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Original Contributions

Calcifying fibrous tumor and inflammatory myofibroblastic tumor are epigenetically related: A comparative genome-wide methylation study☆

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ABSTRACT

Based on histological findings, calcifying fibrous tumor (CFT) may be a late (burned out) stage of inflammatory myofibroblastic tumor (IMT). This concept, however, has not been proven by molecular means.

Five CFTs were analyzed for IMT-related rearrangements in *ALK*, *ROS1* and *RET* using fluorescence in situ hybridization (FISH). Additionally, genome-wide methylation patterns were investigated and compared with IMT (n = 7), leiomyoma (n = 7), angioleiomyoma (n = 9), myopericytoma (n = 7) and reactive soft tissue lesions (n = 10) using unsupervised hierarchical cluster analysis and t distributed stochastic neighbor embedding.

CFT patients, 4 females and 1 male, had a median age of 20 years ranging from 7 to 43 years. Two patients were younger than 18 years old. The tumors originated in the abdomen (n = 4) and axilla (n = 1). Histologically, all lesions were (multi) nodular and hypocellular consisting of bland looking (myo)fibroblasts embedded in a collagenous matrix with calcifications.

FISH analysis brought up negative results for *ALK*, *RET* and *ROS1* rearrangements. However, genome-wide methylation analysis revealed overlapping methylation patterns of CFT and IMT forming a distinct homogeneous methylation cluster with exception of one case clustering with myopericytoma/angioleiomyoma.

In conclusion, DNA methylation profiling supports the concept that CFT and IMT represent both ends of a spectrum of one entity with CFT being the burn out stage of IMT.

1. Introduction

Calcifying fibrous tumor (CFT) is a rare benign mesenchymal lesion occurring in both children and (young) adults [1-5]. The most common location of CFT is the abdominal cavity including the gastrointestinal tract, but its origin varies with occurrence in soft tissues and at other visceral sites [1,2,5-8]. Histologically, CFT is characterized by a hypocellular bland-looking (myo)fibroblastic proliferation associated with dense collagen, chronic inflammation and variably prominent psammomatous or dystrophic calcifications [2,9,10]. Some hybrid lesions with histological features of CFT and inflammatory myofibroblastic tumors (IMT) have been reported suggesting a link between CFT and IMT [11-13]. However, this concept is controversially discussed because strong evidence was missing so far. This study investigated the relation between CFT and IMT by comparing the genome-wide methylation patterns of both CFT and IMT alongside myopericytoma,

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angioleiomyoma, leiomyoma and reactive soft tissue lesions. Furthermore, we performed FISH for *ALK*, *ROS1* and *RET* on CFTs as rearrangements of these genes are the genetic hallmark of IMT.

2. Material and methods

The study was conducted in accordance with the Code of Conduct of the Federation of Medical Scientific Societies.

Patients with CFT were selected using the World Health Organization (WHO) criteria [10]. A total of five cases were obtained from our (referral) files. Clinical details were obtained from the records. H&E slides were reviewed.

2.1. FISH ALK, ROS1 and RET

Fresh cut 4 μm sections of formalin-fixed paraffin embedded tissue were submitted to dual-color fluorescence in situ hybridization (FISH) analysis using three break-apart probes for *ALK*, *ROS1* and *RET*. The probe sets were developed at Cytocell (Cambridge, UK). FISH was performed according the standard methods. At least 50 nuclei per sample were counted.

2.2. DNA isolation, genome-wide DNA methylation data generation and pre-processing

Representative tumor tissue with highest available tumor content was chosen for DNA extraction. The Maxwell® 16 FFPE Plus LEV DNA Kit or the Maxwell® 16 Tissue DNA Purification Kit (for frozen tissue) was applied on the automated Maxwell device (Promega, Madison, WI, USA) according to the manufacturer's instructions. All tumors had a total amount of > 100 ng DNA and were suitable for the array-based DNA methylation analysis. All tumors were subjected to Illumina Infinium HumanMethylation450 (450k) BeadChip or the successor EPIC/850k BeadChip (Illumina, San Diego, USA) analysis at the Genomics and Proteomics Core Facility of the German Cancer Research Center (DKFZ) Heidelberg. DNA methylation data were normalized by performing background correction and dye bias correction (shifting of negative control probe mean intensity to zero and scaling of normalization control probe mean intensity to 20,000, respectively). Probes targeting sex chromosomes, probes containing multiple single nucleotide polymorphisms and those that could not be uniquely mapped were removed. Probes from the EPIC array were excluded if the predecessor Illumina Infinium 450k BeadChip did not cover them, thereby making data generated by both 450k and EPIC feasible for subsequent analyses. In total, 438,370 probes were kept for analysis.

