

## CASE REPORT

# Alveolar rhabdomyosarcoma with unusual cytogenetic findings: one more case and review of the literature

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## Abstract

Alveolar rhabdomyosarcoma (ARMS), a histological subtype of rhabdomyosarcoma (RMS), is characterized by an unfavorable clinical outcome. In most ARMS cases, an indicative chromosomal alteration is identified. The recurrent translocation of FKHR with either PAX3 or PAX7 genes results in the encoding of chimeric transcription factors that boost tumorigenesis. Besides structural mutations, the copy number of these genes also contributes to the oncogenic activity. In our case, a 12-year-old female patient was diagnosed with a 4 cm pelvic mass. Histopathological examination indicated an alveolar type of RMS. Subsequent FISH analysis with a dual color break-apart probe identified positive signals of FKHR3 gene break, as well as the rare event of a synchronous aneuploidy and gene deletion of FKHR. Our findings lead to the conclusion that a systematic break-apart probe FKHR FISH analysis in ARMS, confirms the diagnosis and elucidates the full spectrum of genomic alterations of this malignancy.

## INTRODUCTION

Rhabdomyosarcoma (RMS) is the most common pediatric soft tissue sarcoma and accounts for 3–4% of all pediatric malignancies [1]. Among the various histological subtypes that have been described, are the embryonal (ERMS), alveolar (ARMS), spindle cell, mixed-type, pleomorphic and ganglionic differentiated rhabdomyosarcoma [1]. Although ERMS is the most common RMS form, ARMS is characterized by an increased metastatic potential (25.9%), a lower 10-year survival rate (35.3%) and a worse overall prognosis [1].

Cytogenetic studies of ARMS have identified a characteristic chromosomal alteration [2–4]. More specifically, the majority of ARMSs display a recurrent translocation between genes encoding for transcription factor FKHR with either PAX3 or PAX7 (fusion-positive ARMS) [2]. The resulting chimeric genes encode

transcription factors that promote tumorigenesis [2]. Besides these, in a notable portion of fusion-positive tumors, chimeric genes are amplified, thus confirming the significance of the somatic copy number alterations in ARMS tumorigenesis [5]. However, the concurrent chromosomal amplification and gene deletion in fusion-positive ARMSs is still a rare entity [5].

Therefore, in our study, we present the case of a pediatric ARMS, with evidence of chromosomal translocation on FISH analysis, as well as with FKHR3 gene amplification and deletion. We also review the relevant literature.

## CASE REPORT

A 12-year-old Caucasian female, with no significant medical history and without pathological laboratory results, presented to

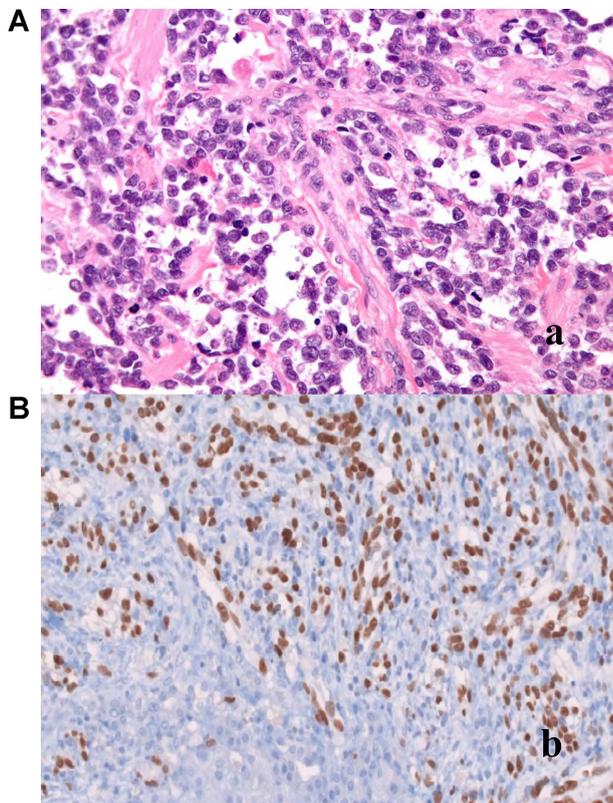
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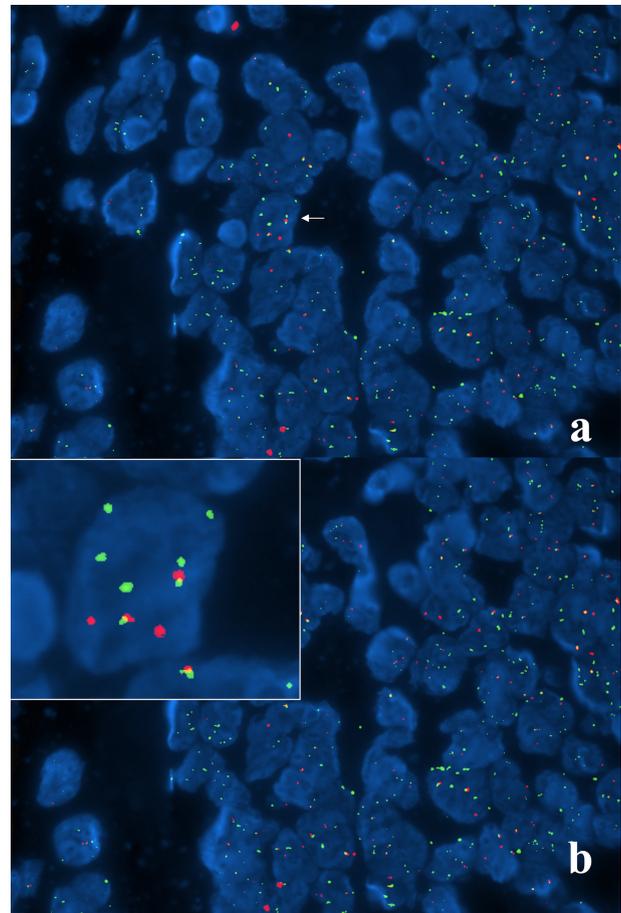


**Figure 1:** (a) Neoplastic cells with medium to large nuclei, homogeneous chromatin and a variable amount of cytoplasm are separated by fibrovascular septae (Hematoxylin and Eosin stain, original magnification  $\times 20$ ). (b) Neoplastic cells displaying strong nuclear positivity for myogenin.

our tertiary hospital emergency department, with dull inguinal pain symptoms. An abdomen CT scan revealed a 4 cm right pelvic mass. Initial diagnostic work-up included a chest CT scan, abdomen MRI and a  $^{99}\text{Tc}$  bone scintigraphy. Since the lesion did not infiltrate any regional structures and there were no metastases, the patient was submitted to a local mass excision and pelvic lymph node dissection. Postoperative recovery was eventless.

The excised tissues were fixed in a formalin solution and processed in paraffin. Microscopic evaluation validated a R0 excision and excluded the presence of lymph node metastatic foci. In hematoxylin and eosin light microscopy, patternless sheets of discohesive tumor cells with fibrovascular septae were identified (Fig. 1a). Polylobated and multinucleated tumor cells and many mitotic figures were also observed. Immunohistochemistry showed diffuse strong cytoplasmic and membranous positivity for desmin, vimentin and strong nuclear staining for MyoD1 and myogenin (Fig. 1b). Other immunostains including cytokeratins AE1/AE3, LCA, S-100, HMB-45 and CD34 were negative. Therefore, the diagnosis of an alveolar type of rhabdomyosarcoma was suggested.

FISH analysis was performed using a dual color break-apart probe for FKHR (also known as FOXO1). A Zeiss Axio Imager Z1 microscope (Carl Zeiss, Jena, Germany) with a Plan Neofluar  $\times 100/1.3$  oil objective lens was introduced for the fluorescence image capture. The recording of the analysis results was achieved by using a monochrome progressive scan CV-M4/CL camera (Applied Imaging Corp., CA, USA) and CytoVision software, 3.93.1 release (Applied Imaging Corp.).



**Figure 2:** (a and b) FISH analysis shows one normal fusion signal (yellow), two split signals for the telomeric region of FKHR (red) and multiple split signals for the centromeric region of FKHR (green). Many of cells show more green than red signals (insert).

Our analysis (Fig. 2a and b) on the sarcoma tissue, revealed one normal fusion signal (yellow), two split signals for the telomeric region of FKHR (red) and multiple split signals for the centromeric region of FKHR (green). These results indicated a rearrangement at FKHR3 locus. Moreover, the multiple green and red signals confirmed gene amplification. The uniqueness of our case is based on the fact that besides gene translocation and aneuploidy, FKHR3 gene deletion (Fig. 2 a and b) was recorded. This observation was suggested by the different and not even number of green and red signals in a cell nuclei during FISH analysis.

