [7]. Not all adenomas will progress to malignant tumours; however, due to the abundance of adenomas carcinoma development is virtually assured [8].

Apart from the apparent loss of APC function, little is known about the molecular processes involved in

adenoma initiation [6]. Similarly, the molecular events occurring during the transformation of adenomas into carcinomas are poorly understood, as are the mechanisms that underlie the development of extra-colonic disease in FAP.

It is well established that FAP patients are susceptible to benign extra-colonic tumours, including desmoid tumours [3]. Although rare in the general population, desmoids occur in approximately 10% of FAP patients and they are the second most common cause of death [9]. Desmoid tumours are poorly encapsulated and consist of spindle-shaped fibroblast cells with varying quantities of collagen [10]. Despite their apparent inability to metastasize, desmoid tumours can be extremely aggressive [11].

It has been speculated that desmoid formation is a result of an abnormal wound healing response [12]. Desmoids can affect surrounding viscera, causing potentially fatal complications [13]. FAP-associated desmoid tumours are usually associated with germline *APC* mutations [14], but somatic *APC* mutations have been detected in sporadic desmoid tumours [15].

Microarray technology has an enormous potential for applications in the endeavour to better understand tumours and their development [16]. The ability to detect expression levels of thousands of genes can identify particular genes that are either up- or down-regulated in different tumour types [17]. Tumours that are currently categorized by similar morphology, such as desmoid tumours, may be more usefully divided into subtypes according to their expression profiles [18]. Particular expression profiles in tumours may also be capable of predicting the clinical outcome in specific patients in the early stages of tumour development [18]. In colorectal cancer, gene expression profiles of adenomas and adenocarcinomas have been compared and subsets of genes expressed at common levels in both lesions have been identified as well as expression patterns that are unique to each [19]. Gene expression profiling has the potential to identify factors involved in the malignant transformation of adenomas, and may aid in the diagnosis of benign versus malignant disease.

Although genome-wide expression studies have been reported on FAP adenomas and desmoid tumours, the present one of the first to compare the two tissue types. The first aim of this study was to identify distinct gene expression profiles for colorectal and stomach FAP adenomas and desmoid tumours. The second aim was to determine the similarity between the gene expression profiles in FAP adenomas and desmoid tumours to identify mechanisms important in regulating formation of these lesions. To achieve this, mRNA from normal colon, FAP stomach and colon adenomas and desmoid tumours was measured using whole human genome expression BeadChips (Illumina). The findings of this study further our understanding of the mechanisms underlying FAP and desmoid tumour formation.

Materials and methods

FAP adenoma and tumour tissue and controls

Frozen adenoma tissue from 4 FAP patients was available for this study. Colorectal FAP adenoma A was from an individual aged 40 at the time of surgery. Genetic testing revealed a heterozygous A5465T change in the APC gene, causing a missense change from aspartic acid to valine at position 1822 in the amino acid sequence. The specimen obtained for this study was obtained as a result of a proctocolectomy. The pathology report indicated that over 100 tubulovillous adenomas were present in the original specimen, with no evidence of invasive tumour. Patients B, C and D harboured the same frameshift mutation, a 4 base pair deletion at position 3462-3465 of the APC gene. Patient B was diagnosed with FAP at the age of 11 years, patient C at 13 years of age, and patient D at the age of 37 years. One gastric adenoma was obtained from patient D, in addition to a colonic adenoma. Normal colon tissue from 7 healthy individuals with no history of FAP or desmoid disease was used as a mixed reference sample for this study.

Desmoid Disease Tissue

Desmoid tumour tissue from two individuals was available for this study. Patient A had FAP-associated desmoid disease. There was a family history of FAP, but no known history of desmoid disease. The individual harboured a 1bp deletion in exon 15 of the APC gene resulting in a frameshift that introduced a premature stop codon at amino acid position 964. Patient B had a family history of FAP and desmoid disease. This patient harboured a 17bp duplication in exon 15 of the APC gene, which introduced a premature stop codon at amino acid position 1969. A previously established fibroblast cell line from a healthy individual with no history of FAP or desmoid disease was used as a control for this study. The fibroblast cell line was cultured in 1x Complete DMEM media at 37°C (5% CO_2).

RNA Extraction

2-3 mm^2 pieces of fresh frozen FAP adenoma and desmoid tumour tissue were cut from the original sample and transferred immediately to 1ml Trizol reagent (Invitrogen, USA). Similarly, approximately 1-10 x 10^6 control fibroblast cells were lysed in 1 ml Trizol reagent

(Invitrogen, USA). RNA was extracted per manufacturer's instructions. The RNA pellet was washed with 75% ethanol, before being dissolved in 20 µl water.

The total RNA was purified using a Qiagen RNeasy MiniElute Cleanup Kit as per manufacturer's instructions. The concentration of the purified total RNA samples was measured using a Quant-It RiboGreen RNA Assay Kit (Invitrogen, USA) and a fluorometer (Fluostar OPTIMA) as per manufacturer's instructions.

RNA amplification

To synthesise first and second strand cDNA and amplify biotinylated cRNA from the total RNA, an Illumina Totalprep RNA Amplification Kit was used as per manufacturer's instructions.

The purified cRNA samples were quantified to determine the volume required for the BeadChip hybridisation step via the Quant-iT RiboGreen RNA Assay Kit as described previously.

Illumina BeadChip Procedure

Hybridisation to the Illumina Sentrix 8 BeadChip was performed according to the manufacturer's instructions without modification. The Sentrix 8 BeadChips were read using an Illumina Beadarray reader (San Diego, CA, USA).

Data Analysis

Analysis and normalisation of expression data from the 24,000 transcripts was carried out using BeadStudio 2.0 (Illumina, San Diego, CA, USA). The t-test error model and cubic spline normalisation was used for all samples. A differential analysis was applied to all adenoma and tumour samples using the Illumina custom test of significance, utilising the mixed normal colon control as the reference group. GeneSpring 5.0 (Agilant, Santa Clara, CA, USA) used standard correlation and distance to create dendrograms (Experiment trees) to show relationships between gene expression profiles. A second dendrogram (Gene tree) was created for each gene list using standard correlation and distance to show relationships between the expression levels of genes across the groups.

Results

Gene expression data from over 23,000 genes on Illumina HumRef-8 BeadChips was analysed and normalised using Illumina BeadStudio 2.0 software. Cubic spline normalisation and the t-test error model were employed for all the FAP adenoma, normal colon and desmoid tumour samples. Correlation analyses identified the average R^2 value of the duplicates for each sample as 0.950 ± 0.04. An average of each duplicate pair was then taken before additional analysis was carried out.

Table 1. Genes commonly up-regulated more than 2-fold in all FAP polyps compared to normal colon

Symbol	Gene Name
Transcription/Transcriptional Regulation	
TBPL1	TBP-like 1
Other	
ZCWCC2	Zinc finger, CW-type with coiled-coil domain 2
KIAA1324	Maba1
FLJ20366	Hypothetical protein FLJ20366
ATOH8	Atonal homolog 8 (*Drosophila*)

Differential gene expression analysis in FAP adenomas and healthy colon tissue

Differential analysis using the mixed normal colon control as the reference group was applied to all adenoma and tumour samples. Genes in each analysis were excluded if their fluorescence detection score was less than 0.99, and if their differential score was less than 13 ($p > 0.05$). From the genes that met the exclusion criteria, according to detection and differential scores, lists were generated for genes both up- and down-regulated more than 2-fold in the FAP adenoma samples compared to the mixed normal colon control. The genes commonly up- and down-regulated across all the FAP adenomas are shown in Tables 1 and 2 and genes that were commonly up- or down-regulated across the 4 colorectal FAP adenomas only are shown in Tables 3 and 4 respectively.

Cluster analysis was performed using GeneSpring 5.0 software in order to further characterise the similarity across the FAP samples and to determine if there was differential gene expression compared to healthy colon tissue. The stomach FAP duplicates display profiles slightly distinct from the other FAP adenomas. The normal colon duplicate profiles are unique to all other profiles (Figure 1).

Differential gene expression analysis in desmoid tumours and control fibroblasts

The average expression in the desmoid tumours was compared to the control fibroblast cell line and significantly altered expression identified by differential gene expression analysis. Genes in each analysis were excluded if their fluorescence detection score was less

Table 2. Genes commonly down-regulated more than 2-fold in all FAP polyps compared to normal colon

Symbol	Gene Name
Cell Cycle Control	
PPP3CB	Protein phosphatase 3 (formerly 2B), catalytic subunit, beta isoform (calcineurin A beta)
Transport	
SLC20A1	Solute carrier family 20 (phosphate transporter), member 1
P2RX4	Purinergic receptor P2X, ligand-gated ion channel, 4, tv-2
Metabolism	
PC	Pyruvate carboxylase, nuclear gene encoding mitochondrial protein, tv-2
PRSS3	Protease, serine, 3 (mesotrypsin)
ST6GALNAC6	CMP-NeuAC: (beta)-N-acetylgalactosaminide (alpha) 2,6-sialyltransferase member IV
Signal Transduction	
IL2RG	Interleukin 2 receptor, gamma (severe combined immunodeficiency)
TJP3	Tight junction protein 3 (zona occludens 3)
Cell Adhesion	
CDC42	Cell division cycle 42 (GTP binding protein, 25kDa), tv-2
GSN	Gelsolin (amyloidosis, Finnish type), tv-2
TAGLN	Transgelin
Apoptosis	
DAPK3	Death-associated protein kinase 3
Structural	
KRT19	Keratin 19
TPM2	Tropomyosin 2 (beta)
Other	
CTGF	Connective tissue growth factor
EPS8L2	EPS8-like 2
LRRC1	Leucine rich repeat containing 1
NS5ATP13TP2	NS5ATP13TP2 protein
PTPRR	Protein tyrosine phosphatase, receptor type, R, tv-2
RICH1	RhoGAP interacting with CIP4 homologs 1
SMTN	Smoothelin, tv-2

than 0.99, and if their differential score was less than 13 ($p>0.05$). Genes with differential expression and up- or down-regulated more than 2-fold in the desmoid tumour samples compared to the normal fibroblast cell line were compiled into lists (Tables 5 and 6).

To reveal any correlation between the expression profiles of desmoid tumours and FAP adenomas, the data from each group were compared. In the upper dendrogram (Figure 2) it can be seen that all the FAP adenomas cluster in the same group. The desmoid tumours and the normal fibroblast cell line clustered in an entirely different group to the FAP samples. The FAP adenomas and the normal colon have distinct gene profiles compared to the desmoid tumours and the normal fibroblasts. Within the FAP adenomas, the stomach adenoma and the normal colon have slightly different gene profiles compared to the colorectal adenomas.

Discussion

In this study, 24K Illumina HumRef-8 BeadArrays were used to compare gene expression of FAP adenomas, desmoid tumours and normal fibroblasts. To date there have been a number of small scale gene expression studies on FAP adenoma tissue, the vast majority of which have employed immunohistochemistry (IHC). Most of these studies have been performed on individual genes

Table 3. Genes commonly up-regulated 2-fold or more in colorectal FAP polyps compared to normal colon

Symbol	Gene Name
Cell Cycle Control	
CCNB2	Cyclin B2
CDKN3	Cyclin-dependent kinase inhibitor 3
AURKB	Aurora kinase B
Cell Cycle	
HCAP-G	Chromosome condensation protein G
PRC1	Protein regulator of cytokinesis 1, tv-1
KIF2C	Kinesin family member 2C
CHC1	Chromosome condensation 1
SMC4L1	SMC4 structural maintenance of chromosome 4-like 1 (yeast)
Pfs2	DNA replication complex GINS protein PSF2
RNASEH2A	Ribonuclease H2, large subunit
Transcription/Transcriptional Regulation	
FLJ20315	Hypothetical protein FLJ20315
TBPL1	TBP-like 1
LOC89958	Hypothetical protein LOC89958
HMGN1	High-mobility group nucleosome binding domain 1
ZNF22	Zinc finger protein 22 (KOX 15)
PTTG1	Pituitary tumour-transforming 1
NFE2L3	Nuclear factor (erythroid-derived 2)-like 3
SOX9	SRY (sex determining region Y)-box 9 (campomelic dysplasia, autosomal sex-reversal)
Transport	
SLC12A2	Solute carrier family 12 (sodium/potassium/chloride transporters) member 2
CLCA1	Chloride channel, calcium activated, family member 1
LCN2	Lipocalin 2 (oncogene 24p3)
Metabolism	
SORD	Sorbitol dehydrogenase
TPRT	Trans-prenyltransferase
QTRT1	Queuine tRNA-ribosyltransferase 1 (tRNA-guanine transglycosylase)
PAICS	Phosphoribosylaminoimidazole carboxylase, Phosphoribosylaminoimidazole succinocarboxamide synthetase
DPH2L2	DPH2-like 2 (S. cerevisiae), tv-1
ALOX5	Arachidonate 5-lipoxygenase
IARS	Isoleucine-tRNA synthetase, tv-short
BRIX	BRIX
TK1	Thymidine kinase 1, soluble
Oncogenesis	
EPHB2	EphB2 (EPHB2), tv-1
BCL11A	B-cell CLL/lymphoma 11A (zinc finger protein) tv-1
MAP17	Membrane-associated protein 17
GDF15	Growth differentiation factor 15
Signalling	
RACGAP1	Rac GTPase activating protein 1
mRNA Processing	
LSM5	LSM5 homolog, U6 small nuclear RNA associated (*S. cerevisiae*)
THOC3	THO complex 3
Cell Adhesion	
C20orf42	Chromosome 20 open reading frame 42

Table 3. Genes commonly up-regulated 2-fold or more in colorectal FAP polyps compared to normal colon

Symbol	Gene Name
Translation	
UK114	Translational inhibitor protein p14.5
Other	
ZCWCC2	Zinc finger, CW-type with coiled-coil domain 2
KIAA1324	Maba1
FLJ10514	Hypothetical protein FLJ10514
ENC1	Ectodermal-neural cortex (with BTB-like domain)
PTTG2	Pituitary tumour-transforming 2
C21orf59	Chromosome 21 open reading frame 59
WDR12	WD repeat domain 12
LXN	Latexin protein
Other	
KIAA1892	KIAA1892
KIAA1797	KIAA1797
GLCE	Glucuronyl C5-epimerase
KIAA0101	KIAA0101 gene product
RRP46	Exosome component Rrp46
S100P	S100 calcium binding protein P
PRDX4	Peroxiredoxin 4
FLJ20366	Hypothetical protein FLJ20366
F12	Coagulation factor XII (Hageman factor)
IGFBP2	Insulin-like growth factor binding protein 2 (36kD)
GW112	Differentially expressed in hematopoietic lineages
C10orf3	Chromosome 10 open reading frame 3
ATOH8	Atonal homolog 8 (*Drosophila*)
MFN1	Mitofusin 1, nuclear gene encoding mitochondrial protein, tv-2
QPCT	Glutaminyl-peptide cyclotransferase (glutaminyl cyclase)
UBE2S	Ubiquitin-conjugating enzyme E2S

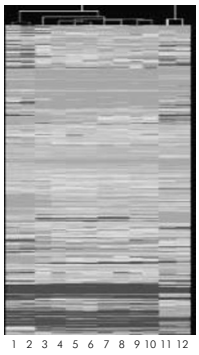

Fig. 1. Cluster analysis of FAP polyps and mixed normal colon. The columns represent the gene expression profiles of each sample. Green – low expression level, yellow – medium expression level, red – high expression level. The relationships between each sample are shown by the upper dendrogram. The colouring in the upper dendrogram represents the sample type: green (left) – normal colon; blue – colorectal FAP polyps; yellow – stomach FAP. 1 – Normal Colon Duplicate; 2 – Normal Colon Duplicate; 3 – Colorectal FAP Polyp A Duplicate; 4 – Colorectal FAP Polyp A Duplicate; 5 – Colorectal FAP Polyp D Duplicate; 6 – Colorectal FAP Polyp D Duplicate; 7 – Colorectal FAP Polyp B Duplicate; 8 – Colorectal FAP Polyp B Duplicate; 9 – Colorectal FAP Polyp C Duplicate; 10 – Colorectal FAP Polyp C Duplicate; 11 – Stomach FAP Polyp D Duplicate; 12 – Stomach FAP Polyp D Duplicate

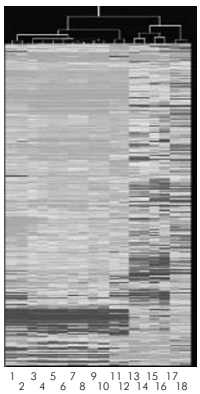

Fig. 2. Cluster analysis of FAP polyps, normal colon, desmoid tumours and normal fibroblasts. The columns represent the gene expression profiles of each sample. Green – low expression level, yellow – medium expression level, red – high expression level. The relationships between each sample are shown by the upper dendrogram. The colouring in the upper dendrogram represents the sample type: green (left) – normal colon; blue – colorectal FAP polyps; orange – stomach FAP polyp; green (right) – desmoid tumours; purple – fibroblast cell line. 1 – Normal Colon Duplicate; 2 – Normal Colon Duplicate; 3 – Colorectal FAP Polyp A Duplicate; 4 – Colorectal FAP Polyp A Duplicate; 5 – Colorectal FAP Polyp D Duplicate; 6 – Colorectal FAP Polyp D Duplicate; 7 – Colorectal FAP Polyp B Duplicate; 8 – Colorectal FAP Polyp B Duplicate; 9 – Colorectal FAP Polyp C Duplicate; 10 – Colorectal FAP Polyp C Duplicate; 11 – Stomach FAP Polyp D Duplicate; 12 – Stomach FAP Polyp D Duplicate; 13 – Desmoid Tumour A Duplicate; 14 – Desmoid Tumour A Duplicate; 15 – Desmoid Tumour C Duplicate; 16 – Desmoid Tumour C Duplicate; 17 – Fibroblast Cell Line Duplicate; 18 – Fibroblast Cell Line Duplicate

Table 4. Genes commonly down-regulated 2-fold or more in colorectal FAP polyps compared to normal colon

