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BÚSQUEDA DE CANDIDATOS A BIOMARCADORES MOLECULARES EN TUMORES EPENDIMARIOS EN PACIENTES PEDIÁTRICOS

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PRESENTA:

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Sin otro particular, me es grato enviarle un cordial saludo.

A T E N T A M E N T E
"POR MI RAZA HABLARÁ EL ESPÍRITU"
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1. Resumen

Introducción. Los Ependimomas son tumores del sistema nervioso central que comprenden el 5% de las neoplasias intracraneales, con una esperanza de vida de 5 años en el 43% al 50% de los casos diagnosticados. La Organización Mundial de la Salud clasifica a los ependimomas para la edad pediátrica en grado II y grado III, recientemente se ha aceptado dentro de esta clasificación a los ependimomas supratentoriales RELA fusión-positiva. Se conoce que la pérdida en 22q y la ganancia en 1q son importantes en estos tumores, así como el cambio de expresión génica de *EGFR* y *NOTCH*. Hoy en día se han tenido avances sobre estos tumores, pero aún falta mucho por conocer sobre la formación, establecimiento y mantenimiento tumoral, así como las características moleculares de los ependimomas. **Metodología.** Se colectaron muestras de tejido fresco y embebidas en parafina, para la realización de ensayos de microarreglos e inmunohistoquímica, respectivamente. Además, se consultaron bases de datos para la realización de la revisión sistemática y meta-análisis. **Resultados.** A partir de los ensayos de microarreglos se encontró que los genes: *ASAH1*, *IPO7*, *IMMT*, *CISD3*, *ZWINT*, *GNAO1* son importantes en el desarrollo de los ependimomas. Con la evaluación de la expresión proteica se observó que ASAH1 y Ki-67 son sobreexpresadas; p53, Ciclina D1, GNAO1, IMMT e IPO7 están subexpresadas en un alto porcentaje de los ependimomas. Además, se encontró que algunos genes y proteínas reportadas pueden ser consideradas como características de lo ependimomas pediátricos y se sugiere que tienen potencial como biomarcadores. **Conclusión.** Las regiones cromosómicas 7q34, 14q32, 12p, 16p13.3 y 5p15.33 juegan un papel importante en la tumorigénesis, pero sólo las alteraciones 22q y 1q25 son características de estas neoplasias. Se sugiere que la sobreexpresión génica de *hTERT, ERBB* y la sobreexpresión proteica de EGFR, EMA, GFAP, NeuN y p53 son características de los ependimomas pediátricos. Las proteínas Ki-67, p53 y Ciclina D1 correlacionan con el grado histopatológico del tumor y la reincidencia. IPO7 e IMMT correlacionan con la sobrevida.

2. Abstract

Introduction. Ependymomas are tumors of the central nervous system that comprise 5% of intracranial neoplasms. The World Health Organization classifies ependymomas for pediatric age in grade II and grade III, recently it has been accepted within this classification the supratentorial ependymomas RELA fusion-positive. It is known that the loss in 22q and the gain in 1q are important in these tumors, as well as the change in gene expression of EGFR and NOTCH. Today there have been advances on these tumors, but there is still much to know about the formation, establishment and maintenance of tumors and about the molecular features of ependymomas. **Methodology**. The samples were collected of fresh tissue and formalin-fixed paraffin-embedded tissue, for the microarray assays and immunohistochemistry, respectively. the databases were consulted for the realization of Systematic Review and Metanalysis. **Results.** It is found that the genes ASAH1, IPO7, IMMT, CISD3, ZWINT, GNAO1 are necessary for the development of ependymomas. With the evaluation of protein expression, we observed that ASAH1 and Ki-67 are overexpressed; p53, Cyclin D1, GNAO1, IMMT, and IPO7 are underexpressed in a high percentage of ependymomas. It is found that some genes and reported proteins can be considered characteristics of pediatric ependymomas suggesting that they are potential biomarkers. **Conclusion.** The chromosomal regions 7q34, 14q32, 12p, 16p13.3, and 5p15.33 play an important role in tumorigenesis, the chromosomal aberration in the 22q and 1q25, the gene overexpression of hTERT, ERBB and protein overexpression of EGFR, EMA, GFAP, NeuN, and p53 are features of pediatric ependymomas. Ki-67, p53 and Cyclin D1 proteins correlate with the histopathological grade and progression-free survival. IPO7 and IMMT correlate with overall survival.

