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Detection of Common Chromosomal Translocations in Small Round Blue Cell Pediatric Tumors

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Background and Aims. Recurrent and specific chromosomal translocations have been described in four pediatric sarcomas belonging to the small round blue cell (SRBC) group of tumors. Identification of mRNA chimeras using RT-PCR discriminates among alveolar rhabdomyosarcoma (ARMS), Ewing's sarcoma (ES/pPNET), synovial sarcoma (SS) and desmoplastic small round cell tumor (DSRCT); however, frequencies of these translocations are variable. We present a retrospective study comparing histological examination and occurrence of major chromosomal translocations to validate the diagnosis and to assess the frequency of these molecular markers in a group of 92 small round blue cell (SRBC) tumor samples from Hospital Infantil de Mexico.

Methods. We tested a panel of RT-PCR assays to each RNA tumor sample from formalin-fixed, paraffin-embedded tumors to detect specific mRNA chimeras in 47 ES/pPNET, 19 ARMS, four SS, three DSRCT, and 19 other SRBC tumors.

Results. After excluding poor RNA quality samples, we found translocations in 17/31 ES/pPNET (54.8%), 10/19 ARMS (52.6%), 4/4 SS (100%) and 4/4 DSRCT (100%). We found disagreement in only three samples: one ES/pPNET and one embryonal rhabdomyosarcoma harbor a PAX3-FOXO1 translocation (for ARMS), and one neuroepithelioma harboring a EWS-WT1 (for DSRCT). Unsuitable RNA was found in 20/92 samples (21.7%) and was related to necrosis, small amount of tumor tissue, and use of nitric acid in bone biopsies, but was not related to age of the block.

Conclusions. We found a significantly lower occurrence of chromosomal translocations in ES/pPNET compared to reports from other groups. Differences may exist in the frequencies of these molecular markers among different populations. © 2014 IMSS. Published by Elsevier Inc.

Key Words: Alveolar rhabdomyosarcoma, Chimeric transcripts, Chromosomal translocations, Ewing, RT-PCR, Sarcomas.

Introduction

Small round blue cell (SRBC) tumors is a morphological description for a broad category of highly aggressive and poorly differentiated malignant tumors. They represent a challenging group for pathologists and oncologists alike due to a considerable morphological overlap and because in many instances accurate histopathological classification is critical for chemotherapy treatment decisions (1). Among SRBC pediatric tumors from which differential diagnosis must be established are alveolar rhabdomyosarcoma (ARMS), embryonal sarcoma (ERMS), Ewing sarcoma family of tumors (ES/pPNET), synovial sarcoma (SS),