Symbol	Gene Name
Cell Cycle Control	
FOSB	FBJ murine osteosarcoma viral oncogene homolog B
PPP3CB	Protein phosphatase 3, catalytic subunit, beta isoform (calcineurn A beta)
Cell Cycle	
MXI1	MAX interacting protein 1, tv-2
CABLES1	Cdk5 and Abl enzyme substrate 1
PMP22	Peripheral myelin protein 22, tv-3
DTR	Diphtheria toxin receptor (heparin-binding epidermal growth factor-like growth factor)
Transcription/Transcriptional Regulation	
HLX1	H2.0-like homeo box 1 (*Drosophila*)
NKX2-3	NK2 transcription factor related, locus 3 (*Drosophila*)
SOX18	SRY (sex determining region Y)-box 18
FNBP1	Formin-binding protein 1
COL4A1	Collagen, type IV, alpha 1
SIRT6	Sirtuin (silent mating type information regulation 2 homolog) 6 (*S. cerevisiae*)
SIRT7	Sirtuin (silent mating type information regulation 2 homolog) 7 (*S. cerevisiae*)
AIM1L	Absent in melanoma 1-like
C19orf21	Chromosome 19 open reading frame 21
Transport	
FBXO32	F-box only protein 32, tv-2
KCNMA1	Potassium large conductance calcium-activated channel, subfamily M, alpha member 1
MYADM	Myeloid-associated differentiation marker
AQP8	Aquaporin 8
SLC17A4	Solute carrier family 17 (sodium phosphate), member 4
SLCO2A1	Solute carrier organic anion transporter family, member 2A1
SGK	Serum/glucocorticoid regulated kinase
P2RX4	Purinergic receptor P2X, ligand-gated ion channel, 4, tv-2
SLC20A1	Solute carrier family 20 (phosphate transporter), member 1
VAMP5	Vesicle-associated membrane protein 5 (myobrevin)
Metabolism	
MGC4171	Hypothetical protein MGC4171
LIPH	Lipase, member H
KIAA0992	Palladin
KIAA0828	KIAA0828 protein
SULT1A2	Sulfotransferase family, cytosolic, 1A, phenol-preferring, member 2, tv-1
UPP1	Uridine phosphorylase 1, tv-1
BTNL3	Butyrophilin-like 3, tv-2
KIAA0934	KIAA0934 protein
AK1	Adenylate kinase 1
DPYSL3	Dihydropyrimidinase-like 3
PLCD1	Phospholipase C, delta 1
CA4	Carbonic anhydrase IV
SVIL	Supervillin, tv-1
PC	Pyruvate carboxylase, nuclear gene encoding mitochondrial protein, tv-2
TMPRSS2	Transmembrane protease, serine 2
PRSS3	Protease, serine, 3 (mesotrypsin)
PCK1	Phosphoenolpyruvate carboxykinase 1 (soluble)
ST6GALNAC6	CMP-NeuAC: (beta)-N-acetylgalactosaminide (alpha)2,6-sialyltransferase member IV
RARRES2	Retinoic acid receptor responder (tazarotene induced) 2
Tumour Suppression	
PPAP2A	Phosphatidic acid phosphatase type 2A, tv-1

Table 4. Genes commonly down-regulated 2-fold or more in colorectal FAP polyps compared to normal colon

Symbol	Gene Name
Signalling	
RGL1	Ral guanine nucleotide dissociation stimulator-like 1
EFNA1	Ephrin-A1, tv-1
SDCBP2	Syndecan binding protein (syntenin) 2, tv-2
GUCA2A	Guanylate cyclase activator 2A (guanylin)
BSG	Basigin (OK blood group), tv-4
TRIF	TIR domain containing adaptor inducing interferon-beta
ILK	Integrin-linked kinase
TJP3	Tight junction protein 3 (zona occludens 3)
PRKCD	Protein kinase C, delta
ITPKA	Inositol 1,4,5-trisposphate 3-kinase A
IL2RG	Interleukin 2 receptor, gamma (severe combined immunodeficiency)
LNK	Lymphocyte adaptor protein
Cell Adhesion	
PC-LKC	Protocadherin LKC
DCN	Decorin, tv-E
FLNA	Filamin A, alpha (actin binding protein 280)
MSN	Moesin
SORBS1	Sorbin and SH3 domain containing 1
TAGLN	Transgelin
CDC42	Cell division cycle 42 (GTP binding protein, 25kDa), tv-2
COL4A2	Collagen, type IV, alpha 2
DBN1	Drebin 1, tv-1
GSN	Gelsolin (amyloidosis, Finnish type), tv-2
ACTG2	Actin, gamma 2, smooth muscle, enteric
ACTA2	Actin, alpha 2, smooth muscle, aorta
CGN	Cingulin
Apoptosis	
RIPK3	Receptor-interacting serine-threonine kinase 3
FOSL2	FOS-like antigen 2
DAPK3	Death-associated protein kinase 3
LGALS1	Lectin, galactoside-binding, soluble, 1 (galactin 1)
GADD45B	Growth arrest and DNA-damage-inducible, beta
Structural	
CLDN5	Claudin 5 (transmembrane protein deleted in velocardiofacial syndrome)
KRT19	Keratin 19
TPM2	Tropomyosin 2 (beta)
Other	
DUSP5	Dual specificity phosphatase 5
CLIPR-59	CLIP-170-related protein
PTPRR	Protein tyrosine phosphatase, receptor type, R, tv-2
SMTN	Smoothelin, tv-2
CEACAM1	Carcinoembryonic antigen-related cell adhesion molecule 1 (biliary glycoprotein)
EPS8L2	EPS8-like 2
RICH1	RhoGAP interacting with CIP4 homologs 1
PDZK2	PDZ domain containing 2
CHKL	Choline kinase-like, tv-1
DIP13B	DIP13 beta
NS5ATP13TP2	NS5ATP13TP2 protein
M-RIP	Myosin phosphatase-Rho interacting protein
MTMR9	Myotubularin related protein 9
LRRC1	Leucine rich repeat containing 1
CTGF	Connective tissue growth factor

