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 1 x Complete DMEM media at 37°C (5% CO₂).

RNA Extraction

2-3 mm² 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 10⁵ control fibroblast cells were lysed in 1 ml Trizol reagent.
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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 R² 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.

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)          |
| TJ3P      | 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| N5ATP13TP2 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                                                 |
| SMG4L1     | SMC4 structural maintenance of chromosome 4-like 1 (yeast)                |
| Plk2       | DNA replication complex GINS protein PSF2                                 |
| RNAESEH2A  | 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                                                    |
| TRPT       | Trans-prenyltransferase                                                   |
| QTTR1      | Queuine RNA-ribosyltransferase 1 (RNA-guanine transglycosylase)           |
| PAICS      | Phosphoribosylaminomimidazole carboxylase, Phosphoribosylaminomimidazole succinocarboxamide synthetase |
| DPH2L2     | DPH2-like 2 (S. cerevisiae), tv-1                                         |
| ALOX5      | Arachidonate 5-lipoxygenase                                               |
| IARS       | Isoleucine-RNA 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**       |                                                               |
| ZCWC2           | Zinc finger, CW-type with coiled-coil domain 2                |
| KIAA1324        | Maba1                                                         |
| FLJ10514        | Hypothetical protein FLJ10514                                 |
| ENC1            | Ectodermal-neural cortex (with BTB-like domain)               |
| PTTC2           | Pituitary tumour-transforming 2                               |
| C21orf59        | Chromosome 21 open reading frame 59                           |
| WDR12           | WD repeat domain 12                                           |
| LINN            | Latsenin 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           | Atal 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 | FB1 murine osteosarcoma viral oncogene homolog B |
| PPP3CB | Protein phosphatase 3, catalytic subunit, beta isoform (calcineurin A beta) |
| **Cell Cycle** |
| M01 | MAX interacting protein 1, tv-2 |
| CABLE51 | Cdk5 and Abl enzyme substrate 1 |
| PPP22 | 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 | Fmn-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) |
| AML1 | 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 |
| SLC20A1 | 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 (myobravin) |
| **Metabolism** |
| MGC4171 | Hypothetical protein MGC4171 |
| LIPH | Lipase, member H |
| KIAA0992 | Palladin |
| KIAA0828 | KIAA0828 protein |
| SULT1A2 | Sulfoconjugate family, cytosolic, 1A, phenol-preferring, member 2, tv-1 |
| UPP1 | Uridine phosphorylase 1, tv-1 |
| BTLN3 | Buthyrophilin-like 3, tv-2 |
| KIAA0934 | KIAA0934 protein |
| AK1 | Adenylyl kinase 1 |
| DPYSL3 | Dihydropyrimidinase-like 3 |
| PLCDL1 | 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) |
| PKC1 | 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-trisphosphate 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 |
| CEAAM1 | Carcinoembryonic antigen-related cell adhesion molecule 1 (biliary glycoprotein) |
| EPSL2 | EPS8-like 2 |
| RICH1 | RhoGAP interacting with CIP4 homologs 1 |
| PDZK2 | PDZ domain containing 2 |
| CHKL | Choline kinase-like, tv-1 |
| DIP13B | DIP13 beta |
| NSSATP13TP2 | NSSATP13TP2 protein |
| M-RIP | Myosin phosphatase-Rho interacting protein |
| MTMR9 | Myotubularin related protein 9 |
| LRRC1 | Leucine rich repeat containing 1 |
| CTGF | Connective tissue growth factor |
that include E-cadherin, 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) |
| GAD7 | Growth arrest-specific 7, tv-b |
| CDKN1C | Cyclin-dependent kinase inhibitor 1C (p57, Kip2) |
| TGFβ3 | Transforming growth factor, beta 3 |
| **Cell Cycle** | 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 |
| ELF3 | E74-like factor 3 (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 | c-jun sarcoma virus 17 oncogene homolog (avian) |
