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FAP-related desmoid tumors: a series of 44 patients evaluated in a cancer referral center

Chiara Colombo[1,2*], Wai Chin Foo[3*], David Whiting[1,2], Eric D. Young[1,2], Kristelle Lusby[1,2], Raphael E. Pollock[1,2], Alexander J. Lazar[2,3] and Dina Lev[2,4]

Departments of [1]Surgical Oncology, [3]Pathology, [4]Cancer Biology and the [2]Sarcoma Research Center at the University of Texas MD Anderson Cancer Center, Houston, Texas, USA

*These authors made equal contributions to the manuscript

Summary. Desmoid tumors (DTs), the commonest extra-intestinal manifestation of familial adenomatosis polyposis (FAP), are monoclonal neoplasms demonstrating fibroblastic - myofibroblastic differentiation; they are locally invasive without metastatic capacity. FAP-associated DT natural history knowledge is limited; we examined patient and tumor characteristics for a FAP-DT cohort and evaluated anti-DT therapy molecular target expression levels (immunohistochemical analyses, FAP-DT tissue microarray; TMA). Forty-four patients were classified as intra-abdominal (IA; n=26), abdominal wall (AW)/extra-abdominal (EA; n=12) or concomitant IA/AW (n=6) based on DT primary diagnosis location. Positive family histories were found in 62% of FAP versus 10% of DT patients. Surgery was the mainstay therapy for AW/EW patients, whereas IA DTs received surgery, chemotherapy, radiotherapy, tamoxifen, NSAIDs, and/or imatinib. Eight of 20 completely resected DTs in the IA and AW/EA groups recurred; 12 of 38 patients in these groups (33%) developed secondary lesions elsewhere. Two intestinal mesenteric DT patients died of disease, three from other cancers, 27 are alive with disease and 12 are alive without disease. All evaluable FAP-DT exhibited nuclear ß-catenin, 65% were positive for cyclin D1, and 66% expressed nuclear p53. No ERα expression was observed, but ERß was expressed in 72%. COX2 was expressed in all evaluable FAP-DTs. KIT was rarely found in DTs but both PDGFRs and their ligands were expressed. Comparing biomarker expression (IA vs. EA DTs), only nuclear ER-ß staining was significantly higher in EA lesions (p=0.0070); no other markers were site informative. Enhanced knowledge of FAP-DT molecular underpinnings will facilitate development of novel therapeutic strategies.

Key words: Gardner syndrome, Desmoid tumors, APC, ß-catenin, Targeted therapy

Offprint requests to: Dina Lev, MD, Department of Cancer Biology, M.D. Anderson Cancer Center, 8515 Fannin, Unit 1104, Houston TX 77054, USA. e-mail: dlev@mdanderson.org

Introduction

Familial adenomatous polyposis (FAP) is a genetically inherited autosomal dominant disorder defined by germline mutations in the *APC* tumor suppressor gene, manifesting in the formation of multiple polyps, mostly in the colon and rectum (Vasen, 2000). Colorectal cancer is an inevitable consequence when diagnosis and/or treatment are delayed, developing in 100% of these patients by the age of 40 (Half et al., 2009). To that end, prophylactic proctocolectomy is considered the treatment of choice (Vasen et al., 2008). In addition to colorectal polyps, a large array of extra-intestinal lesions can commonly develop in FAP patients (Bulow et al., 2006). First described by Gardner in 1951, and what has now been termed 'Gardner's syndrome', is a FAP presentation where colorectal adenomas occur in conjunction with desmoid tumors, osteomas and cutaneous lesions such as fibromas, lipomas, and epidermoid cysts (Gardner and Richard, 1953).

Desmoid tumors (DT) are soft tissue monoclonal neoplasms demonstrating fibroblastic to myofibroblastic differentiation; DT cell of origin of is not known, precursor lesions are not described, and there is a global lack of knowledge regarding risk factors associated with their etiology and development (Kotiligam et al., 2008). This entity, also termed "aggressive fibromatosis" (Sleijfer, 2009), is unique among tumors as being aggressively locally invasive while lacking metastatic capacity (Mendenhall et al., 2005). Approximately 1000

new desmoid diagnoses are made annually in the United States. More than 90% of DTs develop sporadically where they predominantly occur in young adults, especially females, and primarily involve the abdominal wall, trunk or extremities (including proximal loci at the pelvic and shoulder girdles), and less commonly the intestinal mesenteries (de Bree et al., 2009). FAP associated desmoids make up less than 10% of all DTs. However, ~10%-15% of FAP patients develop DTs, making this lesion the most common extra-intestinal manifestation of patients affected by this syndrome, and the second or third most common cause of FAP patients' death following colorectal carcinoma (Arvanitis et al., 1990; Bertario et al., 2001). A recent large meta-analysis identified a family history of DT, APC mutation 3' to codon 1399, previous abdominal surgery, and female gender as statistically significant risk factors for the development of DT in FAP patients (Nieuwenhuis et al., 2010a). Commonly developing early in FAP patients life (2^{nd}-3^{rd} decade), in contrast to sporadic DTs, most FAP-associated tumors occur within the small bowel mesentery and retroperitoneum, although extra-abdominal desmoids have also been reported (Clark et Phillips, 1996).

