Citogenetic-Molecular Alterations in FOXO1 Gene in a Child with Alveolar Rhabdomyosarcoma: Case Report

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Alterações Citogenéticas e Molares no Gene FOXO1 em uma Criança com Rabdomiossarcoma Alveolar: Relato de Caso

Caso de Estudo: Paciente do sexo feminino, de 7 anos de idade, apresentou ao diagnóstico RMSa parameningeo, sem metástases ao diagnóstico. O estudo por meio de FISH mostrou a translocação envolvendo o gene FOXO1 e uma cópia extra desse gene. A paciente foi incluída no protocolo de tratamento do EpSSG, classificado como grupo de alto risco e recebeu quimioterapia e radioterapia. No final do tratamento, foi observada resposta parcial e iniciada quimioterapia de segunda linha. A paciente foi incluída no protocolo de tratamento do EpSSG, apresentando resposta parcial e iniciação de quimioterapia de segunda linha. Não houve resposta clínico-radiológica e a paciente evoluiu com progressão de doença local refratária ao tratamento e obteve um ano do diagnóstico.

Conclusão: De acordo com nosso conhecimento, é a primeira descrição de um caso de RMSa apresentando translocação do gene FOXO1 e uma cópia extra desse gene em clones separados. São necessários ainda mais estudos, a fim de compreender melhor o significado prognóstico da presença dessas alterações.

Keywords: Rabdomiossarcoma; Child; Translocation, Genetic; In Situ Hybridization, Fluorescence; Forkhead Box Protein O1.

Resumen

Introducción: El rabdomiossarcoma (RMS) es el tumor de tejidos blandos más común de la infancia. El RMS puede clasificarse en dos subtipos principales, el rabdomiossarcoma alveolar (RMSa) y el embrionario (RMSe). El RMSa presenta un pronóstico desfavorable si se compara al RMSe, habiendo así necesidad de intensificación del tratamiento. De esta forma, la distinción entre RMSa y RMSe es fundamental. Citogenéticamente, el RMSa presenta en cerca del 80% de los casos de translocación cromosómica que involucra el gen FOXO1. La metodología de Hibridación fluorescente in situ (FISH) ha sido muy utilizada para caracterizar el RMSa. Caso de estudio: Paciente del sexo femenino, de 7 años de edad presentada con un diagnóstico RMSa parameningeo, sem metástasis. El análisis a través del FISH mostró la translocación envolviendo el gen FOXO1 y una copia extra de este gen. La paciente fue incluida en el protocolo de tratamiento del EpSSG, clasificado como grupo de alto riesgo y recibió quimioterapia y radioterapia. Al final del tratamiento fue observada una respuesta parcial y se inició la quimioterapia de segunda línea. No hubo respuesta clínico-radiológica y la paciente evolucionó con progresión de enfermedad local, refractaria y obtuvo un año del diagnóstico. Conclusión: De acuerdo con nuestro conocimiento, este es el primer caso de un niño con RMSa presentando la translocación del gen FOXO1 y una copia extra de este gen en clones separados. Se necesitan nuevos estudios para comprender mejor el significado pronóstico de la presencia de estos cambios.

Palabras clave: Rabdomiossarcoma; Niño; Translocación Genética; Hibridación in situ Fluorescente; Proteína Forkhead Box O1.

Resumo

Introdução: O rabdomiossarcoma (RMS) é o tumor de tecidos moles mais comum da infância. Pode ser classificado em dois subtipos principais: o rabdomiossarcoma alveolar (RMSa) e o embrionário (RMSe). No RMSa, o prognóstico é desfavorável quando comparado ao RMSe, necessitando de tratamento intensificado; dessa forma, a distinção entre ambos os subtipos é fundamental. Citogeneticamente, o RMSe apresenta translocações cromossômicas envolvendo o gene FOXO1 em 80% dos casos. A metodologia de hibridização in situ por fluorescência (FISH) tem sido muito utilizada para caracterizar o RMSa. Relato do caso: Paciente do sexo feminino, com 7 anos de idade, apresentou ao diagnóstico RMSa parameningeo, sem metástases ao diagnóstico. O estudo por meio de FISH mostrou a translocação envolviendo o gene FOXO1 e uma cópia extra desse gene. A paciente foi incluída no protocolo de tratamento do EpSSG, classificado como grupo de alto risco e recebeu quimioterapia e radioterapia. No final do tratamento, foi observada resposta parcial e iniciada quimioterapia de segunda linha. Não houve resposta clínico-radiológica e a paciente evoluiu com progressão de doença local refratária ao tratamento e obteve um ano do diagnóstico. Conclusão: De acordo com o nosso conhecimento, é a primeira descrição de um caso de RMSa apresentando a translocação do gene FOXO1 e uma cópia extra desse gene em clones separados. São necessários ainda mais estudos, a fim de compreender melhor o significado prognóstico da presença dessas alterações.