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desmoplastic round cell tumor (DSRCT), undifferentiated sarcoma, neuroblastoma, non-Hodgkin's lymphoma and others. Some of these tumors can be identified using ancillary techniques such as electron microscopy (2) or immunohistochemistry through identification of myogenic, neural differentiation, specific membrane receptors or other differentiation biomarkers (3–5). Cytogenetic studies have identified recurrent and specific chromosomal translocations in four of these tumors: ARMS involving t(2;13)(q35;q14), ES t(11;22)(q24;q12), SS t(X;18)(p11;q18), DSRCT t(11;22)(q33;q12) (6–10). Molecular cloning of the chromosomal breakpoints demonstrated that these translocations result in juxtaposition of coding regions from otherwise separated genes, creating chimeric genes with novel functions (11–14). Because these fused genes are transcriptionally active, identification in tumor RNA of the fused mRNA using RT-PCR or qRT-PCR has been successfully used as a diagnostic marker when present to discriminate tumors harboring the chromosomal rearrangement in difficult cases. Use of fluorescence in situ hybridization (FISH) is another DNA based technique employed to identify and confirm these chromosomal rearrangements (15,16). In practical terms, however, use of RNA-based enzymatic methods has an important advantage over FISH because it allows testing for the presence of the main translocations mentioned when available tissue is very limited. For this reason, RT-PCR has been accepted as an important ancillary technique to confirm the diagnosis and to exclude other similar appearing round cell tumors. It is important to note that RT-PCR as well as FISH allow the detection only of a few variant types. In addition for specific probes for FISH or specific primers for RT-PCR to detect reported variants, more important technical difficulties such as lack of adequate positive controls for both techniques make the specific and thorough detection of rare variants a very challenging goal to attain. In Mexico there is only one report from our institution in this field comparing FISH and RT-PCR in a group of 30 cases diagnosed with ARMS (17). There is a significant lack of information related to the incidence of these important genetic events in other sarcomas in this pediatric population. Frequencies reported for these chromosomal translocations are relevant for this work because incidence of these genetic events across affected human populations is still incomplete and variable particularly for ES/pPNET and ARMS. This variability in frequencies depends on the technique used, the number of reported cases analyzed by each group of researchers, the criteria used to analyze cases and also with the increasing number of discovered variable partner genes involved in the translocations. Overall translocation frequencies in informative cases reported for SS is 95% (18), for DSRCT 92% (19), for ES/pPNET 75% and for ARMS 76%. Interestingly, frequencies reported for ES/pPNET and ARMS are more variable from almost 100–67% (20–24) for ES/pPNET and from 50–100% for ARMS (25–27). To assess the frequencies and the correlation between the most common chromosomal translocations through molecular detection and histopathology criteria in Mexican pediatric patients, we analyzed RNA from 92 SRBC tumors from formalin-fixed and paraffin-embedded tumors diagnosed and treated at the Hospital Infantil de Mexico from 1993–2007. We performed a blinded assay in each sample to detect the following major translocations for ES/pPNET EWS-FLI1, EWS-ERG, for ARMS PAX3/7-FOXO1, for SS SYT-SSX1 and for DSRCT EWS-WT1.

Materials and Methods

Case Selection

To explore the presence of chromosomal translocations, a group of 92 tumors with diagnosis of SRCT were retrieved by pathologists (L.C.M. and S.S.P) from the files of the Pathology Department at Hospital Infantil de Mexico. Criteria for selection included cases diagnosed as ES, ARMS, SS, DSRCT, undifferentiated sarcoma, SRBC not otherwise specified (NOS) and cases with formalin-fixed paraffin-embedded tissue blocks available. These cases span the small round blue cell sarcoma diagnostic categories where these chromosomal alterations may be found. The formalin-fixed, paraffin-embedded tissue blocks were obtained corresponding to cases diagnosed and treated at this hospital from 1993–2007. Specific diagnostic categories included 47 cases ES/pPNET (peripheral primitive neuroectodermal tumor), 19 ARMS, 4 SS, 3 DSRCT, 4 ERMS (embryonal rhabdomyosarcoma), six rhabdomyosarcomas (RMS), four SRBC not otherwise specified (NOS) tumors, four undifferentiated sarcomas, and one neuroepithelioma. The ethics committee at Hospital Infantil de Mexico approved the testing of specimens for this work.

RNA Extraction

Depending on tissue size, 3–8 full face 10-μm sections were used to extract RNA after deparaffinization with two xylene washes and three 100% ethanol washes. After overnight proteinase K treatment with 500 μg/mL at 55°C in 20 mM Tris HCl, pH 8, 10 mM EDTA, 1% SDS, RNA was isolated using the modified guanidine thiocyanate method with Tripure reagent and chloroform precipitated with isopropyl alcohol according to manufacturer recommendations and resuspended in 30 μl of DEPC-treated water.

Molecular Assays

The assay conditions and primers used were standardized and previously published (28,29). RT-PCR was carried out using the Titan system from Roche Diagnostics GmbH. To assess RNA adequacy, 3.5 μl from each RNA sample was first subjected to detection of the housekeeping gene phosphoglycerate kinase (PGK) with the following primers: