Identification of t(17;22)(q22;q13) (COL1A1/PDGFB) in dermatofibrosarcoma protuberans by FISH in paraffin-embedded tissue microarrays. Clinical and histopathological correlation in 40 cases

Sonia Segura[1], Rocío Salgado[2], Agustí Toll[1], Blanca Espinet[2], Gemma Martín[1], Mireia Yébenes[3], Jesús Luelmo[3], Empar Sàez[4], Carlos Barranco[2], Pablo Umbert[5], Ramon M Pujol[1]

Department of Dermatology[1] and Molecular Cytogenetics Laboratory, Pathology Department[2], Hospital del Mar, IMAS. Barcelona.
Department of Dermatology[3] and Pathology[4], Hospital Parc Taulí, Sabadell. Department of Dermatology[5], Hospital Sagrat Cor, Barcelona.

Introduction

Dermatofibrosarcoma protuberans (DFSP) is characterized by the presence of the translocation t(17;22)(q22;q13)[1]. The detection of the fusion gene COL1A1/PDGFB by RT-PCR is difficult due to the presence of multiple breakpoints of the gene COL1A1 and the problem of extracting RNA from paraffin-embedded tissue[2]. Fluorescence in situ hybridation (FISH) appears as a more effective technique to detect COL1A1/PDGFB rearrangement in DFSP that may have diagnostic and therapeutic interest in clinical practice[3,4]. We aimed to analyze the presence of the translocation t(17;22)(q22;q13) by FISH in paraffin-embedded tissue microarrays (TMA) in a series of 40 DFSP, and correlate with clinical and histopathological features.

Methods

We constructed two TMA with 40 DFSP and 20 dermatofibromas (DF). FISH was performed using a dual fusion COL1A1/PDGFB non-commercial probe. BAC clones mapping the COL1A1 and PDGFB loci on chromosomes 17 and 22, respectively, were obtained from Children's Hospital Oakland Research Institute. DNA was labeled using nick translation kit, with the Spectrum Red for COL1A1 and Spectrum Green for PDFGB loci. The presence and number of fusion signals were assessed in 100 nuclei per case. A fusion-signal pattern was considered positive for the COL1A1-PDFGB rearrangement if the distance between the red and the green signals was lower than the diameter of any one signal.

Results

Clinical and histopathological features of the 40 cases are described in **Table 1** and **Table 2**. FISH technique on TMA was successful in 33 of 40 (83%) DFSP and in 16 (80%) of 20 DF. A fusion-signal pattern was detected in 29 of 33 DFSP (88%) whereas was absent in all the DF. Six of the positive cases showed a simple rearrangement consisting in one or two fusion signals (**Figure 1e**). The remaining 23 cases presented multiple copies of the translocation (three or more). Four of these cases showed six or more copies in a proportion of cells within the tumor (**Figure 1f**). Tumor size positively correlated with the presence of a higher proportion of cells presenting the fusion signal (p= 0,039) and with the presence of multiple copies of the translocation (p=0.014). In five cases of DFSP with fibrosarcomatous areas (DFSP-FS) (Figure 1d) the number of positive cells was significantly higher and exhibited a higher number of copies of the COL1A1-PDGFB gene (**Figure 1f**). Within negative cases one tumor exhibited overlapping features with DF, whereas other two were highly undifferentiated and raised the diagnosis of other fibrohistiocytic malignant soft tissue tumors.