3. Introducción

Los tumores de sistema nervioso central (TSNC) son la primera causa de neoplasias sólidas. Cada año en el mundo se diagnostican alrededor de 4,000 casos nuevos en menores de 20 años, con una esperanza de vida de cinco años. Los TSNC son considerados especialmente problemáticos al localizarse en una región de difícil intervención. Hoy en día el diagnóstico del tumor es realizado con pruebas de imágenes obtenidas a partir de resonancia magnética o tomografía computarizada, posteriormente a la toma de biopsia el diagnóstico histopatológico es llevado a cabo.

Los TSNC comprenden entre el 15 al 20% de todas las neoplasias ocurridas en la niñez y la adolescencia [1]; manifiestan una preferencia para determinados tipos tumorales, con diferencias en la distribución geográfica [2]. El registro central de Tumores Cerebrales de Estados Unidos (CBTRUS, por sus siglas en inglés) estimó que el promedio de la tasa anual de incidencia en la niñez ajustada por edad fue de 5,26 por 100,000 individuos en la población [3]. En México los tumores intracraneales pediátricos son las neoplasias sólidas más frecuentes durante la infancia, con 540 nuevos casos por año, de los cuales el 32% son astrocitomas, 19% meduloblastomas, 11% craneofaringiomas, 5% ependimomas y 4% germinomas (Fig. 1) [4].

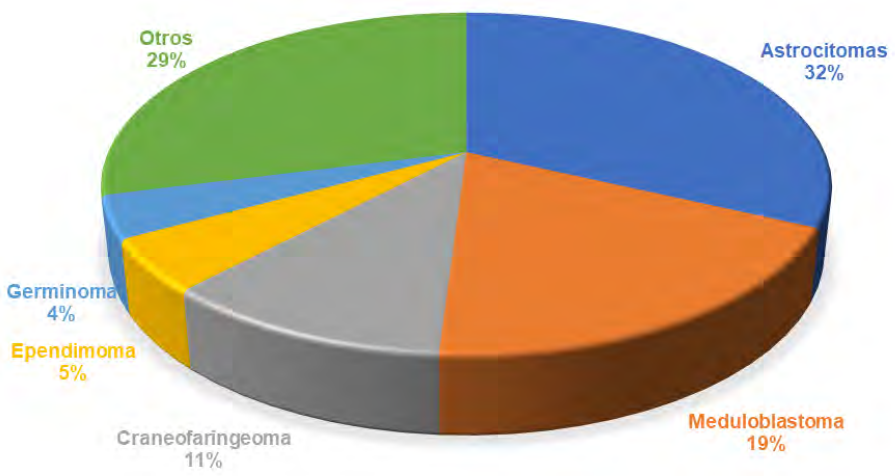

Figura 1. Frecuencia de tumores de Sistema Nervioso Central.

Actualmente, se desconocen los motivos del desarrollo del cáncer infantil, pero existe el conocimiento teórico suficiente, para poder afirmar que dentro de las posibles causas se encuentran la alimentación, los factores ambientales y las causas genéticas y epigenéticas [4].

3.1 Los tumores ependimarios.

En la clasificación de los TSNC se encuentran los gliomas que son las neoplasias más frecuentes y representan casi la mitad de todos los tumores cerebrales. Los gliomas con base en los fenotipos patológicos y dependiendo del tipo celular predominante se encuentran divididos en: ependimoma (EP), astrocitoma, oligodendroglioma, glioma del tronco encefálico, glioma del nervio óptico y gliomas mixtos. El EP de acuerdo con su localización anatómica puede ser de dos tipos: infratentorial o supratentorial con una incidencia del 50.9% y 49.1%, respectivamente (Fig. 2); estos tumores presentan un índice de supervivencia del 43 al 55% de los casos [5,6].