While surgery is considered by most to be the standard of sporadic DT care, it is often not a viable option for FAP-DTs, where encasement of the superior mesenteric vessels precludes complete excision. Furthermore, it has been suggested that surgical trauma itself can even enhance DT development in FAP patients (Gurbuz et al., 1994; Rodriguez-Bigas et al., 1994; Clark et al., 1999; Soravia et al., 2000). At the molecular level, it has been hypothesized that the second hit *APC* mutations resulting in DT development may be caused by misalignment of DNA strands during wound healing subsequent to abdominal surgery (Latchford et al., 2007). Given the above surgical limitations, additional approaches such as radiotherapy, systemic chemotherapy, hormonal blockade, non-steroidal anti-inflammatory agents, and most recently imatinib mesylate are being used with salutary effects (Knudsen and Bulow, 2001). However, due to FAP desmoid rarity there are no currently available randomized trials to help inform management of this tumor and treatment decisions are frequently made empirically.

We have previously evaluated the management and outcome of DT patients at our institution (Lev et al., 2007). However, these studies focused mainly on sporadic desmoids, which are more common, and no specific attention to the natural history of FAP DTs was given. Although exhibiting similar histopathological features, there are possible clinical and molecular differences between these two tumor cohorts warranting FAP-DT specific studies. Enhanced knowledge of FAP-DT will hopefully result in improved patient management and outcome. To that end, with the inherent limitations of a retrospective study, here we aimed to examine patient, tumor, management and outcome variables of a cohort of FAP-DTs evaluated at a single cancer center. Furthermore, we constructed a FAP-DT tissue microarray (TMA), albeit limited due to the small number of samples available (n=22), to enhance the feasibility of molecular-based studies for these rare tumors, and as an initial proof of principle evaluated the expression levels of several targets of currently used anti-DT therapies.

Materials and methods

Clinical database

This study was conducted under a University of Texas MD Anderson Cancer Center (UTMDACC) institutional review board approved protocol enabling retrospective analyses of patients with soft tissue tumors; a waiver of consent was granted for patient record review. FAP-DT patients seen at UTMDACC from 1/1993 through 6/2010 were identified through a search of our desmoid tumor database, institutional tumor registry, and pathology archives. FAP diagnosis was based on clinical parameters and endoscopic findings (Vasen et al., 2008); DT diagnosis was established based on imaging and histological features as determined by a dedicated soft tissue pathologist. Only patients with sufficient clinical information and follow up data were included, amounting to 44 patients. A database containing demographic, tumor, treatment, and outcome variables was constructed. Of note, patients were registered at UTMDACC at different junctures of their DT disease course, and data was recorded from time of initial diagnosis. Consequently, treatment regimens do not simply reflect management at a single institution. Few patients had information regarding APC mutational analysis recorded, and therefore this variable could not be included. The patient population was divided into three groups based on primary lesion site: 1) intra-abdominal/mesenteric (IA); 2) abdominal wall (AW)/extra-abdominal (EA), including tumors located in the abdominal or chest wall, trunk and head/neck; and 3) concomitant IA and AW lesions. No patients with primary extremity DT were identified in our cohort.

Tissue samples and tissue microarray (TMA)

Formalin fixed paraffin embedded (FFPE) specimens of FAP-DTs were retrieved from the Pathology Department archives. H+E stained slides corresponding to each sample were reviewed by two soft tissue pathologists (WCF and AJL) who confirmed the diagnosis and determined the availability of sufficient tumor tissue for study purposes. Twenty-two DT samples from 17 FAP patients were found suitable for study; 8 were IA lesions and 14 AW/EA. For five patients, two separate samples were included, representing lesions in different sites. Sixteen of the patients whose samples were included on the TMA were also included in our clinical database (two additional samples were from patients treated prior to 1993 and

thus excluded from clinical analysis). Four sporadic DTs and 11 specimens representing other fibroblastic tumors (two desmoplastic small round cell tumors [DSCR], four dermatofibrosarcoma protuberans [DFSP], and 5 solitary fibrous tumors [SFT]) were included as controls; the TMA was constructed as previously described (Lazar et al., 2008). In brief, using an automated TMA apparatus (ATA-27, Beecher Instruments), 0.6 mm punch samples (2 per case) were obtained from each donor block and formatted into a recipient block. Sections (4 μm) were cut and verified by H&E.

