Increased midkine expression correlates with desmoid tumour recurrence: a potential biomarker and therapeutic target

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Abstract

Desmoid tumours (DTs) are soft tissue monoclonal neoplasms exhibiting a unique phenotype, consisting of aggressive local invasiveness without metastatic capacity. While DTs can infrequently occur as part of familial adenomatosis polyposis, most cases arise sporadically. Sporadic DTs harbour a high prevalence of CTNNB1 mutations and hence increased β-catenin signalling. However, β-catenin downstream transcriptional targets and other molecular deregulations operative in DT inception and progression are currently not well defined, contributing to the lack of sensitive molecular prognosticators and efficacious targeted therapeutic strategies. We compared the gene expression profiles of 14 sporadic DTs to those of five corresponding normal tissues and six solitary fibrous tumour specimens. A DT expression signature consisting of 636 up- and 119 down-regulated genes highly enriched for extracellular matrix, cell adhesion and wound healing-related proteins was generated. Furthermore, 98 (15%) of the over-expressed genes were demonstrated to contain a TCF/LEF consensus binding site in their promoters, possibly heralding direct β-catenin downstream targets relevant to DT. The protein products of three of the up-regulated DT genes: ADAM12, MMP2 and midkine, were found to be commonly expressed in a large cohort of human DT samples assembled on a tissue microarray. Interestingly, enhanced midkine expression significantly correlated with a higher propensity and decreased time for primary DT recurrence (log-rank p = 0.0025). Finally, midkine was found to enhance the migration and invasion of primary DT cell cultures. Taken together, these studies provide insights into potential DT molecular aberrations and novel β-catenin transcriptional targets. Further studies to confirm the utility of midkine as a clinical DT molecular prognosticator and a potential therapeutic target are therefore warranted. Raw gene array data can be found at: http://smd.stanford.edu/

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Introduction

Desmoid tumours (DTs) are monoclonal neoplasms demonstrating fibroblastic to myofibroblastic differentiation [1]. DTs are unique as locally aggressive lesions devoid of metastatic capacity [2]. Approximately 1000 new desmoid diagnoses/year are made in the USA. DTs predominantly affect young adults, especially females, and primarily involve the trunk (mainly abdominal wall), extremities (especially pelvic and shoulder girdles) and intestinal mesenteries [2]. DTs infrequently occur as part of familial adenomatosis polyposis (FAP) or familial infiltrative fibromatosis, due to germ-line mutations in the adenomatosis polyposis gene (APC) [3]. However, most DTs (>90%) are sporadic; cell of origin and precursor lesions are unknown. The DT molecular hallmark is nuclear accumulation of β-catenin, possibly resulting in currently undefined DT-specific transcriptional programme(s) driving inception and progression. Sporadic DT β-catenin deregulation
is often due to gain-of-function mutations in exon 3 of the **CTNNB1** gene (encoding for β-catenin), observed in up to 85% of tumours [4]; **APC** mutations driving β-catenin stabilization have been also identified in ~10% of sporadic cases.

Sporadic DT diagnosis is based on clinical, radiological and histological parameters. However, distinguishing desmoids from reactive processes (eg scar or other benign fibroblastic neoplasms) or from low-grade sarcoma can challenge even expert pathologists [5–7]. Surgical excision is the treatment of choice if feasible [8]. Nonetheless, desmoids are very infiltrative, locally destructive lesions possessing high local recurrence rates in the range 19–38% [9–11]. Repeat excisions, if necessary, often result in significant treatment-related morbidity, i.e. amputation, loss of foregut structures. DT natural history is highly variable (including cases of spontaneous regression [12,13]); consequently, ‘watchful waiting’ to determine the biology of a particular tumour is increasingly utilized [14,15]. Given the possible surgical morbidities, radiotherapy, systemic chemotherapy, hormonal blockade, non-steroidal anti-inflammatory agents, imatinib mesylate and sorafenib are utilized [16,17]. However, the lack of prospective randomized trials to inform DT management means that therapeutic decisions are frequently empirical. There is an obvious need for: (a) better diagnostic tools; (b) prognostic markers; and (c) more efficacious DT therapies.