Palavras-chave: Rabdomiossarcoma; Criança; Translocação Genética; Hibridização in situ Fluorescente; Proteína Forkhead Box O1.
INTRODUCTION

Rhabdomyosarcoma (RMS) is the most common soft tissue tumor in childhood and is very rare in adults, with 4.7 new cases/million persons/year in the United States\(^1,2\). Approximately 25% of the children present metastatic disease at diagnosis, and the principal sites involved are lungs, bone marrow, bones, and lymph nodes. In pediatric patients, RMS can be classified in two main subtypes, alveolar rhabdomyosarcoma (ARMS) and embryonal rhabdomyosarcoma (ERMS), comprising 20% and 80% of cases, respectively\(^3\). ARMS and ERMS present distinct clinical and biological manifestations. ARMS occurs mainly in older children and is associated with poor prognosis. The distinction between ARMS and ERMS is extremely important, since ARMS displays higher incidence of metastases and worse prognosis than ERMS, thus requiring differential treatment\(^4\). Correct diagnosis of RMS can be challenging in routine histopathology\(^5\).

Cytogenetically, in about 80% of cases, ARMS presents chromosomal translocations involving the FOXO1 gene, with t(2;13)(q35;q14) in 60% of patients. The fusion proteins are transcription factors and determinants of tumorigenesis, and a predictor of worse prognosis\(^6\). Thus, fluorescent in situ hybridization (FISH) has been widely used to study rearrangement involving the FOXO1 gene, as a biomarker in RMS, characterizing the alveolar subtype of the disease\(^8\). The aim of this study is to report a pediatric case that presented, on FISH, cells with the rearrangement and cells with an extra copy of the FOXO1 gene, with diagnosis of ARMS, besides the patient’s clinical evolution.

CASE REPORT

A 7-year-old female patient complained of progressive nasal obstruction, initially diagnosed as tonsillitis, and was started on antibiotic therapy. Computerized tomography (CT) of the face showed a mass with soft tissue density occupying the entire sphenoidal sinus, with destruction of the anteroinferior wall, nasal cavities, and extension to the nasopharynx. Patient presented weight loss, apathy, limited acceptance of diet, and evolution to left ophthalmoplegia and palpebral ptosis. Patient was enrolled in the Pediatric Oncology Service on May 19, 2005. Cranial and facial magnetic resonance imaging (MRI) showed an infiltrative expansive formation with a lobulated contour, predominantly hypointense on T1, with irregular contrast uptake and diffusion restriction, with isocenter in the nasal cavity bilaterally, involving the nasal septum. The lesion extended laterally to the maxillary sinuses, notably on the right, above the medial and posterior ethmoidal cells, and posteriorly to the sphenoidal sinus and nasopharynx, causing complete obliteration of the cavum (Figure 1A). The soft palate was pushed downward. Patient was submitted to rhinoscopy with biopsy. Histopathology revealed the presence of ARMS, with the primary site defined as parameningeal, without metastasis at diagnosis (negative CSF). Immunohistochemistry showed cytoplasmic positivity for desmin and nuclear positivity for myogenin in 90% of the cells and negativity for pancytokeratin, CD99, and NB84. FISH, using the LSI FOXO1 (13q14) Dual Color, Break Apart Rearrangement Probe (Vysis, Abbott), according to the International System for Human Cytogenomic Nomenclature (ISCN, 2016)\(^9\), showed the following result: nuc ish (FOXO1x2) (5’FOXO1 sep 3’FOXO1 x1)[132/200]/nuc ish (FOXO1x3)[64/200]/ nuc ish (FOXO1x2) [4/200]. This methodology thus allowed detecting two abnormal clones involving the FOXO1 gene. The first, in 66% of the 200 cells analyzed, showed translocation involving the FOXO1 gene, while the clone in 32% of the 200 cells analyzed presented an extra copy of the FOXO1 gene. In addition, 2% of all 200 cells showed the presence of normal cells. Figure 2 shows the cytogenetic-molecular alterations involving the FOXO1 gene.

Patient was enrolled in the EpSSG treatment protocol as non-metastatic RMS, classified as a high-risk group, and received chemotherapy with ifosfamide, actinomycin, and vincristine, and radiotherapy at a dose of 50.4 Gy as local treatment. Patient presented partial response, but since a residual lesion was maintained at the primary site (Figure 1B) at the end of treatment, second-line chemotherapy was initiated with carboplatin, doxorubicin, and cyclophosphamide. There was no clinical or radiological response, and the patient evolved with treatment-resistant progression of the local disease and death a year after diagnosis. The study was approved by the Institutional Review Board of the Brazilian National Cancer Institute José Alencar Gomes da Silva (INCA) under protocol number 119/07.