Immunohistochemistry

The polymeric biotin-free horseradish peroxide method on a Microsystems Bond Max stainer (Beecher Instruments, Bannockburn, IL) was used for ß-catenin, p53, cyclin D1, KIT, epidermal growth factor receptor (EGFR), platelet derived growth factor (PDGF)-α and PDGF-ß, platelet derived growth factor receptor (PDGFR)-α, PDGFR-ß, estrogen receptor (ER)-α and ER-ß, and COX2 immunohistochemistry. Staining was performed in the UTMDACC Clinical Immunohistochemistry Core Facility. Appropriate positive and negative controls were used throughout. Immunoreactivity was evaluated and scored for staining intensity. Cytoplasmic intensity was graded as none (=0), weak (=1), moderate (=2), and strong (=3); and nuclear intensity was graded as low or high. Of note, p53, cyclin D1, and KIT immunostains were only scored as positive or negative.

Results

Intra-abdominal (IA) desmoid tumors

Intra-abdominal location was the initial site of FAP-DT in 26 patients, representing 59% of our total FAP-DT patient cohort (Table 1). Median age at presentation was 32 years (range, 15-51) and a slight male gender predilection was noted (15 males vs. 11 females). Fifteen (62%) patients had a family history of FAP, three (12%) had a family history of DT, and all patients exhibited multiple colonic polyps prior to DT diagnosis. Sixteen patients (62%) underwent prophylactic colectomy prior to DT diagnosis within a median time of 55.8 months (range 18-178), while in ten patients (38%) DT developed within an unoperated abdomen. Intestinal mesentery was the most frequent intra-abdominal DT site (21; 81%), retroperitoneal tumors were found in four patients (15%), and one patient exhibited multiple lesions in both the mesentery and retroperitoneum. Size was evaluable in 12 cases and ranged from 3-26 cm (median: 10.5 cm; range, 3-26). Treatments were highly variable. Surgery was attempted as the first modality in nine patients (35%). In seven of these cases complete resection was achieved and no additional treatment was administered. In two patients only partial resection was attainable: one patient was further treated with tamoxifen, and the second patient received intra-operative radiotherapy and later was given chemotherapy and imatinib. One patient underwent complete surgical resection as a second modality after tamoxifen treatment, resulting in stabilization of disease. Tamoxifen was utilized as a first line single agent therapy in eight patients (30%) and in combination with other therapies (chemotherapy, sulindac, radiofrequency ablation and ethanol injection, partial surgical resection) in four patients. One patient received tamoxifen as a second line treatment after chemotherapy. Responses to tamoxifen, as can be ascertained from cases where used as a single agent (n=9), included partial response (n=1; 11%), stabilization of disease (n= 3; 33%), and disease progression (n=5; 56%). NSAIDs (sulindac) were administered to only one patient, in combination with tamoxifen. Two patients received imatinib as second and third line therapies and experienced disease progression and stabilization, respectively.

A variety of chemotherapy regimens were administered as first line treatment to five patients (19%) and as a second or third line therapy to six others (23%). Five patients (55%) receiving chemotherapy alone experienced partial response; four other patients (45%) sustained stabilization of disease. One patient who received chemotherapy in combination with tamoxifen exhibited a partial response, and complete response was observed in one patient in whom chemotherapy was combined with radiotherapy. Only four patients received radiotherapy; in two of these cases radiation was administered intraoperatively. Two patients were simply observed without additional therapy.

During a median follow up of 6.9 years (range 0.2-27), four of eight (50%) completely resected DTs exhibited local recurrence. Interestingly, eight patients (30%) developed a second lesion outside the abdominal cavity (abdominal wall-5, chest wall-1, back-1, and breast-1). Median survival was not reached. Two patients (8%) died of DT and two patients (8%) died of other FAP complications, namely colon and duodenal carcinomas. Sixteen (62%) are alive with DT and six (23%) are free of DT. This last patient cohort includes three patients who underwent complete surgical excision with no evidence of recurrence, two that exhibited complete response to radiation therapy with no later failure, and one patient who experienced spontaneous DT regression.

Abdominal wall (AW) and other extra-abdominal (EA) desmoid tumors

Twelve (27%) of FAP patients (8 females and 4 males), with a median age of 24 years (range, 13-69), exhibited an extra-abdominal DT at time of initial diagnosis (Table 2). Six patients had a family history of FAP, three did not, and for three individuals this information was not available. Only one patient was known to have a family history of DT. The vast majority of patients (66%) exhibited a lesion in the abdominal

wall; in five cases (63%) the tumor developed within a previous surgical scar. Other locations included chest wall, breast, back, and infra-temporal fossa. All patients were treated with surgery as the initial modality. Radiotherapy ± chemotherapy as an adjuvant was administered in two cases. During a median follow up of 11.35 years (range, 1.1-50.2), four patients (33%) experienced local disease recurrence. Interestingly, six patients (50%) developed a second lesion in the mesentery (n=4) or extra-abdominal location (n=2). Six

Table 1. Intra-abdominal DTs.

