Study of *FOXO1* Break-Apart Status and Amplification by Fluorescence *in situ* Hybridization in Pediatric Patients with Alveolar Rhabdomyosarcoma

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INTRODUCTION

Rhabdomyosarcoma (RMS) is a rare tumor, presenting an incidence of 10-15% of pediatric solid tumors and 6% of all malignancies in children. RMS is divided in two main subtypes, alveolar rhabdomyosarcoma (aRMS) and embryonal rhabdomyosarcoma (eRMS), which present distinct clinical and biological manifestations. Alveolar RMS is associated with poor prognosis, whereas eRMS is usually associated with a better prognosis. Cytogenetically, aRMS presents chromosomal translocations involving the *FOXO1* gene in about 80% of the cases, with t(2;13) (q35;q14) in 60% of patients and t(1;13)(p36;q14) in 20% of cases. These translocations result in increased expression of the chimeric genes *PAX3-FOXO1* and *PAX7-FOXO1*, respectively. Due to the relevance of the biomarker *FOXO1* for the diagnosis and treatment of pediatric patients with aRMS and knowing that in our institution there are no studies with cytogenetic and molecular approaches in this pediatric tumor, we intend to initiate a molecular cytogenetic study in aRMS.

OBJECTIVE

The aims of this study were to analyze the frequency of the cyto-molecular alterations in *FOXO1* gene in pediatric patients treated in our institution (INCA); characterize the type of cytogenetic alteration, translocation or amplification of *FOXO1* gene and its association with clinical features.

MATERIAL AND METHODS

A retrospective and prospective study was carried out between 2008 and 2017 in 21 pediatric patients with aRMS. The mean age of the patients was 8 years, ranging from 1 to 17 years. In relation to gender, 8 patients were males and 13 females. Initially the FISH methodology was standardized (Figure 1A). The FISH technique was performed from paraffin blocks using the LSI FOXO1 (13q14) Dual Color, Break Apart probe (Vysis) (Figure 1B). This project was approved by the Research Ethics Committee. The workflow of this study is in Figure 2.

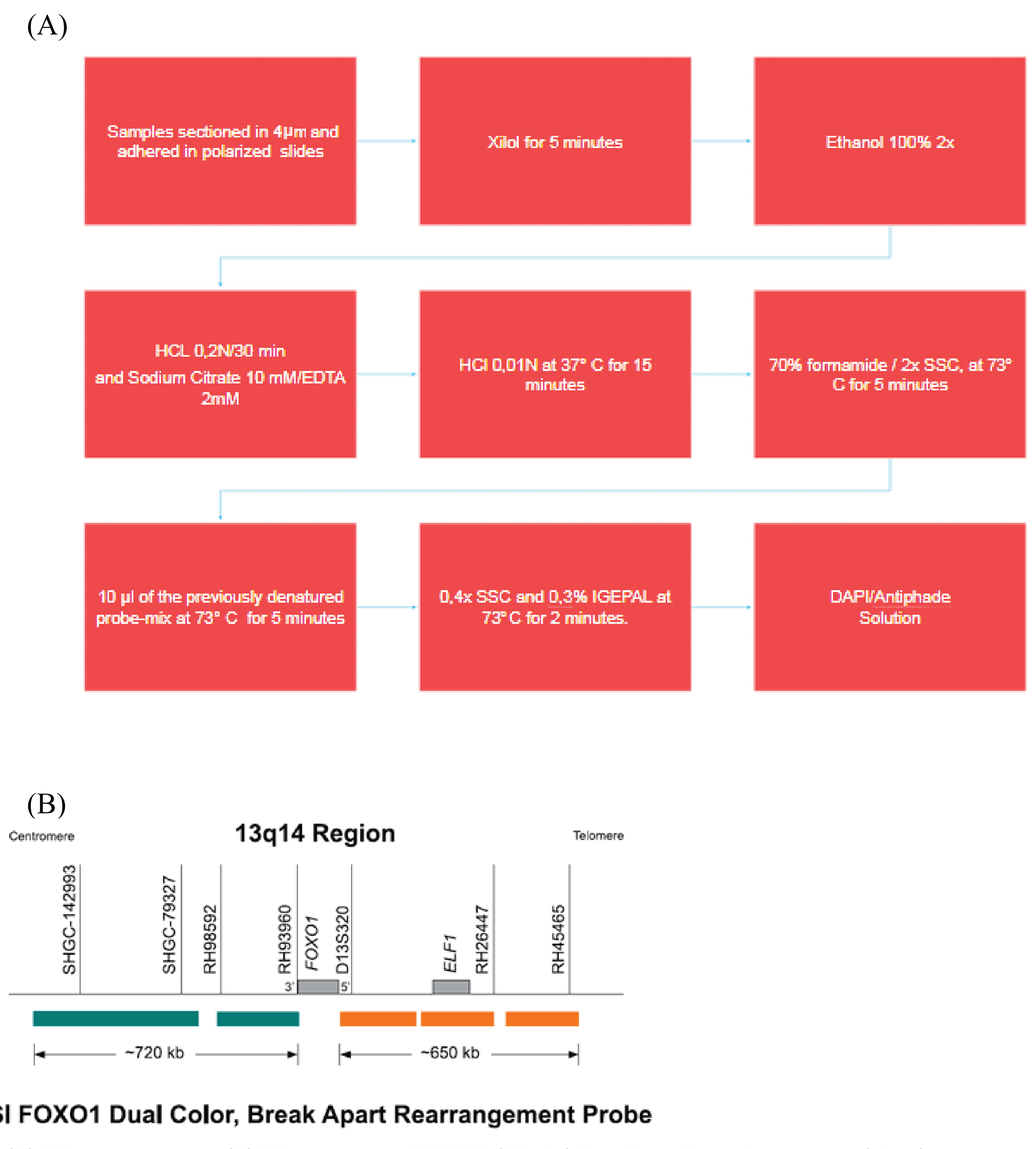

Figure 1: (A) FISH methodology; (B) FISH using the LSI FOXO1 (13q14) Dual Color, Break Apart probe (Vysis).