| KIF1C | Kinesin family member C2 |
| NUCKS | Nuclear ubiquitous casein kinase and cyclin-dependent kinase substrate |
| PRK | Pre-B-cell leukemia transcription factor 2 |
| PPRI | 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 |
| PCDH1 | Pcdh family, member 1 |
| AQP1 | Aquaporin 1 (channel-forming integral protein, 28kDa) tv-1 |
| SCN1D | Sodium channel, nonvoltage-gated, delta |
| SLC22A22 | Solute carrier family 22 (organic anion transporter), member 22 |
| **Metabolism** | |
| SULT1A1 | Sulphotransferase 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 S2                                            |
| RARRES2     | Retinoic acid receptor responder (tazarotene induced) 2                  |
| ADAM19      | A disteintegrin 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        | Calphosine, 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                                      |
| PTMR1       | Parathyroid hormone receptor 1                                            |
| TMEPEI      | Transmembrane, prostate androgen induced RNA, tv-4                       |
| ARH4U       | 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                             |
| PRKCBP      | 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       | AXEN1 up-regulated 1                                                     |
| CASP10      | Caspase 10, apoptosis-related cysteine protease, tv-B                    |
| MX1         | Myxovirus (influenza virus) resistance 1, interferon-inducible protein p78 (mouse) |
| PCB4        | Poly(C) 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 |
| PEL1 | Pelirna 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 | Phosphoinositol-3-kinase, regulatory subunit, polypeptide 1 (p85 alpha), tv-2 |
| KIAA1223 | KIAA1223 |
| STAR9 | START domain containing 9 |
| LOC375786 | Hypothetical gene supported by AL713796 |
| SR140 | U2-associated SR140 protein |
| MIDN | Midolin |
| SEC31L2 | SEC31-like 2 (S. cerevisiae), tv-1 |
| FLJ12178 | Hypothetical protein FLJ12178 |
| LOC157567 | Hypothetical protein LOC157567 |
| FLJ25005 | FLJ25005 protein |
| WARP | van 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 |
| MAP23B | Matrix metalloproteinase 23B |
| AKAP8L | A kinase (PRKA) anchor protein 8-like |
| FLJ11029 | Hypothetical protein FLJ11029 |
| DDI4 | DNA-damage-inducible tv-4 |
| APCDD1 | Adenomatous Polyposis Coli down-regulated 1 |
| CDW92 | CDW92 antigen |
| Symbol | Gene Name |
|--------|-----------|
| Cell Cycle | |
| GRN | Granulin |
| GSCN6 | 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 | |
| HST1H2BK | 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, DHHHC 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 |
| CTS1 | Cathepsin L, tv-2 |
| GCLM | Glutamate-cysteine ligase, modifier subunit |
| GNPD1A2 | 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 |
| SPP1 | Putative intramembrane cleaving protease |
| STS | Steroid sulfatase (microsomal), arylsulfatase C, isozyme 5 |
| UBE2G1 | Ubiquitin-conjugating enzyme E2G 1 (UBE7 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 |
| DIRR1S | 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) |
| DFKZp56411922 | Adlican |
| mRNA Processing | |
| CSTF1 | Cleavage stimulation factor, 3' pre-RNA, subunit 1, 50kDa |
| Cell Adhesion | |
| CNTPNAP1 | 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                                          |

| 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        | Benzoazapine receptor (peripheral), tv-PBR-5 |
| 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 |
| LYL1        | Lysophospholipase 1      |
| MGC12992    | Hypothetical protein MGC12992 |
| MGST1       | Microsomal glutathione S-transferase 1, tv-1a |
| MOCOS       | Molybdenum cofactor sulfatase |
| NNT         | Nicotinamide nucleotide transhydrogenase |
| PKM2        | Pyruvate kinase, muscle, tv-1 |
| PPAP2B      | Phosphatic 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 (Drasophila) |
| 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 upregulated 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 tumours are 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 downregulation of angiogenesis-associated genes could account for the poor vascularity 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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