Case no. [1]	Sex, age at DT diagnosis (years)[2]	Family history of FAP/DT[3]	Surgery prior DT/time from surgery to DT (mo.)[4]	Size (cm)[5]	Treatment/response for patients treated medically[6]	Recurrence if surgery/ TTR (mo.)[7]	Site of second lesion[8]	FU (years)	Status at FU[9]
#1	M/41	No/No	Yes/178.8	Unk	Observation/SD	-	AW	6.6	DID*
#2*	M/33	Unk	Yes/58.8	Unk	Complete resection	No	AW	21.1	AWD
#3*	M/34	Unk	No/-	Unk	Tamoxifen/SD Complete resection + IORT	No	-	0.7	ANED
#4	M/33	Yes/No	No/-	Unk	Complete resection	Yes/10	-	16.1	DID**
#5	F/19	Yes/No	No/-	11	Tamoxifen/PD Adria + DTIC/PR Etoposid/SD	-	-	0.8	AWD
#6	F/20	No/No	Yes/56.4	Unk	Partial resection Tamoxifen/PD	-	-	7.2	ANED
#7	M/24	Yes/Yes	Yes/24	Unk	Tamoxifen/PD MTX + Vin/PR	-	-	11.0	AWD
#8	F/28	Yes/No	Yes/55.2	7	Adria + DTIC/PR Tamoxifen/SD	-	-	11.5	AWD
#9	M/30	No/No	No/-	25	Complete resection	Yes/19	-	21.8	AWD
#10	F/30	No/No	Yes/70.8	Unk	Complete resection	No	-	13.2	ANED
#11*	M/36	No/No	Yes/24	Unk	Adria + DTIC/SD	-	-	5.5	AWD
#12	F/51	Yes/No	Yes/18	Unk	Tamoxifen /PD Imatinib/PD Adria + DTIC/SD	-	Breast	8.0	AWD
#13*	M/23	Yes/No	No/-	Unk	Partial resection + IORT Vin+ Adria/PR Imatinib/PR	-	Back	12.6	DOD
#14	F/23	Yes/Yes	Yes/108	Unk	Adria + Actinomicin + Vin + Tamoxifen/SD	-	-	15.9	AWD
#15	F/35	Yes/Yes	Yes/116.4	3	Sulindac + Tamoxifen/SD	-	-	0.8	AWD
#16	M/21	Yes/No	No/-	4.4	Tamoxifen/PR	-	-	10.7	AWD
#17	M/15	Yes/No	No/-	Unk	Complete resection	Yes/17	AW	6.5	DOD
#18	F/32	Yes/No	Yes/31.2	6	RFA + Ethanol injection + Tamoxifen/SD RT/CR	-	-	8.6	ANED
#19*	M/29	No/No	Yes/99.6	20	Tamoxifen/SD	-	-	0.2	AWD
#20	F/15	No/No	No/-	Unk	Complete resection	No	-	1.6	ANED
#21	M/36	Yes/No	No/-	14	Observation	-	-	1.4	AWD
#22*	M/36	No/No	No/-	4	Tamoxifen/PD Adria + DTIC/PR	-	AW	1.3	AWD
#23*	F/39	Yes/No	Yes/63.6	14	Tamoxifen/SD	-	-	1.6	AWD
#24	M/49	Yes/No	Yes/51.6	26	Complete resection	Yes/37	AW	3.8	AWD
#25	M/44	Yes/No	Yes/30	10	MTX + Vin/SD	-	-	1.1	AWD
#26*	F/36	Yes/No	Yes/36	Unk	Adria + DTIC + RT/CR	-	CW	27	ANED

[1] *included in TMA; [2] M: male; F: female; [3] Unk, unknown; [4] mo.: months; [5] Unk: unknown: the size of intra-abdominal DT is often difficult to assess due to the infiltrative growth of pattern in the mesentery or in the retroperitoneum; [6]Adria: adriamycin; MTX: methotrexate; DTIC: dacarbazine; Vin: vinorelbine; RT: radiotherapy; RFA: radiofrequency ablation; CR: complete response; PD: progressive disease; SD: stable disease; PR: partial response ; [7] TTR: time to recurrence; mo.: months; [8]AW: abdominal wall; CW: chest wall; [9] AWD: alive with disease; ANED: alive no evidence of disease; DOD: deceased for disease; DID*: deceased for intercurrent disease (duodenal carcinoma); DID**: deceased for intercurrent disease (rectal cancer).

patients are alive with no evidence of disease, including one patient who developed a second lesion in the mesentery that was amenable to complete surgical resection. Six patients are alive with disease.