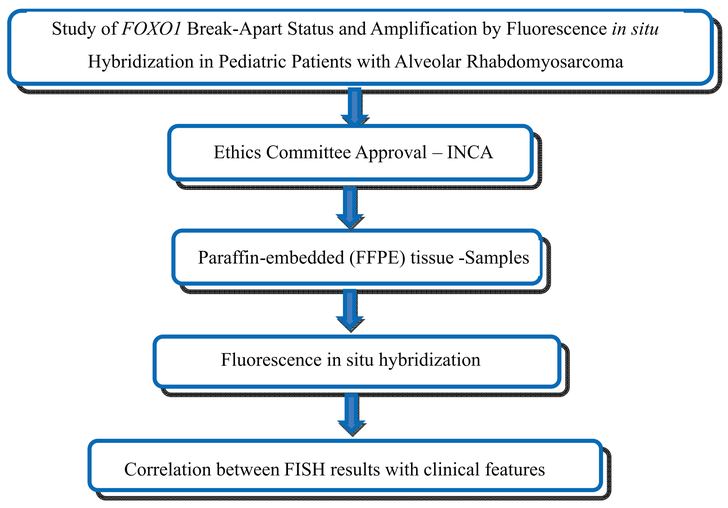

Figure 2: Workflow of this study.

RESULTS

The frequency of cyto-molecular alterations in the *FOXO1* gene was 76% (16/21) (Figure 3). It was observed different types of cytogenetic alterations in *FOXO1* gene like translocation, amplification and translocation/amplification (Figure 4). We observed a higher number of patients with positive FISH and metastases.

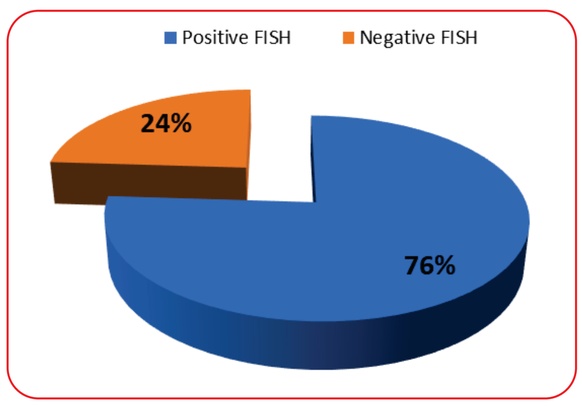

Figure 3: Frequency of cyto-molecular changes in the *FOXO1* gene through fluorescence *in situ* hybridization (FISH) in pediatric patients with alveolar rhabdomyosarcoma.

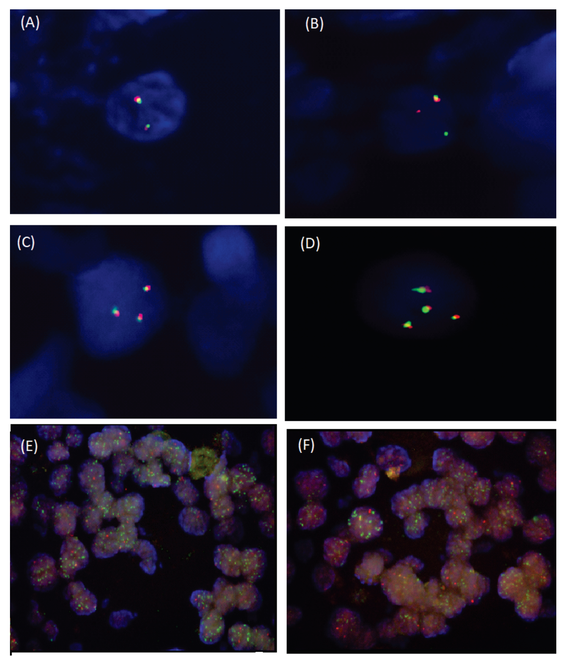

Figure 4: FISH analysis in pediatric patients with aRMS, showing in (A) the *FOXO1* gene with normal signals; (B) The result of *FOXO-1* gene break-apart FISH probe demonstrates divided green and red signals indicating translocation of the affected gene; (C) the interphase nucleus present an extra signal; (D) two extra signals of the *FOXO1* gene; (F) and (G) The result of *FOXO-1* gene break-apart FISH probe demonstrates divided green and red signals indicating translocation and also amplification of this gene.

CONCLUSION

The FISH methodology using the *FOXO1* probe is important for the diagnosis and prognosis of aRMS. The histopathological and mainly genetic classification of RMS has an important clinical application, helping to direct the treatment, reducing the exposure to intensive treatments and its side effects. Our results confirmed the importance of FISH involving the *FOXO1* gene as a diagnostic and prognostic biomarker. Through this translational research (from the bench to the clinic), we established an additional diagnosis test, molecular cytogenetics - FISH for the pediatric patients attended in our institution- INCA. We intend through this translational research to continue this study to contribute to the cytogenetic characterization of aRMS at diagnosis and during treatment, benefiting the children with this type of tumor treated in our institution.

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