Gene expression profiling may help address the above DT knowledge gaps. Expression profiles have already informed studies of multiple malignancies [18–24], including sarcomas [25–29]; although additional mining is warranted, this approach has already led to clinically applicable diagnosis and treatment tools [30,31]. However, only a few studies have evaluated DT gene expression profiles [32–37]; DT molecular fingerprints await further determination. Here we sought to examine molecular aberrations underlying DT inception and progression, hoping to identify diagnostic/prognostic biomarkers and candidate novel DT therapeutic targets. We identified commonly deregulated genes operative in DT by comparing the gene expression profiles of DTs, DT patient normal tissues, and solitary fibrous tumours (SFTs; a different fibroblastic tumour type). Bioinformatic analyses and array validation confirming enhanced expression of selected proteins (using a large clinically annotated tissue microarray) were also performed.

### Materials and methods

#### Gene expression arrays

For gene expression profiling, frozen tissues derived from 14 sporadic desmoid tumours (FAP-associated samples not included), five frozen normal tissues acquired from corresponding DT patients, and six SFTs, were utilized (see Supporting information, Table S1, for sample-related information). Of note, in order to decrease genetic background heterogeneity, we opted to utilize normal tissues derived from the same patients from whom DT samples were retrieved. This objective limited normal controls to those cases where such tissues were retrieved at surgery, were freshly frozen, and where good quality RNA was extractable (n = 5 of 14) and affected the type of tissue available for use in the study (fascia = 3, muscle = 1, small bowel wall = 1). To confirm that the sample was representative of the case, a frozen section from each specimen was first prepared and examined. Tissue was homogenized in Trizol reagent (Invitrogen, Carlsbad, CA, USA) and total RNA isolated according to the manufacturer’s protocol; reference RNA for all hybridizations consisted of Universal human reference RNA (Stratagene, Santa Clara, CA, USA). cDNA was synthesized and labelled as previously described [25] and the samples were loaded onto 4 × 44 K Agilent arrays (Agilent Technology, Santa Clara, CA, USA) for hybridization. Arrays were scanned on an Agilent Microarray Scanner G2565BA (5 μm resolution). Captured images were transformed to data using Feature Extraction Software, v 9.5 (Agilent; raw gene array data can be found at: http://smd.stanford.edu/).

### Immunohistochemistry

A previously described desmoid tissue microarray (TMA) was utilized to evaluate candidate biomarker protein expression [4]. Of 195 DT tissue samples, 152 representing independent patients were relevant for inclusion in the current study; 98 primary lesions, 54 recurrences: sites included 132 extra-abdominal (61 abdominal/chest wall, 57 extremity, 14 head and neck) and 20 intra-abdominal. A comprehensive clinical database containing patient, tumour (including **CTNNB1** mutational status), treatment and follow-up information, and scoring of previously conducted immunohistochemistry (IHC) studies linked to TMA, was updated for this investigation. Commercially available anti-Midkine antibodies (Antigenix America, Huntington Station, NY, USA), ADAM12 (Abcam, Cambridge, MA, USA) and MMP2 (Chemicon, Billerica, MA, USA) were used for immunohistochemistry, conducted as previously described [38]. Positive and negative controls were run in parallel. Immunostaining was scored for both intensity (0 = none, 1 = low; 2 = moderate/strong) and percentage of positively expressing cells by a sarcoma pathologist (AJL). All staining results were integrated into the TMA clinical database.

### Cellular assays

Isolation of DT primary cultures was conducted with approval from the MDACC institutional reviw board (IRB) and informed patient consent [39]. To confirm the tumourigenic origin of these cells, **CTNNB1** genotyping of both cell strains and corresponding tumours...
Desmoid tumour gene expression signature

was conducted as previously reported [4]. Western blot (WB) analyses were performed by standard methods [40]. Normal human dermal fibroblasts (NHDFs; Cambrex Corporation, East Rutherford, NJ, USA) were used as controls. Recombinant midkine (R&D, Minneapolis, MN, USA) was used for stimulation; midkine knockdown was conducted using anti-midkine siRNA constructs. DT cells (5 × 10⁴/well) were cultured in a six-well plate and incubated overnight at 37°C. The following morning SmartPool midkine (MK) siRNA or non-targeting siRNA constructs (Dharmacon) were transfected using Lipofectamine 2000 (Invitrogen) reagents, according to manufacturer’s instructions. MTS assays were performed as described [39] to determine the impact of midkine knockdown on DT cell growth. Migration and invasion assays were conducted using modified Boyden chambers, as previously described [39].