Concomitant IA and AW desmoid tumors

Six patients (14%; three male and three females; median age of 31 years) presented with concomitant intra-abdominal and abdominal wall DT (Table 3). Three of these patients had a family history of FAP and none had a known family history of DT. All patients underwent a prophylactic surgical procedure prior to DT diagnosis. The intra-abdominal lesions were located in the retroperitoneum in three patients (50%) and in the mesentery in the other three individuals (50%). Complete resection of the abdominal wall lesion was successful in two cases; a third patient underwent partial resection of abdominal wall and intra-abdominal DTs. A wide variety of therapeutic combinations, mainly conventional chemotherapeutic agents, as depicted in Table 3, have been used to treat these patients. At a median follow-up of 2.3 years (range, 0.2-17.9), none of the patients have developed a DT in an additional site;

Table 2. Abdominal wall and other extra-abdominal DTs.

Case no.[1]	Sex, age at DT diagnosis[2]	Family history of FAP/DT[3]	Surgery prior DT/time from surgery to DT (mo.)[4]	Site/Size (cm)[5]	Treatment for primary[6]	Recurrence if surgery/ TTR (mo.)[7]	Site of second lesion[8]	FU (years)	Status at FU[9]
#1*	F/30	Yes/No	No/-	CW/10	Complete resection	No	Mesentery	26.5	ANED
#2*	F/22	No/No	No/-	AW/6	Complete resection	Yes/34	Mesentery	26.2	AWD
#3	M/15	Unk	No/-	Infra-temporal fossa/3	Complete resection + CT + RT	No	Mandible	12.2	AWD
#4*	F/22	Yes/No	Yes/20.4	AW/4	Complete resection	No	-	13.1	ANED
#5*	M/69	Unk	Yes/133.2	AW/5	Complete resection	Yes/36	-	4.7	ANED
#6	F/38	Yes/No	Yes/32.4	AW/10	Complete resection	No	-	7.7	ANED
#7*	F/22	Yes/No	No/-	AW/15	Complete resection	No	-	1.1	ANED
#8	F/28	Yes/No	Yes/168	AW/8	Complete resection	Yes/12	-	10.5	AWD
#9	M/41	No/No	No/-	Back/Unk	Complete resection + RT	No	Mesentery	4.7	AWD
#10*	F/26	No/No	Yes/21.6	AW/2.5	Complete resection	No	-	1.3	ANED
#11	F/17	Unk	No/-	Breast/4	Complete resection	Yes/12	AW	15.8	AWD
#12	M/13	Yes/Yes	No/-	AW/9	Complete resection	No	Mesentery	50.2	AWD

[1] *included in TMA; [2] M: male; F: female; [3] Unk: unknown; [4] mo.: months; [5] Unk: unknown; CW: chest wall; AW: abdominal wall; [6] RT: radiotherapy; CT: chemotherapy; [7] TTR: time to recurrence; mo.: months; NA: not applicable; [8] AW: abdominal wall; [9] AWD: alive with disease; ANED: alive no evidence of disease.

Table 3. Synchronous intra-abdominal and abdominal wall DTs.

Case no.[1]	Sex, age at DT diagnosis[2]	Family history of FAP/DT	Surgery prior DT/time from surgery to DT (mo.)[3]	Size-IA/ AW (cm)[4]	Treatment/response for patients treated medically[5]	FU (years)	Status at FU[6]
#1	M/34	Yes/No	Yes/19.2	Unk*/3	Perfinidone/PD Ifosfamide + Etoposide/PD Mitomycine + Adria + Cisplatin/SD Adria + DTIC/PR	10.6	AWD
#2	M/31	No/No	Yes/60	Unk*/Unk	Observation	0.2	AWD
#3*	F/36	Yes/No	Yes/27.6	13/4	Adria + DTIC/PR	1.3	AWD
#4	M/17	No/No	Yes/24	Unk*/Unk	Partial resection (AW and IA lesions) Tamoxifen/SD	17.9	DID*
#5*	F/31	No/No	Yes/61.2	6/4.6	Complete resection (AW lesion)	0.4	AWD
#6	F/22	Yes/No	Yes/10.8	Unk*/5	Complete resection (AW lesion) Tamoxifen + Sulindac/PD MTX + Vinelbine/PR Adria + DTIC/SD	3.3	AWD

[1]: *included in TMA; [2]: M: male; F: female; [3]: mo.: months; [4]: IA: intra-abdominal; AW: abdominal wall; Unk*: unknown: the size of intra-abdominal desmoid tumor is often difficult to assess due to the infiltrative growth of pattern in the mesentery or in retroperitoneum ; [5] Adria: adriamycin, MTX, methotrexate; DTIC: dacarbazine; PD: progressive disease; SD: stable disease; PR: partial response; unk: unknown; [6] AWD: alive with disease; ANED: alive not evidence of disease; DID*: deceased for intercurrent disease (rectal cancer).