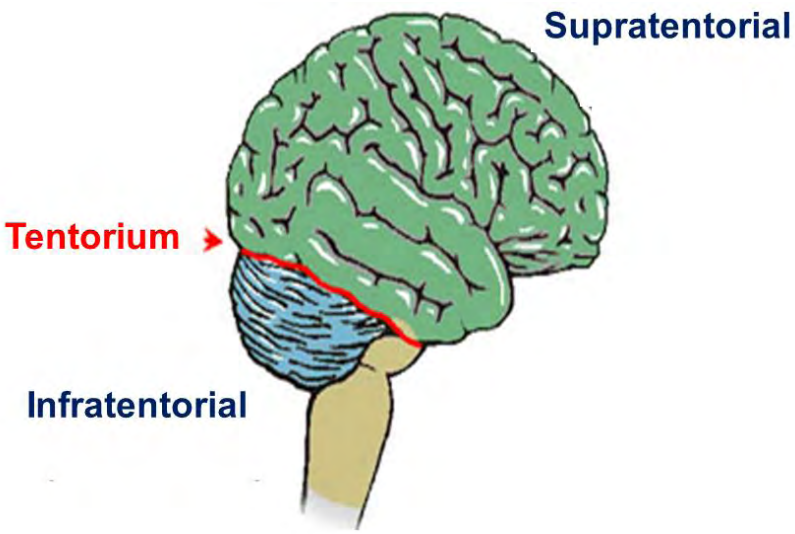

Figura 2. Localización anatómica de las regiones. En rojo se muestra el tentorium, en verde la región supratentorial; en azul y café la región infratentorial.

El tumor ependimario o EP es de crecimiento lento que se origina a partir de las células ependimarias del sistema ventricular, plexos coroides y del canal central de la médula espinal. Se forman principalmente en el cuarto ventrículo y en menor frecuencia del parénquima cerebral como resultado de la migración de las células ependimarias durante la embriogénesis [2,4,7]. La característica histológica de los

EP es la formación de rosetas o pseudorosetas, apariencia adquirida por la formación de células apiladas alrededor de vasos sanguíneos [8]. Es importante mencionar que la Organización Mundial de la Salud (WHO, por sus siglas en inglés) clasifica a los EP por variantes histopatológicas, para la edad pediátrica son: EP clásico (WHO grado II) y anaplásico (WHO grado III) [9]. En 2016 se incorporaron características moleculares a la clasificación del EP con objetivo de subdividir estos tumores, por lo que ha sido aceptado el ependimoma RELA-fusión positiva, esta variante explica el 80% de los tumores supratentoriales en niños [10].

3.2 Importancia de las Alteraciones Cromosómicas en Ependimomas

La citogenética combinada con enfoques moleculares, incluyendo la preparación de cariotipos, hibridación fluorescente *in situ* (FISH) e hibridación genómica comparada (CGH), ha dado lugar a la identificación de regiones del genoma que contienen una variedad de genes supresores de tumores y oncogenes. Se conoce a la inestabilidad genómica como un sello distintivo del cáncer y acelera el proceso de transformación celular (Fig. 3). Este conocimiento puede conducir a la aplicación clínica de estrategias de tratamiento en pacientes con EP, tales como las anomalías genéticas específicas con relación a la ubicación anatómica del tumor (intracraneal o espinal) [11–13].