2.3. Unsupervised clustering, t-SNE analysis and cumulative copy number plotting

For unsupervised hierarchical clustering, we selected 10,000 probes that showed the highest median absolute deviation (MAD) across the beta values. Samples were hierarchically clustered using the Euclidean distance and Ward's linkage method. Hierarchical clustering using Euclidean distance and complete linkage reordered methylation probes. The unscaled methylation levels were shown in a heat map from unmethylated state (blue color) to methylated state (red color). For unsupervised 2D representation of pairwise sample correlations, dimensionality reduction by t distributed stochastic neighbor embedding (t-SNE) was performed using the 10,000 most variable probes, a perplexity of 20 and 2500 iterations. Novel methylation groups were tested for stability by varying the number of the most variable probes.

The control group consisted of IMT (n = 7), leiomyoma (n = 7), angioleiomyoma (n = 9), myopericytoma (n = 7) and reactive soft tissue lesions (n = 10).

Table 1
Clinical data and histopathological diagnoses.

Patient number	Sex	Age at diagnosis	Location of tumor	Tumor type
1	F	7	Axilla	Calcifying fibrous tumor
2	F	20	Abdomen	Calcifying fibrous tumor
3	F	43	Upper abdomen	Myopericytoma/ angioleioma
4	F	38	Abdomen	Calcifying fibrous tumor
5	M	13	Omentum	Calcifying fibrous tumor

3. Results

3.1. Clinical data

The clinical features of the CFT patients studied are summarized in Table 1. The study consisted of 4 females and 1 male. The ages ranged from 7 to 43 years, with a median of 20 years. Two patients were younger than 18 years old. The tumors originated in the abdomen (n = 4) and axilla (n = 1).

3.2. Histological data

All lesions were (multi)nodular with hypocellularity and a high amount of hyalinized collagen. (Myo)fibroblastic cells, haphazardly distributed, showed inconspicuous nuclei and scant cytoplasm. Calcifications and lymphoplasmacytic inflammatory infiltrate, scattered throughout the lesion, were present in all of the cases (Fig. 1). Hyalinized vessels were observed.

3.3. FISH data

All five cases were available for *ALK*, *RET* and *ROS1* FISH, but rearrangements were not observed in any case.

3.4. Genome-wide methylation analysis

Four of the five CFTs formed a homogeneous methylation group with the IMTs by clustering and t-SNE analysis (Fig. 2), which remained stable when varying the number of CpGs (Cytosine-phosphatidyl-Guanine) used. The single CFT outlier assigned to the distinct methylation cluster of myopericytoma/angioleioma. Leiomyoma and reactive lesions clustered separately.

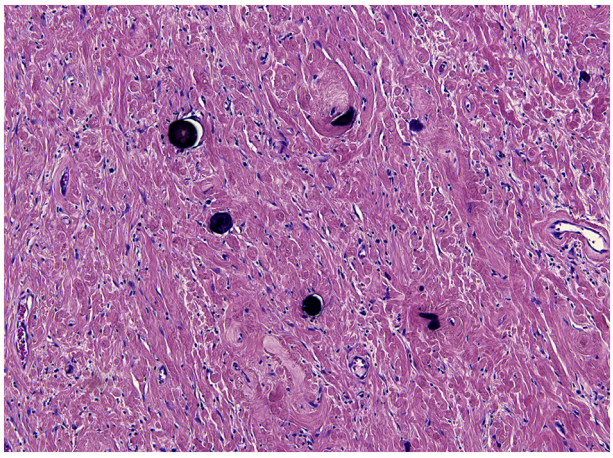

Fig. 1. Histologically, cases were hypocellular and possessed a prominent collagenous matrix with psammomatous calcifications and an inflammatory reaction.