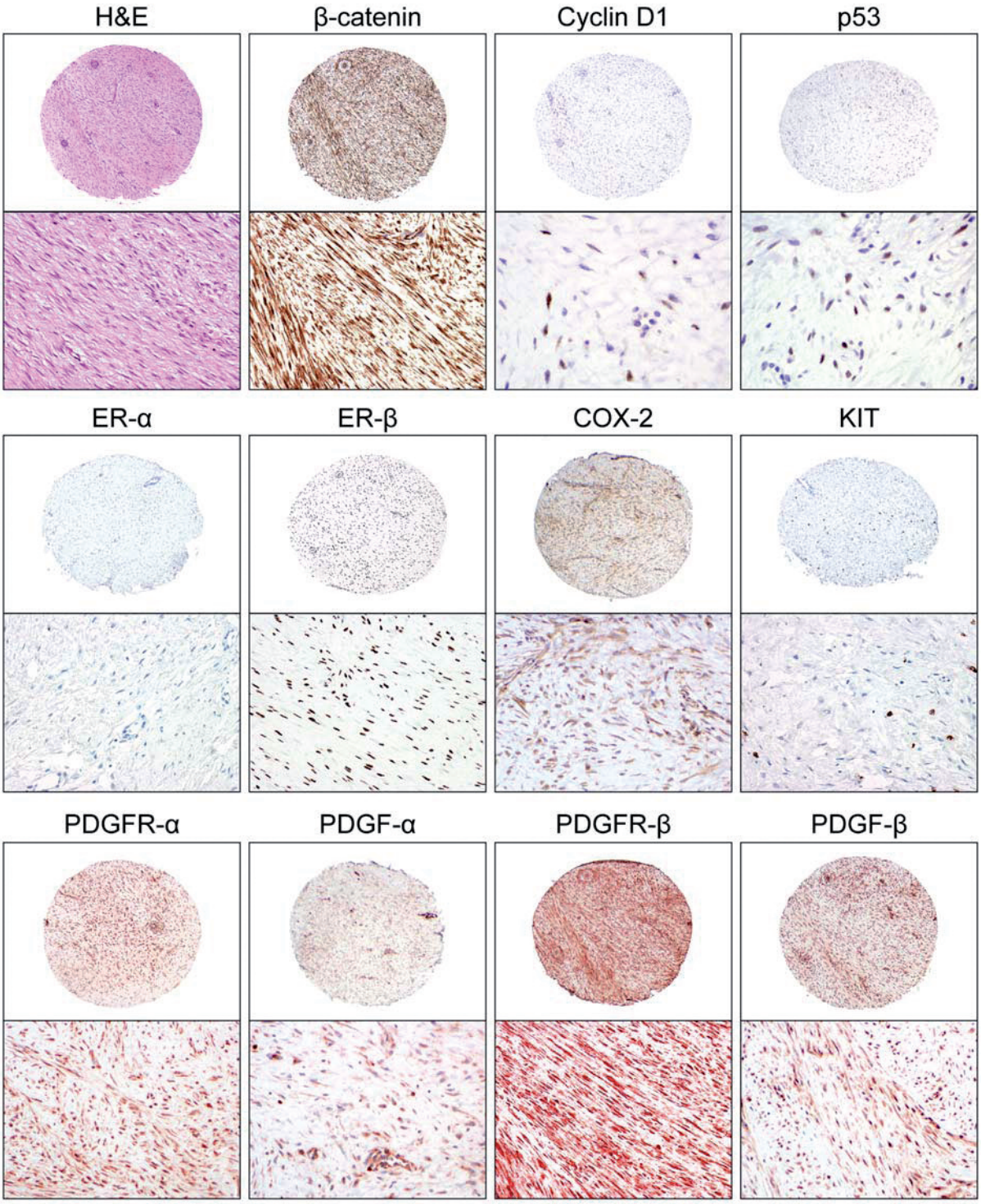

Fig. 1. Representative FAP desmoid tumor samples with H&E staining and immunohistochemistry. Tissue microarray spots are 40x original magnification and the enlarged fields are 200x, except for the high power fields of cyclin D1 and p53, which are at 400x to enhance the demonstration of nuclear staining. The TMA was scored by an expert sarcoma pathologist at x 400 magnification. For each immunohistochemical stain, the samples were evaluated for percent immunoreactivity of the neoplastic cells. In addition, for ß-catenin, ER-ß, COX-2, PDGFR-α, PDGF-α, PDGFR-ß, and PDGF-ß immunohistochemical stains, the samples were also evaluated for intensity of staining in the neoplastic cells. The figure reflects the predominant results for each immunohistochemical stain (see Table 4 for scoring results) as per the following; nuclear ß-catenin expression was observed in all DTs; cyclin D1 was considered positive when identified in >5% of cells although expression level was relatively low; similarly, nuclear p53 expression in >5% of the cells was considered positive; ER-α expression was negative in all cases; high ER-ß expression was noted in 12 samples; COX-2 was positive in all evaluable cases; KIT was negative in all but one evaluable core (in this case weak immunoreactivity was noted). The picture here represents the typical negative staining, immunoreactive cells are mast cells while neoplastic spindle cells are negative; high PDGFR-α expression level was observed in 12 cases; PDGF-α positivity was noted in nine samples, in all but one low expression was observed; PDGFR-ß was strongly expressed in all evaluable samples; similarly, PDGF-ß positivity was noted in all DT samples, although in the majority low expression levels were noted.

Table 4. FAP-DT biomarkers expression.

	Positive	Low intensity	High intensity
ß-catenin	20/20	9/20	11/20
Cyclin D1 (≥5%)	11/17	n/a	n/a
P53 (≥5%)	12/18	n/a	n/a
ER-α	0/14	n/a	n/a
ER-ß	13/18	1/13	12/13
COX-2	13/13	4/13	9/13
KIT	1/19	n/a	n/a
PDGFR-A	13/16	1/13	12/13
PDGF-α	9/13	8/9	1/9
PDGFR-B	17/17	0/17	17/17
PDGF-ß	17/17	13/17	4/17

one patient died of rectal cancer and all five surviving patients are alive with DT and are under surveillance.

FAP DT biomarker expression

APC loss of function mutations result in the activation of ß-catenin; as expected all evaluable FAP DTs exhibited positive ß-catenin nuclear staining (55% high and 45% low intensity; Table 4, Fig 1). High nuclear ß-catenin expression was similarly found in sporadic DTs; with the exception of one SFT all other fibroblastic tumors exhibited low or no ß-catenin expression. Positive nuclear cyclin D1 and P53 staining (in ≥5% of cells) were observed in 65% and 67% of FAP-DT samples, respectively. Only four evaluable samples originated from patients who later received chemotherapy (i.e.; samples were chemo-naive); one patient exhibited complete response with highly positive (>80% of cells) p53 tissue sample staining; two patients experienced a partial response and one had stabilization of disease; for these latter three patients p53 staining was negative; however, this is too small a cohort to enable meaningful conclusions.