DSCR1L1	Down syndrome critical region gene 1-like 1
TU12B1-TY	TU12B1-TY protein
MYH11	Myosin, heavy polypeptide 11, smooth muscle, tv-SM1
FLJ23471	MICAL-like 2, tv-2
DKFZP434B044	Hypothetical protein DKFZp434B044
MUCDHL	Mucin and cadherin-like, tv-2
MMP28	Matrix metalloproteinase 28, tv-1
TRIM15	Tripartite motif-containing 15, tv-1
COL6A2	Collagen, type VI, alpha 2, tv-2C2
SELM	Selenoprotein SelM
ZAK	Sterile alpha motif and leucine zipper containing kinase AZK
SMTN	Smoothelin, tv-3
TNXB	Tenascin XB, tv-XB-S
EPS8L1	EPS8-like 1, tv-3
FLJ10350	Hypothetical protein FLJ10350
DKFZP762C186	Tangerin
TBC1D1	TBC1 (tre-2/USP6, BUB2, cdc16) domain family, member 1
KIAA1145	KIAA1145 protein
PKIG	Protein kinase (cAMP-dependent, catalytic) inhibitor gamma, tv-2
PKIB	Protein kinase (cAMP-dependent, catalytic) inhibitor beta, tv-3
IGSF9	Immunoglobulin superfamily, member 9
LOC90313	Hypothetical protein BC004507
FLJ22582	Hypothetical protein FLJ22582
KIAA0063	KIAA0063 gene product
FSTL1	Follistatin-like 1
PRNP	Prion protein (Creutzfeld-Jakob disease, Gerstmann-Strausler-Scheinker syndrome, fatal familial insomnia), tv-2
ANKRD25	Ankyrin repeat domain 25
STOM	Stomatin, tv-2
FLJ46603	FLJ46603 protein
RAIN	Ras-interacting protein
DHRS9	Dehydrogenase/reductase (SDR family) member 9, tv-1
LIMS2	LIM and senescent cell antigen-like domains 2
ARHGEF18	Rho/rac guanine nucleotide exchange factor (GEF) 18
KIAA0285	KIAA0285 gene product
PDLIM7	PDZ and LIM domain 7 (enigma), tv-1
CXX1	CAAX box 1
MGP	Matrix Gla protein
PTPRH	Protein tyrosine phosphatase, receptor type, H
SPARC	Secreted protein, acidic, cysteine-rich (osteonectin)
FLJ90022	Hypothetical protein FLJ90022
SERPING1	Serine (or cysteine) proteinase inhibitor, clade G (C1 inhibitor), member 1, (angioedema, hereditary)
CSRP1	Cysteine and glycine-rich protein 1
KIAA0513	KIAA0513 gene product
OAS1	2',5'-oligoadenylate synthetase 1, 40/46kDa

that include E-cadhein, α-, β- and γ-catenin, COX-1, COX-2, and c-myc [20-25]. In addition, one study used semi-quantitative RT-PCR to study GKLF [26]. The only report examining global gene expression in human FAP adenoma tissue identified 84 differentially expressed genes in adenomas compared to normal colon tissue [27].

In this study, the gene expression profiles obtained from the FAP adenomas indicate that colorectal adenomas are similar but distinctly different to the stomach adenomas. There were a large number of commonly expressed genes identified across the colorectal FAP adenomas, but when the differentially expressed genes from the stomach adenoma were included in the analysis the number of commonly expressed genes decreased dramatically. The genes that were differentially expressed in the four colonic adenomas and one stomach adenoma were investigated more closely in an attempt to identify common genetic features in FAP. From this analysis genes involved in the cell cycle, transcription and metabolism were the most frequently up-regulated. The most frequently down-regulated genes included those involved in metabolism, cell adhesion, signal transduction, transcription and transport. Since adenomas develop due to a breakdown in the fidelity of the Wnt signalling pathway it was not surprising to

Table 5. Genes commonly up-regulated 2-fold or more in desmoid tumours compared to normal fibroblast cells

Symbol	Gene Name
Cell Cycle Control	
PTN	Pleiotrophin (heparin binding growth factor 8, neurite growth-promoting factor 1)
GAS7	Growth arrest-specific 7, tv-b
CDKN1C	Cyclin-dependent kinase inhibitor 1C (p57, Kip2)
TGFB3	Transforming growth factor, beta 3
Cell Cycle	
NEK3	NIMA (never in mitosis gene a)-related kinase 3, tv-2
Transcription/Transcriptional Regulation	
BHLHB2	Basic helix-loop-helix domain containing, class B, 2
COL4A1	Collagen, type IV, alpha 1
COL4A2	Collagen, type IV, alpha 2
DNAJB2	DnaJ (Hsp40) homolog, subfamily B, member 2
ELF2	E74-like factor 2 (ets domain transcription factor), tv-1
EVI1	Ecotropic viral integration site 1
FKBP1A	FK506 binding protein 1A, 12kDa, tv-12A
FLJ10404	Hypothetical protein FLJ10404
HDAC8	Histone deacetylase 8
JUN	v-jun sarcoma virus 17 oncogene homolog (avian)
KIF2C	Kinesin family member C2
NUCKS	Nuclear ubiquitous casein kinase and cyclin-dependent kinase substrate
PBX2	Pre-B-cell leukemia transcription factor 2
PPIE	Peptidylprolyl isomerase E (cyclophilin E), tv-2
PRR3	Proline-rich polypeptide 3
TEAD2	TEA domain family member 2
TLE2	Transducin-like enhancer of split 2 (E(sp1)) homolog, *Drosophila*
TLE4	Transducin-like enhancer of split 4 (E(sp1)) homolog, *Drosophila*
ZNF22	Zinc finger protein 22 (KOX15)
ZNF254	Zinc finger protein 254
TDRD3	Tudor domain containing 3
ZNF300	Zinc finger protein 300
MEF2C	MADS box transcription enhancer factor 2, polypeptide C (myocyte enhancer factor 2C)
NAB1	NGFI-A binding protein 1 (EGR1 binding protein 1)
Hes4	bHLH factor Hes4
C19orf13	Chromosome 19 open reading frame 13
ARNT	Aryl hydrocarbon receptor nuclear translocator, tv-2
ZNF266	Zinc finger protein 266
ZNF26	Zinc finger protein 26 (KOX 20)
MGC51082	Hypothetical protein MGC51082
TGIF2	TGFB-induced factor 2 (TALE family homeobox)
MYST3	MYST histone acetyltransferase (monocytic leukemia) 3
M96	Likely ortholog of mouse metal response element binding transcription factor 2
BAZ2B	Bromodomain adjacent to zinc finger domain, 2B
Transport	
NXT1	NTF2-like export factor 1
ABCA1	ATP-binding cassette, sub-family A, member 1
SLC25A29	Solute carrier family 25, member 29
SLC16A9	Solute carrier family 16 (monocarboxylic acid transporters), member 9
PSCD1	Pleckstrin homology, Sec7 and coiled-coil domains 1(cytohesin 1), tv-2
AQP1	Aquaporin 1(Channel-forming integral protein, 28kDa) tv-1
SCNN1D	Sodium channel, nonvoltage-gated, delta
SLC22A17	Solute carrier family 22 (organic cation transporter), member 17, tv-2
Metabolism	
SULT1A1	Sulfotransferase family, cytosolic, 1A, phenol-preferring, member 1, tv-1
CH25H	Cholesterol 25-hydroxylase

Table 5. Genes commonly up-regulated 2-fold or more in desmoid tumours compared to normal fibroblast cells