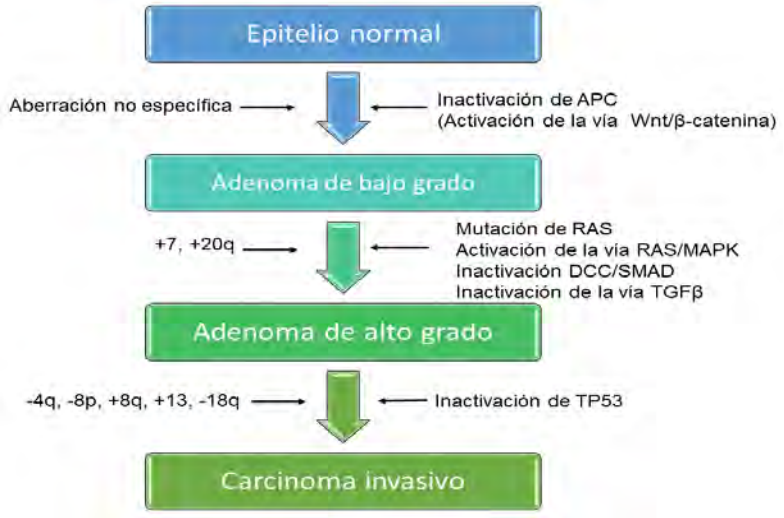

Figura 3. Modelo de progresión en cáncer colorrectal. Se observa que a mayor grado histopatológico mayor número de alteraciones cromosómicas (Tomado y modificado de Grade et al., 2015 [12]).

Las aberraciones cromosómicas se relacionan frecuentemente en eventos de tumorigénesis y se han visto asociadas con la localización anatómica [14], estas alteraciones pueden ser de amplificación o de pérdida cromosómica principalmente. Algunas de las encontradas frecuentemente en los EP son: ganancia en los cromosomas 1q, 7p, 9q,12,15q y las pérdidas de los cromosomas 6q, 14q [5,14,15]. Con menor frecuencia, se han detectado pérdidas cromosómicas en 2q, 4q, 5q, 6q, 7q, 9p, 10q, 15q, 16, 17p, 19p, y 21 [16]. Las aberraciones cromosómicas pueden ser las causantes de la pérdida de expresión de genes como EGFR y sobreexpresión de genes de las vías de NOTCH y Sonic Hedgehog en EP intracraneales [17,18].

3.3 Metilación

La metilación es considerada una modificación epigenética que provoca un cambio fenotípico en las células con consecuencias funcionales; lo cual es importante durante los procesos biológicos como el desarrollo embrionario, diferenciación de células madre, expresión de genes tejido-específico [19]. El DNA metilado es un prerrequisito para la expresión de genes o diferenciación de tejido y un mecanismo significativo de silenciamiento génico [20,21]. La pérdida global de metilación de DNA es conocida como una aberración epigenómica asociada a carcinogénesis y progresión de cáncer que afecta predominantemente a elementos repetidos [22]. En estudios recientes sobre metilación en tumores cerebrales se definieron modificaciones epigenéticas, que afectan múltiples genes de regulación celular como reparación del DNA, migración celular y apoptosis [23].

3.4 Metilación en Ependimomas

Actualmente, en la investigación molecular de los EP, como en muchos otros tipos de cáncer, se ha pensado que en la epigenética se puede encontrar las respuestas a las diferentes preguntas planteadas, así como entender la formación, el establecimiento y el mantenimiento tumoral, la búsqueda de blancos terapéuticos, factores pronóstico y respuesta al tratamiento, por lo que en los últimos años se ha tenido un creciente número de publicaciones que abordan los aspectos epigenéticos de los EP. Además del conocimiento sobre las características biológicas, se ha logrado proporcionar nueva información sobre la patogénesis para facilitar el

diagnóstico y contribuir a una mejor estratificación de grupos de riesgo durante la terapia [8].