STUDY OF CYTOGENETIC-MOLECULAR ALTERNATIONS IN THE FOXO1 GENE BY THE FISH METHOD

FISH was used to study the cytogenetic-molecular alterations involving the FOXO1 gene, with the LSI FOXO1 (13q14) Dual Color, Break Apart Rearrangement Probe (Vysis, Abbott) and the material in paraffin, according to manufacturer’s instructions. The tissue samples were prepared in 4 micrometer sections and adhered to polarized...
Fusion proteins are considered transcription factors and PAX7-FOXO1 in 70% to 80% of the cases. or t(1;13)(p36;q14), resulting in the fusion of gene P...tions involving the FOXO1 gene are intact (on microscopy, the signal is represented by the yellow color, with juxtaposition of the gene’s green and red signals); (A2) and (A3) two separate signals, showing the gene’s rearrangement (microscopy reveals two separate signals, green and red); (B1), (B2), and (B3) three fusion signals of the FOXO1 gene, with one signal representing an extra copy of the gene (on microscopy, represented by yellow, with juxtaposition of the gene’s green and red signals).

DISCUSSION

RMS is the most common soft tissue sarcoma in children and adolescents11. ARMS is characterized by the chromosomal translocations t(2;13)(q35;q14) or t(1;13)(p36;q14), resulting in the fusion of gene PAX3-FOXO1 or PAX7-FOXO1 in 70% to 80% of the cases. Fusion proteins are considered transcription factors and determinants of tumorigenesis8,12. Cytogenetic-molecular analysis, that is, application of FISH, has been used as an important diagnostic tool for cases of RMS that are difficult to differentiate into subtypes by histopathology. The most frequently used probe is FOXO1 gene dual color break-apart, which can detect both translocations involving the FOXO1 gene. There is a current proposal to include this molecular alteration as one of the prognostic factors for the disease14.

This case report was based on the FISH technique using the FOXO1 gene dual color, break apart probe, showing the presence of cells with the translocation involving the FOXO1 gene and other cells presenting an extra signal for this gene. The patient presented ARMS, a subtype with worse prognosis and the primary tumor defined as having a parameningeal location. This location is considered unfavorable and represents a distinct group, with worse local control of the disease than the other sites, and an overall ten-year survival of 66%. Parameningeal location and alveolar subtype are independent prognostic factors for death following disease relapse15.

The finding of a translocation involving the FOXO1 gene in association with an extra copy of this gene suggests molecular evolution of the disease with distinct clones. Studies that used FISH to investigate the presence of FOXO1 gene rearrangements in ARMS mainly showed positive cases for the gene’s translocation. The separate green and red signals indicate the translocation and another fusion signal in the same cell; green and red juxtaposed indicate the normal allele of the FOXO1 gene. Another widely reported alteration is amplification, with the gene’s centromeric region presenting (10-20) extra copies of the green signal, with a frequency of up to 50% in ARMS cases16,17. Amplification of the FOXO1 gene was described by the Children’s Oncology Group (COG) and identified as a possible prognostic factor for RMS8. A literature review showed that the current article was the first case report of a child with ARMS presenting both
translocation of the \textit{FOXO1} gene and an extra copy of this gene in distinct cells, representing separate clones.

**CONCLUSION**

The patient reported here underwent unfavorable evolution, with disease progression and death. However, further studies are needed to better understand the prognostic significance of the presence of clones with cytogenetic-molecular alterations involving both translocation and an extra copy of the \textit{FOXO1} gene.

**CONTRIBUTIONS**

Nicolas Cabral Cunha participated in performance of the FISH technique, the manuscript's development, and revision of the final version for publication. Arissa Ikeda Suzuki contributed to the research, interpretation, design, and critical analysis of the intellectual content and approval of the version submitted for publication. Fernanda Ferreira da Silva Lima participated in the interpretation of the results and case description and elaboration of the manuscript for submission. Priscila Valverde Fernandes contributed to the selection and processing of the histopathology material. Paulo Antônio Silvestre de Faria contributed to the identification, revision, and reanalysis of the anatomical pathology reports, essential for analysis of this case, in addition to the computation and analysis of the results. Teresa de Souza Fernandez participated in the study design, was responsible for analysis and interpretation of the FISH result, and participated in the manuscript's development and analysis and revision of the final version for submission. Sima Esther Ferman participated in the study design, elaboration of the manuscript, and analysis and revision of the article for submission.

**CONFLICT OF INTEREST**

None.

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None.

**REFERENCES**


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