Symbol	Gene Name
QTRTD1	Queuine tRNA-ribosyltransferase domain containing 1
FLJ23749	Hypothetical protein FLJ23749
FLJ10706	Hypothetical protein FLJ10706
USP52	Ubiquitin specific protease 52
RARRES2	Retinoic acid receptor responder (tazarotene induced) 2
ADAM19	A distintegrin and metalloproteinase domain 19 (meltrin beta), tv-2
AUTS2	Autism susceptibility candidate 2
GALNT3	UDP-N-acetyl-alpha-D-galactosamine:polypeptide N-acetylgalactosaminyltransferase 3 (GalNAc-T3)
KIAA0140	KIAA0140
ODC-p	Ornithine decarboxylase-like
PCSK5	Proprotein convertase subtilisin/kexin type 5
Oncogenesis	
AKAP13	A kinase (PRKA) anchor protein 13, tv-3
MGP	Matrix Gla protein
EWSR1	Ewing sarcoma breakpoint region 1, tv-EWS-b
SFRP4	Secreted frizzled-related protein 4
SRPUL	Sushi-repeat protein
Signalling	
GABBR1	Gamma-aminobutyric acid (GABA) B receptor, 1, tv-2
CAPS	Calcyphosine, tv-2
NET1	Neuroepithelial cell transforming gene 1
PRKCH	Protein kinase C, eta
PPP2R2B	Protein phosphatase 2 (formerly 2A), regulatory subunit B (PR52), beta isoform, tv-4
RGS16	Regulator of G-protein signalling 16
PTHR1	Parathyroid hormone receptor 1
TMPEI	Transmembrane, prostate androgen induced RNA, tv-4
ARHU	Ras homolog gene family, member U
CHN1	Chimerin (chimaerin) 1
EFNB3	Ephrin-B3
GFRA2	GDNF family receptor alpha 2
GNB4	Guanine nucleotide binding protein (G protein), beta polypeptide 4
IL11RA	Interleukin 11 receptor, alpha, tv-1
ITPKB	Inositol 1,4,5-trisphosphate 3-kinase B
KIF13B	Kinesin family member 13B
MAP4K1	Mitogen-activated protein kinase kinase kinase kinase 1
MLP	MARCKS-like protein
PDGFRL	Platelet-derived growth factor receptor-like
PRKCABP	Protein kinase C, alpha binding protein
RASD1	RAS, dexamethasone-induced 1
TNFAIP6	Tumour necrosis factor, alpha-induced protein 6
Cell Adhesion	
COL7A1	Collagen, type VII, alpha 1 (epidermolysis bullosa, dystrophic, dominant and recessive)
ISLR	Immunoglobulin superfamily containing leucine-rich repeat, tv-1
Apoptosis	
PPP1R13B	Protein phosphatase 1, regulatory (inhibitor) subunit 13B
AXUD1	AXIN1 up-regulated 1
CASP10	Caspase 10, apoptosis-related cysteine protease, tv-B
MX1	Myxovirus (influenza virus) resistance 1, interferon-inducible protein p78 (mouse)
PCBP4	Poly(rC) binding protein 4, tv-4
TNFRSF19	Tumour necrosis factor receptor superfamily, member 19, tv-2
TNFRSF25	Tumour necrosis factor receptor superfamily, member 25, tv-7
Tumourigenesis	
BARD1	BRCA1 associated RING domain 1
LOH11CR2A	Loss of heterozygosity, 11, chromosomal region 2, gene A

Table 5. Genes commonly up-regulated 2-fold or more in desmoid tumours compared to normal fibroblast cells

Symbol	Gene Name
Immune Response	
HLA-DPA1	Major histocompatibility complex, class II, DP alpha 1
C1R	Complement component 1, r subcomponent
CXCL14	Chemokine (C-X-C motif) ligand 14
IFI27	Interferon, alpha-inducible protein 27, tv-a
MX2	Myxovirus (influenza virus) resistance 2 (mouse)
RNA Processing	
DHX8	DEAH (Asp-Glu-Ala-His) box polypeptide 8
HNRPA1	Heterogeneous nuclear ribonucleoprotein A1, tv-1
SFRS11	Splicing factor, arginine/serine-rich 11
Structural	
ACTL6	Actin-like 6
FBLN1	Fibulin 1 (FBLN1), tv-C
FBLN1	Fibulin 1 (FBLN1), tv-D
SMTN	Smoothelin, tv-2
Other	
MT1H	Metallothionein 1H
C12orf14	Chromosome 12 open reading frame 14
PELI1	Pellino homolog 1 (*Drosophila*)
IFI44	Interferon-induced protein 44
C10orf6	Chromosome 10 open reading frame 6
C2orf11	Chromosome 2 open reading frame 11
FLJ31951	Hypothetical protein FLJ31951
ISYNA1	Myo-inositol 1-phosphate synthase A1
FLJ31614	Hypothetical protein FLJ31614
AD031	AD031 protein
CASC3	Cancer susceptibility candidate 3
GBA2	Glucosidase, beta (bile acid) 2
CGI-85	CGI-85 protein, tv-2
C14orf80	Chromosome 14 open reading frame 80
ACAS2L	Acetyl-Coenzyme A synthetase 2 (AMP forming)-like, nuclear gene encoding mitochondrial protein
DTX3	Deltex 3 homolog (*Drosophila*)
FLJ23059	Hypothetical protein FLJ23059
PIK3R1	Phosphoinositide-3-kinase, regulatory subunit, polypeptide 1 (p85 alpha), tv-2
KIAA1223	KIAA1223
STARD9	START domain containing 9
LOC375786	Hypothetical gene supported by AL713796
SR140	U2-associated SR140 protein
MIDN	Midnolin
SEC31L2	SEC31-like 2 (*S. cerevisiae*), tv-1
FLJ12178	Hypothetical protein FLJ12178
LOC157567	Hypothetical protein LOC157567
FLJ25005	FLJ25005 protein
WARP	von Willebrand factor A domain-related protein, tv-1
KIAA1036	KIAA1036
LOC374969	Hypothetical protein LOC374969
LOC155435	Hypothetical protein LOC155435
MGC9913	Hypothetical protein MGC9913
CASKIN2	CASK interacting protein 2
CFDP1	Craniofacial development protein 1
SPAG5	Sperm associated antigen 5
MMP23B	Matrix metalloproteinase 23B
AKAP8L	A kinase (PRKA) anchor protein 8-like
FLJ11029	Hypothetical protein FLJ11029
DDIT4	DNA-damage-inducible tv-4
APCDD1	Adenomatous Polyposis Coli down-regulated 1
CDW92	CDW92 antigen