En algunos trabajos de investigación se identificaron genes potencialmente importantes en la patogénesis del EP independientemente del subtipo clínico e histológico del tumor, entre éstos se encuentra *RASSF1A* que es transcripcionalmente silenciado por la metilación en la mayoría de los EP, sugiriendo que *RASSF1A* tiene función de gen supresor de tumor. El hecho de que la metilación es casi del 100% en todos los sitios CpG sugiere a la inactivación de *RASSF1A* como un evento temprano en la tumorigénesis. Por otra parte, *CASP8*, *TFRSF10C*, y *TFRSF10D* son genes implicados en la vía de la apoptosis por TRAIL. Lo anterior propone a la metilación de *CASP8* como característica de los EP mixopapilares de bajo grado, mientras *MGMT* está implicado en la reparación del DNA en los diferentes subtipos de EP [8]. También se ha demostrado que la hipermetilación de genes como: *HIC1*, *RASSF1A*, *CDKN2A*, *CDKN2B*, *MCJ MGMT*, *p73* y los genes relacionados con la vía de apoptosis TRAIL (*CASP8*, *TNFRSF10B* y *TNFRSF10D*) son un mecanismo importante en la patogénesis de EP pediátrico espinal y supratentorial. La hipermetilación de los promotores de *BEX1*, *CCND2*, *BAI2* y *CDKN2A* se correlaciona fuertemente con la disminución de los niveles de expresión de estos genes, además *BEX1* se considera un candidato a gen supresor de tumor en EP intracraneal [18,24]. Otros genes metilados comúnmente en EP son *BLU*, *GSTP1*, *DAPK*, *FHIT*, *RARB*, *TIMP3*, *THBS1* [19]. Los tumores ependimales pueden compartir patrones de metilación similares donde, generalmente, los ependimomas supratentoriales y espinales tienen perfiles hipermetilados, similares al fenotipo metilador de islas CpG (CIMP, por sus siglas en inglés) descrito en otros tumores malignos [25].

3.5 Expresión Génica en Ependimomas

La expresión génica es el resultado de diversos procesos de regulación como son las alteraciones cromosómicas y la metilación, por lo que entre las anomalías cromosómicas, los investigadores comenzaron a identificar genes que mostraron expresión alterada en EP. El objetivo de estos estudios fue correlacionar la expresión génica alterada con la agresividad del tumor, se estableció a *BCL-2* como

un biomarcador vinculado al comportamiento agresivo del tumor. Los bajos niveles de expresión de caspasa-3 en EP no están asociados con el comportamiento biológico o pronóstico de EP [26]. La pérdida de *RAC2* en 22q13 o la amplificación de *TPR* en 1q25 se asoció significativamente con la supervivencia global en pacientes más jóvenes [27], se han reportado cambios en la expresión de los genes *EGFR*, *CDKN2A*, *p16INK4A* y *p14ARF* [28,29]. Además, el análisis de EP ha revelado un perfil de expresión genética anormal y rutas moleculares aberrantes que conducen al desarrollo y la progresión de estos tumores, dichos análisis pueden contribuir a la identificación de objetivos terapéuticos potenciales. La sobreexpresión de *TNC* en ependimomas pediátricos está asociada con el aumento de la vascularización tumoral, disminución del intervalo de recaída y un mal pronóstico general en otros tumores cerebrales [29]. Datos recientes muestran que la microinvasión tumoral en tejidos cerebrales adyacentes y la expresión tumoral de *MMP2* y *MMP14* son predictores significativos en la supervivencia libre de progresión en EP pediátricos [30]. Por otra parte, AIF1, múltiples alelos MHC de clase II (HLADMA, HLA-DMB, HLA-DPB1, HLA-DRB5 y CD74) y LILRB1 se han asociado con la actividad de microglia/macrófagos, los componentes celulares clave del sistema inmune han correlacionado con un buen pronóstico en EP [31].

3.6 Biomarcadores tumorales

En los últimos años se originó una cadena de estudios sobre perfiles moleculares que muestran anomalías genéticas únicas para los tumores cerebrales pediátricos para ayudar a un mejor diagnóstico. Se ha sugerido a los perfiles epigenéticos como una manera segura de categorizar a los tumores cerebrales y posteriormente la aplicación racional y correcta de la terapia específica. Se sugiere que los marcadores basados en la metilación son una buena estrategia de diagnóstico; este tipo de análisis puede proporcionar información relacionada con el diagnóstico, pronóstico y seguimiento [32,33]. Se debe de tomar en cuenta las condiciones ideales para los procesos de descubrimiento y/o desarrollo de biomarcadores que deben incluir: 1) la necesidad de tales biomarcadores, 2) el uso previsto (por ejemplo, progresión del tumor o respuesta a tratamiento), 3) pacientes bien caracterizados, 4) muestras conservadas en bancos o pueden ser colectados de forma prospectiva y 5) un método específico [32].

