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Genomics and epigenetics: A study of ependymomas in pediatric patients.

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ABSTRACT

Objective: We identify chromosomal alterations, the methylation pattern and gene expression changes in pediatric ependymomas.
Methods: CGH microarray, methylation and gene expression were performed through the Agilent platform. The results were analyzed with the software MatLab, MapViewer, DAVID, GeneCards and Hippie.
Results: Amplification was found in 14q32.33, 2p22.3 and 8p22, and deletion was found in 8p11.23-p11.22 and 1q21.3. We observed 42.387 CpG islands with changes in their methylation pattern, in which we found 272 genes involved in signaling pathways related to carcinogenesis. We found 481 genes with altered expression. The genes *IMMT, JHDMD1D, ASAH1, ZWINT, IPO7, GNAO1* and *CISD3* were found to be altered among the three levels.
Conclusion: The 2p22.3, 8p11.23-p11.22 and 14q32.33 regions were identified as the most important; the changes in the methylation pattern related to cell cycle and cancer genes occurred in *MIB2*, *FGF18* and *ITIH5*. The *IPO7, GNAO1* and *ASAH1* genes may play a major role in ependymoma development.

1. Introduction

Brain tumors represent the second most common pediatric malignancy [1,2]. The number of children, adolescents and young adults (0–19 years) with a brain tumor diagnosis is approximately 4350 per year. In Mexico, it has been reported that there are 560 new cases of brain tumors per year [3]. The cause is unknown for most of the tumors, but it has been recognized that are some predisposing conditions that give rise to certain tumor types. Ependymomas represent approximately 10% of all primary tumors of the central nervous system in children [4].

An ependymoma (EP) is a slow-growing tumor that arises from ependymal cells of the ventricular system, from the choroid plexus or the central canal of the spinal cord [4], and less frequently from the brain parenchyma as a result of cell migration during embryo-

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genesis [5]. These tumors are considered to be of glial origin and may originate from cells derived during the transformation of neural stem cells, which contribute to the initiation and development of brain tumors; these cells share similar properties with the central nervous system (CNS), including self-renewal and proliferative capacity for the generation of offspring cells [6]. Very young children have a tendency towards a less favorable prognosis than older children and even adults, which cannot be entirely explained by differences in resectability, location or adjuvant therapy [7].

Remarkably, the WHO classifies pediatric EP according to the histopathological variants in Grade II (classic) and Grade III (anaplastic). The classification is established by factors such as: cell density, mitotic activity, proliferation, necrosis and microvasculature [8]. It has been observed that chromosomal aberrations (deletion and/or amplification) that contribute to tumor events and the anatomical location of the neoplasia are important for EP development. Amplification observed at chromosomes 1q, 7p, 9q and 12 are chromosomal aberrations associated with a poor prognosis [9]. An important epigenetic factor, methylation, is a unique and significant process involving covalent modification of the genetic material of a cell, which affects genes promoters leading to the regulation of transcription [10]; methylome studies of brain tumors have revealed epigenetic modifications that disturb multiple genes involved in cellular regulation, DNA repair, cell migration and apoptosis [11].

Currently, hypomethylation of DNA in tumors of glial origin affects 10 million dinucleotide CpG islands of the genome, which appear to be associated with the evolution of cellular malignancy through oncogene activation, promotion of genomic instability and the loss of imprinting genes [12]. At the intracranial EP, it has been demonstrated that the gene expression for the Notch and Sonic Hedgehog (*SHH*) pathways is overexpressed; while in the medullar EP, involvement with the *OLIG2, PI3K* and the *HOX* family genes has been identified [13,14]. DNA methylation and chromosomal abnormalities may influence gene expression; however, in many reports these approaches have been studied separately. Aure et al. observed that chromosomal amplifications and hypermethylation in breast cancer stimulated miRNA over-regulation, suggesting that miRNAs are also involved in the regulation of gene expression in ependymal tumors. A review tumor of behavior may facilitate a better understanding of the development, progression and maintenance of tumors [15]. To identify and correlate the chromosomal alterations, methylation pattern and gene expression changes in pediatric ependymomas.

2. Material and methods

2.1. Samples

The incidence of ependymomas accounts for 5–10% of all tumors of the central nervous system. Because of this, sample collection was established during the period of 2012–2013. The samples were collected from the services of the Pediatric Neurosurgery at General Hospital "Dr. Gaudencio González Garza" National Medical Center "La Raza", Mexican Social Security Institute (IMSS) and the Department of Neurosurgery at Childrenís Hospital of Mexico Federico Gómez. Tumor tissue was removed by surgical resection of pediatric patients between 0 and 16 years old. A total of 50 tumor samples were collected and these tumors were diagnosed by histopathologic evidence and images, but six samples were selected, because have confirmed diagnosis of ependymoma (Table S1 DOI: 10.6070/H4RN35WP). The signing of informed consent was requested in writing by the parent/guardian.

A control group (pool) was designed from lobotomy tissue from patients with epilepsy and the surrounding tissue (when necessary). The Ethics Committee of the National Commission approved the study for Scientific Research at IMSS under registration number 2009-785-042. The data were kept strictly confidential and in compliance with national and international standards for research on human health. All experiments were performed in accordance with the Declaration of Helsinki, and all subjects showed good understanding of the process and had given their written consent for the procedures.

2.2. Nucleic acid extraction

The DNA was extracted from tumor tissue with DNeasy Blood & Tissue (Qiagen), following the manufacturer's instructions. Total RNA was extracted from the tumor tissue through RNeasy (Qiagen) following the manufacturer's recommendations. Both nucleic acids were verified using the NanoDrop 1000 (Thermo Scientific) and 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA, USA).

2.3. Microarray-based comparative genome hybridization

Chromosomal aberrations were evaluated through array comparative genome hybridization (aCGH) using the Agilent platform with the CGH array chips G3 Sureprint Human CGH Microarray 400K (G4448A Agilent). Implementation of the recommended protocol by the supplier was followed.

2.4. Methylation microarrays

Methylation assays were performed with the Human CpG Island Microarray Chip 1X244K (G4495A-023795 Agilent). The protocol recommended by the supplier was performed. We used Feature extraction software (Agilent Technologies).

2.5. Expression microarray

Gene expression analysis was performed using a two-color microarray based method (Quick Amp Labeling Kit, Two Color Agilent); cRNA was purified through the RNeasy kit (Qiagen). Human GE 4X44K v2 (G4845A) microarray chips (Agilent Technologies). Data were extracted and preprocessed with the Agilent Feature Extraction Software [16] and GeneSpring software (Agilent technologies).

2.6. Analysis of results

The data obtained from the microarray platform were uploaded to the ArrayExpress Archive of Functional Genomics Data (http://www.ebi.ac.uk/arrayexpress) at the European Bioinformatics Institute (EMBL-EBI) with the access numbers E-MTAB-3846 and E-MTAB-3850 [17]. Tables and and images generated as complements were deposited at the LabArchives Electronic Lab Notebook (http://www.labarchives.com/). The aCGH results were analyzed with MapViewer de National Center for Biotechnology Information. The methylation and expression microarray data analysis was performed with the MatLab software (The MathWorks Inc.). Subsequently, we used DAVID Bioinformatics Resources 6.7 software [18] to identify the functionality of the modified genes as well as BioGrid version 3.3.122 [19] and GeneCards 1996–2015 [20]. The databases Hippie [21] and STRING version 9.1 [22] were used to determine the biological importance.