Statistical and bioinformatics analyses

Differentially expressed genes, based on gene arrays, were identified using a two-sided t-test on log-transformed data and fold change; false discovery rate (FDR) was assessed by permutation testing (n = 1000) of the sample group labels. In brief, the selection approach was to first compute two t-tests and two fold changes for each comparison of desmoid versus one of the two control groups, and then to take genes with nominal p value < 0.01 and fold > 2 for each comparison. To assess FDR, we carried out this same selection 1000 times in permutation testing, where the sample group labels were switched around in each permutation. Java TreeView [41] represented expression values as colour maps. Of note, to unravel a DT-specific gene expression signature, the analysis was set to identify over- and under-expressed genes in DT as compared to all control samples (normal tissue and SFT). Consequently, differences amongst normal tissue types and between normal tissues and SFTs would not be reflected. Using Sigterm [42], our desmoid-associated gene signature was searched for Gene Ontology (GO) annotation terms, gene sets curated by the Molecular Signature Database (MSigDB, v2.5 [43]) and top-enriched transcription factor sequence motifs; enrichment was assessed by one-sided Fisher’s exact test (using the entire set of unique genes included on the array as the population), with FDR estimated using the Story et al. method [44]. TMA immunohistochemical staining results were analysed statistically as previously described [4]. Associations between biomarker expression levels and CTNNB1 mutational status/β-catenin expression were examined using Fisher’s exact test, Kruskal–Wallis test or Spearman’s correlation coefficient, as appropriate. Time to recurrence was examined using Cox proportional hazards regression and the Kaplan–Meier (KM) method. Strata were compared using the log-rank test. p values were two-tailed and considered significant at α < 0.05. Analyses were conducted using SAS for Windows (release 9.1; SAS Institute, Cary, NC, USA). Cell culture-based assays were repeated at least twice and mean ± SD was calculated. Two-sample t-tests were used to assess the differences in DT cell proliferation, migration and invasion.

Results

Desmoid tumours exhibit unique gene expression signatures

To identify those genes uniquely expressed in sporadic DT, we compared the microarray profiling data for DT samples (n = 14) to those of corresponding patient normal tissue (n = 5) and a cohort of SFTs (n = 6) representing a different fibroblastic soft tissue tumour. Genes were included whose expression exhibited a statistically significant (p < 0.01) at least two-fold, same direction change (increase or decrease) in DTS compared to their expression in both other sample groups (FDR = 0, with 99% of permutations yielding < 20 gene probes). Collectively, 754 unique genes (1293 gene probes) comprised the ‘DT signature’, most being over-expressed (n = 636); 119 genes exhibited lower DT expression compared to normal tissues and SFTs (Figure 1A; see Supporting information, Table S2, for a comprehensive list of genes and relevant statistical information; small molecule inhibitors and/or other therapeutic strategies for a specific over-expressed target, if available, are also noted); gene COL1A2 was represented in both over- and under-expressed lists (by different gene probes, possibly representing alternate splice variants). We compared our DT gene expression profile to four previously published gene array-derived signatures [33–35,37]; while these studies utilize different array platforms, statistical approaches and tissue controls, overlap was found for 139 (22%) of the over-expressed and 24 (20%) of the down-regulated genes on the Table S2 list (see Supporting information), possibly reflecting the importance of these specific deregulations in DT.

Next, we conducted gene annotation enrichment analyses to identify over-representation of predefined gene sets as possible indicator(s) of gene classes/pathways operative in DT. Analysis by GO terms demonstrated enrichment of genes belonging to several extracellular matrix- and function-related classes (e.g. LRRC17, midkine, ADAM12, MMP2, MMP11 and MMP19, and multiple collagens), cell adhesion (e.g. cadherin 11, spondin 1 and peristin), WNT pathway (e.g. WNT5a, ROR1 and ROR2), and wound healing (fibromodulin, neuregulin 1 and syndecan 1; see Supporting information, Tables S3, S4). Enrichment analysis for MSigDB pathway-associated gene sets indicated over-representation of several functional gene groups in DT, including those associated with TGFβ and VEGF stimulation (see Supporting information, Tables S4, S6); these data may indicate a role for identified pathways in desmoidogenesis and unique patterns of locally invasive progression.
Figure 1. Genes over-expressed in desmoid tumours are enriched for LEF1/TCF-regulated targets. (A) Heat map representation of genes over-expressed (yellow) and under-expressed (blue) in desmoid tumours (DFTs) as compared to both normal tissue and solitary fibrous tumours (SFTs). Rows, gene transcripts; columns, profiled samples. The top 20 genes are listed, as ranked by fold change. (B) For genes high in desmoid tumours, the fraction with a given DNA motif in the promoter region was tabulated (black) and compared with the chance expected (grey). The top 20 most enriched DNA motifs are shown (ranked by p value; p < 0.02, one-sided Fisher’s exact test, FDR < 0.12).