Next we evaluated the expression of molecular targets of commonly utilized anti-DT therapies. No ERα expression could be identified, but ERß was expressed in 72% of cases. There were only four evaluable samples from patients who later received tamoxifen; three exhibited disease progression, and ERß was highly expressed in one and negative in two individuals. One patient experienced disease stabilization despite negative ERß staining. Again, this small sample number does not allow for definitive conclusions. COX2 was expressed in all evaluable FAP-DTs. However, none of the samples were retrieved from patients who were later treated with sulindac. Lastly, imatinib targets were evaluated; KIT was not commonly found in DTs but both PDGFRs and their ligands were expressed to varying levels. However, none of the evaluated samples were derived from patients treated with imatinib. Comparing biomarker expression in IA vs. EA DTs, only nuclear ER-ß staining was statistically significantly higher in the EA lesions ($p=0.0070$); all other markers did not differentiate between tumor sites.

Discussion

This study joins several previous reports describing the management and outcome of FAP-associated DTs (Sinha et al., 2010). Based on these initial studies we opted to divide our patient cohort into three distinct groups as per the site of the original lesion. Confirming and expanding current knowledge, we found that FAP DTs occur most commonly in the third and fourth decade of life, whereas EA lesions generally develop earlier. In contrast to sporadic desmoids, no gender predilection was observed. A family history of DT has recently been found to be the most significant risk factor for the eventual development of these tumors in FAP patients (Sinha et al., 2010). However, the vast majority of the patients (90%) in the current series (for whom this information was attainable) did not exhibit such family history. Surgical trauma has been suggested as a factor contributing to the development of DTs in FAP patients (Sleijfer, 2009). Consequently, several investigators have proposed delaying prophylactic surgery in patients at high risk for desmoid development (Friedl et al., 2001; Durno et al., 2007). In the present series about 40% of all DTs and 33% of IA DTs developed prior to surgical intervention, suggesting that other factors drive, or at least contribute to, disease inception.

As anticipated, the majority of FAP DTs in our series initially arose intra-abdominally, either as the sole location (59%) or in conjunction with an abdominal wall lesion (14%). This is a unique feature of FAP-associated DTs and differentiates them from sporadic DTs which more commonly develop extra-abdominally (Nieuwenhuis et al., 2010). While extra-abdominal DTs do develop in FAP patients, these are most commonly associated with the abdominal wall (Nieuwenhuis et al., 2010a); in our series only four patients (9%) presented with DTs in other locations (trunk, head and neck), and three additional patients (7.5%) developed such tumors as a second lesion later in the course of their disease. Interestingly, none of the FAP patients of this series had extremity tumors, a relatively common location of sporadic DTs. Our series also further highlights the avid multifocality of FAP-DTs; more than 45% of our patients exhibited synchronous or metachronous IA and EA (most commonly AW) lesions, possibly reflecting the systemic nature of FAP-associated DTs governed by an underlying germline mutation. Taken together, the above insights perhaps suggest different cells of origin and/or molecular aberrations driving FAP-associated versus sporadic DTs, further supporting the continued intensive laboratory- based investigations of these rare tumors.

Based on previous data and our current findings, several comments can be made regarding the management of FAP-associated DTs (Nieuwenhuis et al., 2010). Firstly, IA and EA DTs are usually treated differently. Surgery is the mainstay of therapy for DTs

located in the abdominal wall and other extra-abdominal sites similar to the commonly utilized approach for sporadic DTs. In general, operative morbidity is low and outcome is favorable (Heinskanen and Järvinen, 1996). Of note, while our series is too small to be conclusive, a 33% local recurrence rate was observed for AW/EA DTs after complete surgical resection; two of the 12 resected patients received adjuvant radiotherapy and exhibited no signs of recurrence after 12.2 year and 4.7 year follow up periods. We and others have previously found that combining radiotherapy with surgery might decrease sporadic DT local recurrence. Such an approach should possibly be investigated for the treatment of EA FAP-associated DTs.

Secondly, surgery is less commonly utilized for the treatment of IA FAP DTs; in our series complete resection was possible for only 22% (7/32) of patients initially diagnosed with IA DT. As demonstrated in Tables 1 and 3, a multitude of diverse agents, combinations, and treatment regimens are generally utilized. Patients included in our study were evaluated at UTMDACC at different time points during their course of disease; consequently, treatment for the initial lesion was administered in multiple institutions by different care-givers over the time span of many years and thus reflects personal preferences rather than a unified protocol-based therapeutic approach. Within the limitations of a retrospective based study, it generally appears that chemotherapy (different agents and regimens although mainly adriamycin-based) resulted in the best objective responses as measured by serial imaging. Several studies have reported encouraging effects of adriamycin-dacarbazine-based regimens for the treatment of IA DTs. Of potential importance, however, is the observation that treatment effects of anti-estrogens and NSAIDs on sporadic DTs might not be immediately apparent; in some patients objective responses may only be apparent after several months of continuous therapy. Recently imatinib has joined the repertoire of agents utilized for the treatment of DTs (Chugh et al., 2010; Penel et al., 2011). Only two patients in the current study received imatinib as part of the treatment of their initial tumor, so conclusions regarding the effects of this compound cannot be made.

Prospective, multi-institutional and even multi-national studies to enable sufficient patient accrual are critically needed to determine the best anti-DT therapeutic approach. Moreover, in our current era of "personalized" medicine replete with a growing number of novel therapeutic agents available, insights into FAP-DT molecular deregulations will possibly enhance therapy selection. As an initial "proof of principle", we have evaluated the expression of several potential molecular targets for drugs currently used in DT management and have been able to demonstrate their differential expression. Additional clinically-oriented studies and trials are needed to enable the analysis of these markers for their possible utility as therapeutic response biomarkers. Tools such as the TMA described here are extremely useful for the study of FAP-DT related molecular aberrations and should be further developed.

Thirdly, due to the slow-growing nature of DTs and evidence suggesting that some of these lesions can spontaneously stabilize and even regress, a "wait and see" policy has recently been advocated for sporadic tumors (Fiore et al., 2009; Bonvalot et al., 2008). Three patients with IA DTs in our series were only observed after initial diagnosis, demonstrating disease stability. Furthermore, most of the patients that were alive with disease at the end of the study period had already undergone several types of therapy to obliterate their existing disease. One patient in our study did exhibit spontaneous disease regression. Taken together, and until such time as when highly effective therapeutic modalities are readily available, these findings support an initial observation period for asymptomatic patients harboring IA FAP-DTs to enable determination of their tumor growth dynamics. This approach should be considered as a viable option in the multidisciplinary treatment decision-making setting. If such a management option is selected, patients should be followed up carefully so as to not miss a potentially narrow window of therapeutic opportunity on disease progression.

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