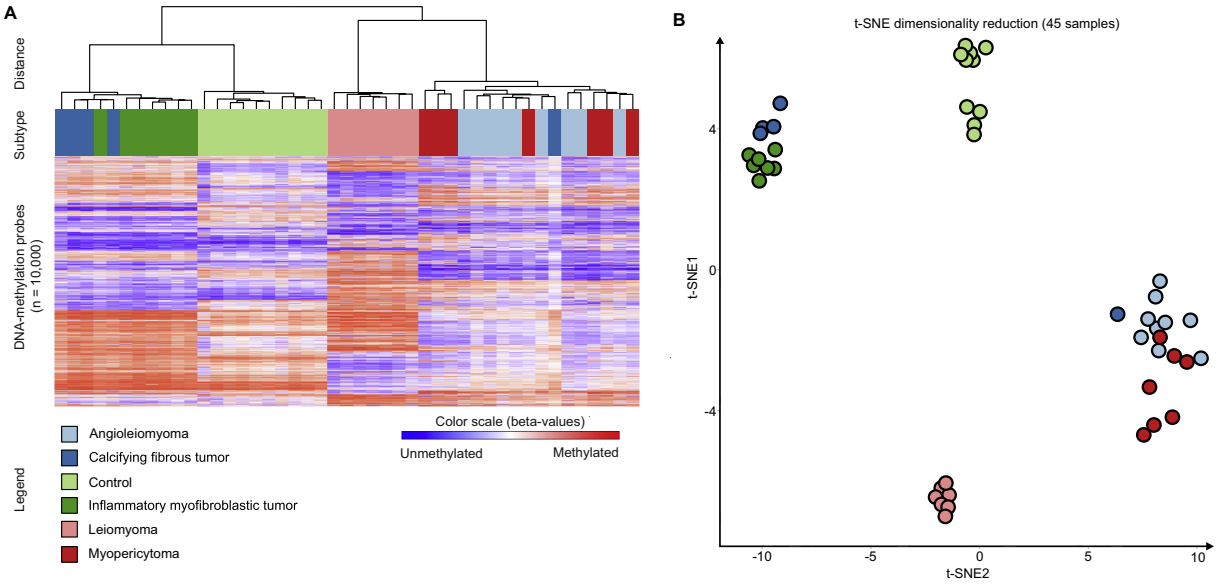

Fig. 2. DNA methylation pattern of CFT were similar to that of IMT, with one exceptional case clustering with myopericytoma/angioleiomyoma.

4. Discussion

Calcifying fibrous tumors (CFT) were first described in 1988 by Rosenthal and Abdul-Karim under the name “childhood fibrous tumor with psammoma bodies”. In 1993 Fetsch et al. reported on several cases of the files of the Armed Forces Institute of Pathology expanding the age range and using the term calcifying fibrous pseudotumor [2,9]. Later on, these lesions were considered neoplastic and designated as calcifying fibrous tumor [6].

Since its original description the pathogenesis is still under debate. Based on clinicopathological characteristics a relationship with inflammatory myofibroblastic tumor (IMT) has been suggested and subsequently controversially discussed [6,10,12-14]. Coffin et al. determined three phenotypes of IMT: 1) myxoid, vascular, inflammatory, 2) compact spindle cells with intermingled inflammatory cells, 3) dense plate-like collagen, reflecting high variability of IMT morphology with the latter being probably a late stage resembling CFT [15]. To the best of our knowledge, three cases have been reported with coexisting histological patterns of CFT and IMT arguing that CFT is a late stage of IMT [11-13]. However, ALK, a consistent marker of IMT, has been found only exceptionally in CFT [16]. *ALK* rearrangement, or alternatively rearrangement of *ROS1* and *RET* shown in 56–61.1%, 5.6–10% and 1.6% of investigated cases, respectively, are the driver genetic events in IMTs and FISH analysis can aid as diagnostic tool to confirm the diagnosis [17,18]. Rearrangements of these genes were not detected in any of our CFT cases. When CFT is the advanced stage of regressed IMT, it is not surprising that the mentioned genetic changes and the corresponding proteins are absent seemingly due to substitution of scar-like tissue and a decreased amount of lesional cells.

The most convincing argument that CFT and IMT form a spectrum with CFT being the burned out end we found by methylation profiling showing overlapping methylation patterns. It appears that this assay is of a higher sensitivity in comparison to rearrangement analysis using FISH, at least when we deal with a lesion with features of marked regression. Another explanation could be that the subset of IMT going into regression shows alternative fusion genes, e.g. *NTRK3*, *PDGFRB* and *IGF1R* [17,19,20].

CFTs are benign with exceptional recurrences whereas IMTs may behave aggressively, even rarely [10]. One could argue that a subset of benign behaving IMTs go into regression offering a CFT appearance. This is also confirmed by cases with morphological aspects of both, IMT and CFT [11-13].

One of our cases (case 3) clustered with myopericytoma/angioleiomyoma. From the morphological point of view this case was not different showing that a late stage myopericytoma/angioleiomyoma may have the same burn-out features as CFT.

In conclusion, we could show by methylation profiling that CFT has the same epigenetic profile as IMT and support a relationship with CFT being probably a late, burned out stage of IMT irrespective of negative fusion gene analysis leading to lack of expression of the corresponding proteins in CFT.

Declaration of Competing Interest

There are no conflicts of interest.

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