Genes containing LEF1/TCF-binding promoter motifs are over-represented in DT

Next, the promoters of DT over-expressed genes were searched to identify the most enriched transcription factor (TF) binding sequence motifs; several predicted TF binding sites were shown to be significantly over-represented in the DT gene panel (Figure 1B; see also Supporting information, Table S7). Most pronounced (p < 1E-05, FDR < 0.001) was the enrichment of LEF1/TCF binding site sequence (CTTTGT)-containing gene promoters, aligning with the fact that β-catenin deregulation is a common DT molecular event. Together, 98 of the 636 genes over-expressed in DT (15.4%) were found to contain a TCF/LEF binding site in their promoter (see Supporting information, Table S8); these genes possibly represent direct targets of β-catenin in the unique DT intracellular milieu. Several of these genes were previously identified as regulated via β-catenin signalling (ie cyclin D1, VCAN, EFNB3, GJA1 and AXIN2 [45–49]), while the majority are potentially novel transcriptional targets.

Increased midkine expression correlates with DT recurrence

Next, we selected three up-regulated genes (ADAM12, midkine and MMP2), seeking to determine the expression levels of their encoded proteins in a large clinically annotated desmoid sample cohort. Selection of these proteins was made based on: (a) their potential cellular invasiveness role, a function critical for DT local aggressiveness; (b) the availability of highly specific antibodies for IHC analysis; and/or (c) their identification as being over-expressed in previous DT gene array studies. Immunohistochemical analysis revealed varying expression levels of these three proteins (Figure 2A). ADAM12 was expressed in 100% of evaluable DT samples; low expression levels were noted in 34% and high levels in 66% of samples. Midkine was expressed in 46% of DTs; 54% of samples exhibited no expression. Interestingly, two expression patterns were noted: 31% of midkine-expressing tumours exhibited diffuse staining throughout the specimen (Figure 2A), whereas in 69% patchy staining was noted (≤25% of DT cells exhibited positive staining) and most noticeably these midkine-positive cells were primarily localized in proximity to vascular structures (see Supporting information, Figure S2). This unique distribution pattern was noted only for midkine, while ADAM12 and MMP2 expression was relatively homogeneous and uniform within each individual sample. MMP2 was positive in 97% of samples, low expression in 40% of cases with moderate to high expression in 57%. No correlation between the expression levels of any of the three biomarkers and tumour site was identified. Similarly, no correlations between ADAM12 or midkine expression and CTNNB1 mutation status or β-catenin expression levels were identified. Interestingly, a significant and direct correlation (Fisher’s exact test, p = 0.0438) between MMP2 intensity and presence of CTNNB1 mutation was found. While no correlation was found between MMP2 and nuclear β-catenin expression, association between MMP2 and cytoplasmic β-catenin (Fisher’s exact test, p = 0.0283) was observed. The potential relevance of cytoplasmic β-catenin generally, and in DT specifically, is currently unknown. Taken together, these findings suggest that DT microarray-identified genes can be demonstrated as expressed at the protein level in human specimens.

We sought to determine whether any of the above protein expression levels correlated with DT patient
Desmoid tumour gene expression signature

Figure 2. The protein products of genes up-regulated in DT are expressed in human samples. (A) Immunohistochemical images demonstrating representative levels of evaluated markers in DT and haematoxylin and eosin (H&E) staining. All original images were captured at ×200 magnification; (B) Kaplan–Meier (KM) plots demonstrating shorter time to local recurrence for DTs diffusely (i.e. in the majority of DT cells) expressing midkine as compared to DTs negative for midkine and those exhibiting patchy staining (<25% of cells: positive cells in proximity to vascular structures) when the entire cohort is evaluated (a, log-rank \( p = 0.0036 \)) and for primary DTs (b, log-rank \( p = 0.0025 \)), but no statistically significant correlation between midkine expression and re-recurrence of already recurrent tumours (c, log-rank \( p = 0.4 \)). KM plots for ADAM12 and MMP2, comparing no expression (\( = 0 \)), low intensity (\( = 1 \); note that > 80% of DT cells/sample exhibited positive staining) and moderate–high intensity (\( = 2 \); note that > 80% of DT cells/sample exhibited positive staining), did not identify any statistical correlation with DT recurrence (graphs not shown).