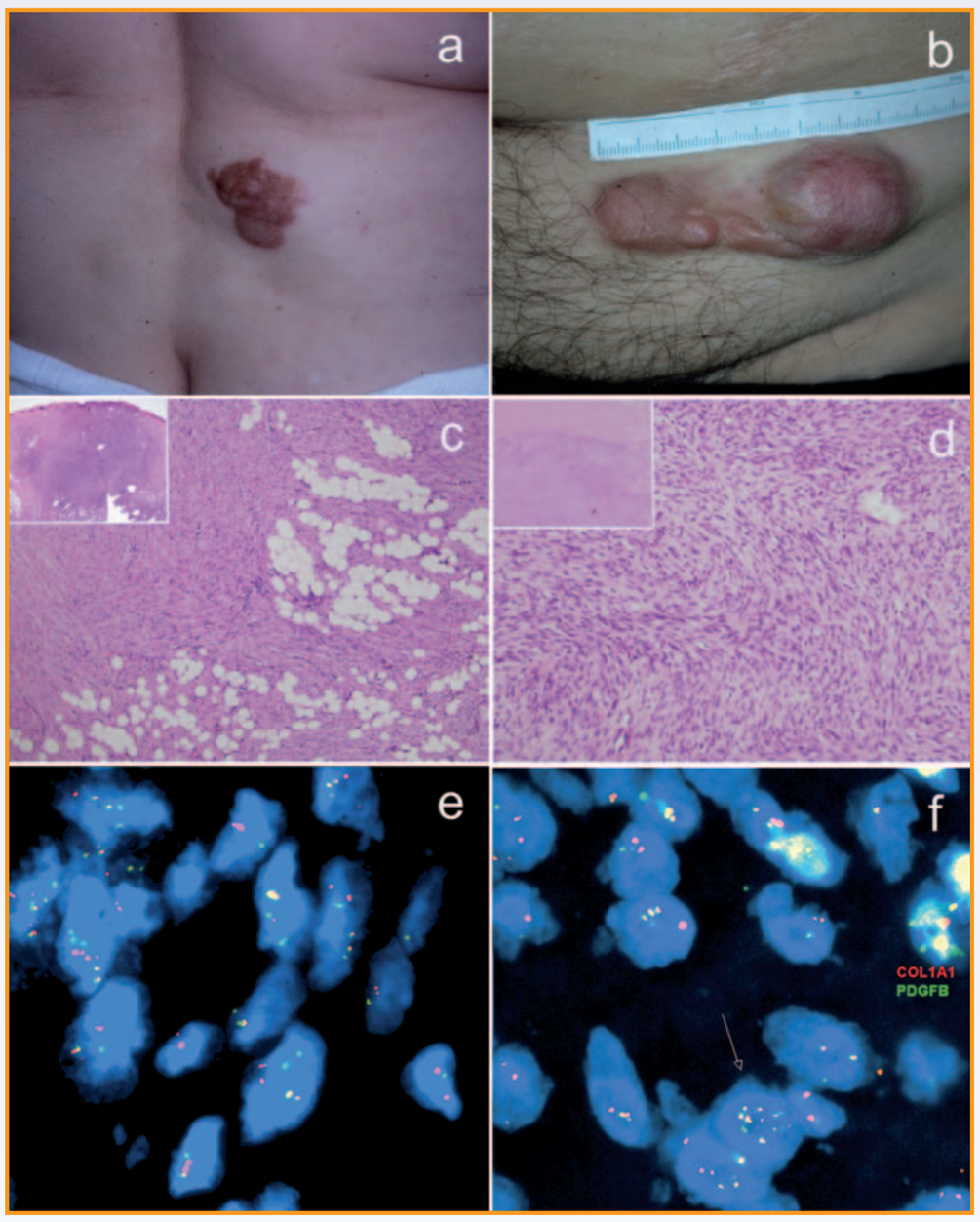

Figure 1. **a:** Clinical aspect of a DFSP on the back showing an indurate plaque; **b:** Typical morphology of DFSP as a mutilobulated plaque on the abdomen; **c:** H&E, original magnification 100x (inset 20x), classical DFSP showing a proliferation of monomorphous spindle-shaped cells with characteristic honeycomb-like infiltration of the subcutaneous tissue; **d:** H&E, original magnification 200x (inset 40x), DFSP-FS exhibiting highly dense areas arranged in fascicles with an herringbone pattern and a higher degree of atypia; **e:** FISH image showing positive cells with two fusion signals in a case of classical DFSP; **f:** FISH image showing multiple copies of the fusion gene COL1A1-PDGFB in a case of DFSP-FS.

Table 1. Clinical features of 40 DFSP

Women/Men **Mean age (range)** **Mean time before dx (range)** **Mean tumor size (range)**	24/16 43y (15-87) 12 mo (3 mo-14 y) 3,4 cm (1-9,5 cm)
Morphology	Nodule 13 cases Multinodular plaque 6 cases Plaque 4 cases Exophytic tumor 4 cases NA 13 cases
Location	Trunk 16 cases Upper limbs 9 cases Lower limbs 9 cases Head 5 cases NA 1 case
Treatment	Primary excision 9 cases Reexcision 27 cases Mohs surgery 4 cases Adjuvant radiotherapy 1 case
Recurrences	3 cases

Table 2. Main histological features of 40 DFSP

Histological subtype	Classic DFSP 27 cases DFSP-FS 5 cases DF overlapping features 4 cases Undifferentiated 2 cases Mixoid 1 case DFSP with giant cells 1 case
Epidermal features	Hiperplasia 15 cases Atrofia 7 cases Normal 12 cases; NV 6 cases
Cellular density	Moderate to intense 34 cases Mild 6 cases
Grade atypia	Low to moderate 31 cases High 9 cases
Mitosis rate HPF (range)	2 (0-10)
Pattern of subcutaneous infiltration	Honeycomb 19 cases Diffuse 10 cases Multilayer 2 cases Others 4 cases NV 5 cases
CD34	Positive 37 Negative 3
Other dermal features	Mild inflammatory infiltrate 7 cases Foci of hemorrhage 8 cases Pseudovascular structures 7 cases

Discussion

The fusion gene COL1A1-PDGFB can be detected by FISH in paraffin-embedded TMA and it is present in most of DFSP[3]. The detection of this translocation may be of interest in cases histologically challenging, in order to confirm or exclude DPSP diagnosis. DFSP-FS cases were associated with the presence of higher number of copies of COL1A1-PDGFB fusion gene. However, the implication of copy gains in fibrosarcomatous transformation of DFSP has to be confirmed in further studies[4].

References

1. Sirvent N, *et al*. Genetics of Dermatofibrosarcoma protuberans family of tumors: from ring chromosomes to tyrosine kinase inhibitor treatment. Genes Chromosomes Cancer. 2003; 37:1–19.
2. Llombart B, *et al*. Dermatofibrosarcoma protuberans: clinical, pathological, and genetic (COL1A1-PDGFB) study with therapeutic implications. Histopathology. 2009; 54: 860-72.
3. Patel KU, *et al*. Dermatofibrosarcoma protuberans COL1A1-PDGFB fusion is identified in virtually all dermatofibrosarcoma protuberans cases when investigated by newly developed multiplex reverse transcription polymerase chain reaction and fluorescence in situ hybridization assays. Hum Pathol.2008; 39:184–93.
4. Abbott JJ, *et al*. Gains of COL1A1-PDGFB genomic copies occur in fibrosarcomatous transformation of dermatofibrosarcoma protuberans. Mod Pathol. 2006;19: 1512-8.

hospitaldelmar

C S B Consorci Sanitari de Barcelona
IMAS
Institut Municipal d'Assistència Sanitària