Postoperatively, the patient received an adjuvant vincristine, dactinomycin and cyclophosphamide regimen. Moreover, a 36 Gy radiotherapy course was also administered. There was no recurrence at 6 months follow-up.

## DISCUSSION

Among the various subtypes, ARMS is considered to be among the pediatric RMSs with the worst overall survival outcomes. Regarding histologic presentation, ARMS is characterized by round cells, clustered in nests and separated by fibrous septa with periseptal rows and peripheral discohesion. Myogenesis-related factors, such as MyoD or myogenin, are also positive [4].

Differential diagnosis of ARMS includes other soft tissue sarcomas with overlapping cytological features and the embry-

onal type of rhabdomyosarcoma which presents overlapping immunohistochemical characteristics. The final diagnosis is reached by identification of one of two transcription factor translocations: t(2;13)(q35;q14), i.e. PAX3- FKHR or t(1;13)(p36;q14), i.e. PAX7-FKHR [6]. PAX3 -FKHR and PAX7-FKHR mutations have been reported in 64% and 18% of ARMS cases, respectively [2]. Other genomic rearrangements that have been reported in ARMS, include the generation of the PAX3-AFX, PAX3-NCOA1 and PAX3-NCOA2 fusion genes [7]. The oncogenic potential of the chimeric proteins, derives from the stimulation of cell proliferation, promotion of cell survival and suppression of terminal differentiation.

The interphase cytogenetic findings of our case are unique, based on the simultaneously observed amplification and deletion of FKHR3 gene.

Early publications have demonstrated genomic amplification in ARMS tumors. Amplification has been detected in 47 of 84 ARMS tumors (56%) and the amplification events were derived from the 1p36, 2p24, 12q13-q14, 13q14 and 13q31 chromosomal regions [8].

In addition to this, the majority of fusion-positive ARMSs, show amplification of the chimeric PAX7-FKHR gene [5]. In our study, we used a break-apart FKHR3 probe, which does not allow the fusion partner to be recognized. However, the multiple signals for the centromeric region of FKHR3 are indicative of gene amplification.

Moreover, in our case, we detected a synchronous deletion of the FKHR gene. Previous publications have reported RMS cases with somatic copy number alterations, detected in parallel to chromosomal structural rearrangements [4, 5]. However, they are generally more prevalent in ERMS than in ARMS [4]. Interestingly, Harrison et al. [9] reported a case of disseminated alveolar rhabdomyosarcoma, where chromosome analysis showed a deletion of chromosome 13(q14).

This was the first reported case, in which this deletion occurred without involvement of chromosome 2 and the authors correlated this cytogenetic feature with the possible oncogenic role of the retinoblastoma (RB1) gene located at the related breakpoint. Recently, Liu et al. [10], using an array comparative genomic hybridization technique, provided a spectrum of gene amplification and deletion regions in RMS. The clinical significance of identifying gene amplification and deletion lies to the fact that these regions contain oncogenes or tumor suppressor genes [5].

In our case FKHR3 gene deletion occurred simultaneously with gene amplification and gene rearrangement. To our knowledge, this cytogenetic profile is reported for first time in the literature.

Identification of ARMS fusion genes can be detected through real-time PCR, or FISH analysis [6]. When comparing FISH FKHR to PCR analysis, FISH displays a higher sensitivity rate, with lower technical and cost requirements [6]. Furthermore, a diagnostic enhancement can be achieved by replacing single-fusion probes with break-apart probes, which present a higher FKHR rearrangement detection rate (34.5% vs 92.3%) including cases with unknown fusion partners [3, 6].

Therefore, FISH analysis with a break-apart probe for FKHR, should be performed on all sarcomas with histological evidence of alveolar features for diagnostic purposes, as well as, to select cases with aberrant cytogenetic findings for further cytogenetic studies. Histological classification of the tumor and identification of genetic aberrations may help the development of diagnostic and predictive biomarkers and possible drug targets

implicated in the diagnosis and the targeted therapy of rhabdomyosarcoma.

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## CONFLICT OF INTEREST STATEMENT

None declared.

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## ETHICAL APPROVAL

Since no experimental process was involved in the diagnosis and treatment of the patient, a local ethics committee approval was not necessary and therefore, not obtained.

## CONSENT

The patient prior to the initiation of the diagnostic and treatment processes completed an institution informed consent form. A consent form was also obtained for the storage, process and anonymous publication of the case data.

## GUARANTOR

The author MARIA IOANNOU (mioan@med.uth.gr) is the guarantor of the present study.

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