Table 6. Genes commonly down-regulated 2-fold or more in desmoid tumours compared to normal fibroblast cells

Symbol	Gene Name
Cell Cycle	
GRN	Granulin
QSCN6	Quiescin Q6
STAT1	Signal transducer and activator of transcription 1,91kDa, tv-α
STAT1	Signal transducer and activator of transcription 1,91kDa, tv-β
TIMP1	Tissue inhibitor of metalloproteinase 1 (erythroid potentiating activity, collagenase inhibitor)
Transcription/Transcriptional Regulation	
HIST1H2BK	Histone 1, H2bk
LOXL1	Lysyl oxidase-like 1
MSC	Musculin (activated B-cell factor-1)
PRRX1	Paired related homeobox 1, tv-pmx-1b
ZDHHC14	Zinc finger, DHHC domain containing 14
Transport	
GLRB	Glycine receptor, beta
PCOLCE2	Procollagen C-endopeptidase enhancer 2
SCAMP3	Secretory carrier membrane protein 3, tv-1
SLC31A2	Solute carrier family 31 (copper transporters), member 2
Metabolism	
AK1	Adenylate kinase 1
AKR1C3	Aldo-keto reductase family 1, member C3 (3-alpha hydroxysteroid dehydrogenase, type II)
C1RL	Complement component 1, r subcomponent-like
COMT	Catechol-O-methyltransferase, tv-MB-COMT
CTSL	Cathepsin L, tv-2
GCLM	Glutamate-cysteine ligase, modifier subunit
GNPDA2	Glucosamine-6-phosphate deaminase 2
IDH1	Isocitrate dehydrogenase 1 (NADP+), soluble
NQO1	NAD(P)H dehydrogenase, quinone 1
PTGIS	Prostaglandin I2 (prostacyclin) synthase
SMPDL3A	Sphingomyelin phosphodiesterase, acid-like 3A
SPPL2A	Putative intramembrane cleaving protease
STS	Steroid sulfatase (microsomal), arylsulfatase C, isozyme S
UBE2G1	Ubiquitin-conjugating enzyme E2G 1 (UBC7 homolog, *C. elegans*), tv-1
UCHL1	Ubiquitin carboxyl-terminal esterase L1 (ubiquitin thiolesterase)
Tumour Suppression	
MADH3	MAD, mothers against decapentaplegic homolog 3 (*Drosophila*)
Signalling	
DEPDC6	DEP domain containing 6
DIRAS1	DIRAS family, GTP-binding RAS-like 1
PDGFRA	Platelet-derived growth factor receptor, alpha polypeptide
PENK	Proenkephalin
SARA2	SAR1a gene homolog 2 (*S. cerevisiae*)
SNTB1	Syntrophin, beta 1 (dystrophin-associated protein A1, 59kDa, basic component 1)
DKFZp564I1922	Adlican
mRNA Processing	
CSTF1	Cleavage stimulation factor, 3' pre-RNA, subunit 1, 50kDa
Cell Adhesion	
CNTNAP1	Contactin-associated protein 1
THBS2	Thrombospondin 2
ZYX	Zyxin

Table 6. Genes commonly down-regulated 2-fold or more in desmoid tumours compared to normal fibroblast cells

Symbol	Gene Name
Apoptosis	
C20orf97	Chromosome 20 open reading frame 97
DAPK1	Death-associated protein kinase 1
MAPK1	Mitogen-activated protein kinase 1, tv-1
Structural	
KRT18	Keratin 18, tv-1
TUBG1	Tubulin, gamma 1
Immune Response	
ANKRD15	Ankyrin repeat domain 15, tv-1
DPP4	Dipeptidylpeptidase 4 (CD26, adenosine deaminase complexing protein 2)
MR1	Major histocompatibility complex, class I-related
Other	
ANGPTL2	Angiopoietin-like 2
ANTXR2	Anthrax toxin receptor 2
BCKDHB	Branched chain keto acid dehydrogenase E1, beta polypeptide (maple syrup urine disease), nuclear gene encoding mitochondrial protein, tv-2
BZRP	Benzodiazapine receptor (peripheral), tv-PBR-S
C11orf17	Chromosome 11 open reading frame 17, tv-2
C6orf32	Chromosome 6 open reading frame 32
C9orf88	Chromosome 9 open reading frame 88
CDC42EP2	CDC42 effector protein (Rho GTPase binding) 2
CRLF1	Cytokine receptor-like factor 1
DIRC2	Disrupted in renal carcinoma 2
EDEM1	ER degradation enhancer, mannosidase alpha-like 1
FLJ20073	FLJ20073 protein
FLJ20272	Hypothetical protein FLJ20272
FLJ22582	Hypothetical protein FLJ22582
HOM-TES-103	HOM-TES-103 tumour antigen-like, tv-3
HSPC157	HSPC157 protein
KIAA0196	KIAA0196 gene product
LOC196463	Hypothetical protein LOC196463
LOC221091	Similar to hypothetical protein
LOC286343	Hypothetical protein LOC286343
LOC387908	Similar to Ferritin heavy chain (Ferritin H subunit)
LOC57168	Similar to aspartate beta hydroxylase (ASPH)
LRRFIP2	Leucine rich repeat (in FLII) interacting protein 2
LYPLA1	Lysophospholipase I
MGC12992	Hypothetical protein MGC12992
MGST1	Microsomal glutathione S-transferase 1, tv-1a
MOCOS	Molybdenum cofactor sulfurase
NNT	Nicotinamide nucleotide transhydrogenase
PKM2	Pyruvate kinase, muscle, tv-1
PPAP2B	Phosphatidic acid phosphatase type 2B, tv-2
PSFL	Anterior pharynx defective 1B-like
PTX3	Pentaxin-related gene, rapidly induced by IL-1 beta
S100A4	S100 calcium binding protein A4 (calcium protein, calvasculin, metastasin, murine placental homolog), tv-2
SLIT3	Slit homolog 3 (*Drosophila*)
SMP1	Small membrane protein 1
TRIM4	Tripartite motif-containing 4, tv-β
UNQ564	UNQ564
ZC3HAV1	Zinc finger CCCH type, antiviral 1, tv-2

observe the over-expression of genes involved in cell cycle progression.

Altered Expression of Wnt/β-catenin Target Genes in Colorectal FAP Adenomas

It has been long established that deregulation of the Wnt signalling pathway due to APC mutations plays a major role in the progression of FAP [5]. The Wnt/β-catenin signalling pathway is involved in the control of expression of Sox9, PTTG1 and EphB2, all of which were found to be up-regulated by more than 2-fold in all the colorectal FAP adenomas compared to the normal colon.