local recurrence propensity, thereby potentially serving as prognostic biomarker(s). Time to recurrence was calculated from date of surgery at MDACC. While no correlation was identified for ADAM12 and MMP2, KM analysis demonstrated that high levels of midkine expression significantly correlate with increased local recurrence risk (log-rank \( p = 0.0036 \); Figure 2B). Median time to recurrence in the diffuse midkine-expressing tumour group was 3.1 years, whereas it had not been achieved in the midkine-negative/focal cohort. Interestingly, when patients with primary or recurrent DT at presentation to MDACC were separately analysed, no statistically significant correlation between time to re-recurrence and midkine expression levels were identifiable for recurrent disease patients (Figure 2B). These data possibly suggest that, with recurrence, other molecular factors contribute to subsequent DT local failure. In contrast, midkine expression remained a strong predictor of outcome for primary DTs (log-rank \( p = 0.0025 \); Figure 2B); the 3-year recurrence-free survival rate was 85.07% (95% CI 74.62–91.46%) among those with negative or focal midkine expression, compared to 61.54% (95% CI 30.83–81.85%) among those with enhanced and diffuse midkine expression. The hazard of local recurrence was increased among those with enhanced midkine expression (HR 3.4; 95% CI 1.5, 8.0; \( p = 0.004 \)), suggesting a potential role for midkine as a DT-associated prognosticator.

Midkine enhances DT cell migration and invasion

Lastly, we examined the potential effect of midkine on DT cell phenotype. The global lack of human DT cell lines is a major investigative limitation, so primary DT cell cultures derived from fresh surgical resections were utilized. To assure that DT cells were used rather than fibroblasts, only cell strains harbouring CTNNB1 mutations corresponding to the mutation of the original tumour were used (see Supporting information, Figure S1). Such cells (as expected according to DT
typically indolent clinical behaviour) grow slowly and senesce after several passages. However, they are useful for initial examination of DT molecular deregulations, and may more accurately reflect DT clinical behaviour compared to putative cell lines growing in culture for decades. WB analysis demonstrated increased midkine expression in a subset of DT cell strains compared to NHDf (Figure 3A), corresponding to the variable expression identified in human samples. Recombinant midkine enhanced the migration and invasion of DT cells lacking constitutive midkine expression ($p < 0.05$; Figure 3B). Midkine knockdown in cells over-expressing this cytokine resulted in decreased cell migration and invasion ($p < 0.05$; Figure 3C-D). No effect of midkine stimulation or knockdown on DT cell proliferation was found (data not shown). Taken together, these data support a midkine role in the DT pro-tumourigenic phenotype.

**Discussion**

Our study joins others in establishing DT gene expression signatures [33–35,37]; at least one-fifth of the genes identified here overlap with those described previously, suggesting the possible importance of these specific molecular aberrations demonstrated across experimental platforms in independent DT cohorts (genes in our signature not previously reported likely represent false negatives of the other studies). DT molecular deregulations associate extracellular matrix, cellular adhesion and wound-healing processes with DT cellular properties.

The high prevalence of DT β-catenin mutations implies that aberrant β-catenin signalling is an important contributor to DT pathophysiology, possibly serving as an ‘oncogenic addiction’. Mutations that affect the serine/threonine-rich region between Ser$^{29}$ and Lys$^{49}$ stabilize β-catenin, resulting in accumulation of non-phosphorylated β-catenin in the cytoplasm [8]. Non-phosphorylated β-catenin escapes recognition by β-TrCP, thereby avoiding degradation, and is translocated into the nucleus, where β-catenin forms a complex with members of the TCF/LEF family of transcription factors. Many of the data regarding cancer-related deregulated β-catenin signalling effects stem from colon cancer studies, where several potential over-expressed downstream transcriptional targets, such as CCND1, c-MYC and MMP-7, have been identified as contributing to tumorigenesis and progression [45,50,51]. However, DT-specific β-catenin-driven altered gene expression is not well characterized. It is likely that the effects of β-catenin stabilization vary between tumour types, as reflected in their significantly disparate biological behaviours. Some β-catenin target genes identified in colon cancer (eg PPAR-$\beta$, c-Myc and c-jun) have been found not to be up-regulated in desmoids [34,52]. Recently, several potential β-catenin-regulated genes, including IGFBP6 and WT1, were found to be repressed and induced, respectively, in desmoids compared to normal fibroblasts [34,53]. Our study further expands this small pool of DT-associated β-catenin-regulated genes, identifying 98 of the over-expressed genes as containing LEF/TCF binding sites in their promoters. These data might have significant therapeutic implications. Switching off crucial β-catenin signalling, on which DT cells depend, could potentially elicit significant anti-desmoid effects; of necessity, such targeting must spare normal cells. However, agents directly down-regulating β-catenin are not yet available, and may not be clinically applicable due to important physiological β-catenin roles [54]; therefore, identifying therapeutically accessible DT-associated β-catenin downstream targets may represent a more useful approach.

Our studies further confirmed varying levels of ADAM12, MMP2 and midkine protein expression in desmoid samples. Most importantly, midkine expression levels were found to directly correlate with DT propensity for local recurrence. Midkine is a heparin-binding growth factor commonly expressed during mid-gestation, when it plays a role in neural differentiation, maturation and survival [55]. Midkine expression is limited and restricted in adult human tissues under normal conditions, but is highly expressed in a diverse group of malignancies of both epithelial and mesenchymal origin [55]. Correlation between midkine expression and clinical outcome is seen in several malignancies [56–58]. Midkine induces broad pro-tumorigenic phenotypes, promoting cell growth and angiogenesis, enhancing tumour cell survival and migration [59–62] and, as shown here, enhanced DT cell migratory and invasive capacities. Commercial anti-MK therapies are not yet available; however, several modalities (i.e. anti-MK siRNA and anti-MK antibodies) have shown effects in preclinical studies [63] and may be relevant in DT treatment. Additional studies to confirm the utility of midkine as a clinical DT molecular prognosticator and potential therapeutic target appear warranted.

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Figure 3. Midkine enhances DT cell migration and invasion. (A) Subset of DT primary cultures exhibit increased midkine expression (WB; NHDFs). (B) Midkine stimulation (100 nM) resulted in a marked increase in the migration and invasion (modified Boyden chamber assays; 3 × 10^5 cells were used for these experiments) of DT cells (average migrating/invading cells per ×200 field are depicted graphically); hrMDK, (Human recombinant Midkine); NC, (negative control). (C) Midkine knockdown was achieved using anti-MK smartpool siRNA [WB confirmed decreased midkine expression as compared to non-targeting (NT) siRNA]. (D) Midkine knockdown resulted in decreased DT cell migration and invasion (modified Boyden chamber assays; 6 × 10^5 cells were used for these experiments; average migrating/invading cells per ×200 field are depicted graphically). NT, (non-target).
Author contributions

CC, MG and SB carried out experiments; CLW, CJC, RBW and AJL participated in data collection, analysis and interpretation; MVD and DL conceived experiments and analysed data. All authors were involved in writing the paper and had final approval of the submitted versions.

Abbreviations

APC, adenomatosis polyposis gene; DTs, desmoid tumours; FAP, familial adenomatosis polyposis; FDR, false discovery rate; GO, Gene Ontology; IHC, immunohistochemistry; IRB, institutional review board; KM, Kaplan–Meier; MSigDB, molecular signature database; NHDF, normal human dermal fibroblasts; SAS, Statistical Analysis Software; SFT, solitary fibrous tumour; TF, transcription factor; TMA, tissue microarray; WB, western blot.

References

Desmoid tumour gene expression signature


SUPPORTING INFORMATION ON THE INTERNET

The following supporting information may be found in the online version of this article:

Figure S1. CTNNB1 exon 3 sequencing of desmoid primary cultures and corresponding tissues.

Figure S2. Midekine expression in DT.

Table S1. Information relating to human tissues used for gene expression profiling.

Table S2. List of all up- and down-regulated DT genes.

Table S3. List of top enriched GO terms in DT gene profile.

Table S4. List of DT genes per GO classification.

Table S5. List of top enriched mSigDb pathways in DT gene profile.

Table S6. List of genes per mSigDb classification.

Table S7. List of top enriched TF sequence motifs (genes high in desmoid tumours).

Table S8. List of over-expressed DT genes containing LEF/TCF transcription factor motifs in their promoters.