PTTG1 is regulated by a TCF binding sequence in its promoter region [28]. The normal function of PTTG1 is to regulate chromosome segregation during cell division [29]. Over-expression of PTTG1 has been reported frequently in various types of cancer, including colorectal, and has been associated with angiogenesis [30-32]. The role of PTTG1 in angiogenesis is thought to be a result of its part in mediating the secretion of the basic fibroblast growth factor into the extracellular matrix, which promotes proliferation and migration of colorectal cancer cells [30, 31].

The Sox9 gene encodes a transcription factor that is required for chondrogenesis and male gonad development [32], which is under the control of the Wnt signalling pathway [33]. The expression of the Sox9 gene in the intestine is dependent on the activity of the β-catenin/TCF-4 complex, although it is unknown whether this complex interacts directly with the Sox9 promoter or through another of its targets [33].

The EphB2 gene encodes the Eph receptor B, which has been shown to be a target of the Wnt signalling pathway [34]. There is evidence to suggest that normal patterning in the epithelium of the intestinal crypts is coordinated by EphB2 and its ligand, ephrin B [34]. Over-expression of EphB2 is often found in colorectal cancers, but there is confusion about its role in tumourigenesis. Many studies on other tumours have reported EphB2 over-expression as a marker of poor prognosis, but recent studies in colorectal cancer have suggested otherwise [35, 36].

Altered Expression of Cell Cycle-Related Genes in Colorectal FAP Adenomas

A number of genes found to be commonly up-regulated in the adenomas used in this study have previously been reported as being over-expressed in various types of cancers. These genes include the cell cycle-related genes Chromosome condensation protein G (HCAP-G), Protein regulator of cytokinesis 1 (PRC1), SMC4 structural maintenance of chromosome 4-like 1 (SMC4L1) and Cyclin B2 (CCNB2) [37-39]. Although these genes are associated with tumour development none have been thoroughly characterized in FAP to date.

Altered Gene Expression in Desmoid Tumours

A limited number of gene expression studies have been performed on desmoid tumours, primarily due to the difficulties in obtaining tissue. Two reports have studied gene expression in desmoid disease using 6.8K, 19K and 33K Affymetrix microarrays [40, 41]. Skubitz and Skubitz (2004) [40] reported that ADAM12, WISP-1, Sox-11 and fibroblast activation protein-α are uniquely expressed in desmoids. Denys et al. (2004) identified 69 differentially expressed genes in desmoid tumour tissue compared to normal fibroblasts, before focusing on the down-regulation of IGFBP-6 [41].

A number of genes that were identified as being differentially expressed in desmoid tumours in this study have been reported previously. The over-expressed genes include transforming growth factor β3 (TGFβ3), a distintegrin and metalloproteinase domain 19 (ADAM19), chimerin 1 (CHN1), and ephrin-B3 (EFNB3) [40, 41]. The under-expressed genes include quiescin Q6 (QSCN6), prostaglandin I2 synthase (PTGIS), proenkephalin (PENK), keratin 18 (KRT18), cytokine receptor-like factor 1 (CRLF1), pentaxin-related gene (PTX3) and endoglin (ENG) [41].

Ephrin-B3, a Wnt Target Overexpressed in Desmoid Tumours

The known Wnt/β-catenin target gene ephrin-B3 [42] has been found in this study to be up-regulated more than 2-fold in desmoid tumours compared to normal fibroblasts. The ephrins are ligands for the EPH receptor family, whose normal function is to organize cell patterning in the intestinal crypts [34]. In addition, more recent observations suggest that ephrins are tumour suppressors, although the mechanism by which this is affected remains to be clarified [3, 43, 44]. Further investigation into the precise role of ephrin-B3 is required before any conclusions can be made regarding its role in desmoid disease.

Wound Healing-Associated Genes Differentially Expressed in Desmoid Tumours

Two genes, transforming growth factor β-3 (TGFβ3) and pleiotrophin (PTN), were found to be differentially expressed in desmoid tumours. Both genes are

associated with wound healing and could potentially explain the growth advantage of desmoid tumours [45].

TGFβ3 is a multifunctional protein, having roles in cell proliferation and differentiation during embryogenesis and wound healing [46]. Pleiotrophin has been reported to be strongly expressed in many human cancers, and is thought to promote malignant transformation and angiogenesis [47]. It is also frequently found to be up-regulated during the wound healing process [48].

In this study, three genes associated with negative regulation of the wound response have been identified as being under-expressed in desmoid tumours. The three genes are: signal transducer and activator of transcription 1 (STAT1), mothers against decapentaplegic homolog 3 (MADH3 or Smad3) and mothers against decapentaplegic homolog 6 (MADH6 or Smad6). STAT1 enhances transcription in response to interferon-γ, an action which has been shown to inhibit the wound healing response by preventing phosphorylation of Smad2 and Smad3 [49]. This in turn inhibits the action of TGFβ on the wound response [50]. The role of Smad3 in the wound response is not entirely understood; however, the absence of Smad3 causes an accelerated healing response, even though its over-expression has also been shown to promote healing [51, 52]. Smad6 is a known inhibitor of TGFβ, and has shown to be down-regulated in keloids [53].

The abundance of wound response-related genes found to be deregulated in the desmoid tumours in this study adds to the notion that desmoid formation is an abnormal wound response. The finding of over-expressed genes involved in fibroblast proliferation and migration could explain the abnormal proliferation and local invasiveness of desmoid tumours. The down-regulation of angiogenesis-associated genes could account for the poor vascularisation of desmoids.

The limiting factor in this study of desmoid tumours is the small number of desmoids available. In order to reach more conclusions regarding the exact molecular nature of desmoids and their growth mechanisms, a much larger sample size would be required.

Comparison of FAP Adenoma and Desmoid Tumour Molecular Profiles

It has long been recognized that desmoid tumours occur with a much higher frequency in FAP patients than in the general population. The apparent role of aberrant Wnt signalling in both diseases could indicate a molecular similarity between the two. Although Wnt target genes were identified as being up-regulated in both tumour types in this study, the specific genes were different in the two groups. The finding of different Wnt targets could be attributed to the use of different control groups for the FAP adenomas and desmoid tumours. Nevertheless, the molecular profiles obtained using cluster analysis clearly demonstrated that FAP adenomas and desmoid tumours display distinctly different gene expression profiles.

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