La comprensión de cómo la arquitectura de la cromatina, la metilación del DNA y la expresión génica afectan el desarrollo del cáncer cambian la opinión sobre la carcinogénesis, así como la clasificación y estadificación del cáncer. Las modificaciones epigenéticas y genéticas podrían convertirse en biomarcadores útiles para el pronóstico y el tratamiento de la enfermedad (Fig. 4) [34].

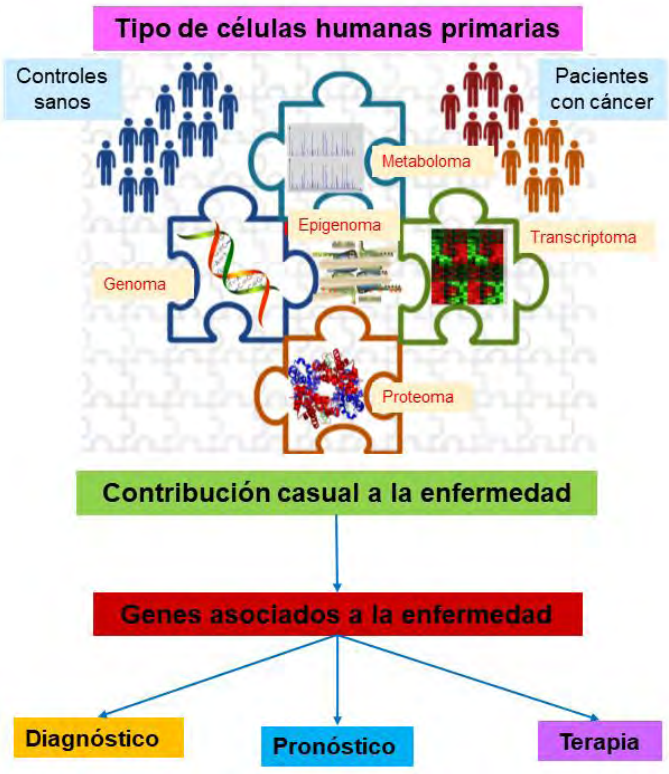

Figura 4. Análisis integrado del genoma completo. Los datos integrados de diferentes plataformas conducen a una mejor comprensión de la base del cáncer y el camino hacia la medicina personalizada (Tomado y modificado de Nebbioso et al., 2018 [34]).

4. Justificación

Los tumores de sistema nervioso central son un problema de salud pública, al considerarse la segunda causa de muerte infantil y la primera en neoplasias sólidas. Los ependimomas representan el 5% de los TSNC, estos tumores en algunos países llegan a ser diagnosticados hasta 200 casos por año, con una reincidencia del 50% de los casos después de 5 años al momento del diagnóstico.

Actualmente, no se tiene el conocimiento suficiente para comprender el desarrollo de los tumores ependimarios y la posible identificación de genes candidatos a biomarcadores moleculares de pronóstico. De acuerdo con la literatura se sabe que las alteraciones cromosómicas, los cambios en la expresión génica y el patrón de metilación puede ayudar a comprender el desarrollo y la progresión tumoral. Este conocimiento permite postular genes candidatos a biomarcadores de pronóstico y en un futuro de diagnóstico.

La generación de biomarcadores en ependimomas permitirá que los pacientes con esta neoplasia puedan acceder a un pronóstico más preciso, al conocer la esperanza de vida y el tiempo libre de reincidencia. Además, estos biomarcadores, podrán permitir conocer el tratamiento adecuado para el paciente.

5. Objetivos

3.1 Objetivo General

Encontrar genes asociados a los ependimomas pediátricos y posibles genes candidatos a biomarcadores moleculares como factor, por medio del análisis de alteraciones cromosómicas, expresión de RNAs mensajeros y el cambio en el estado de metilación.

3.2 Objetivos Particulares

1. Identificar alteraciones cromosómicas y cambios en el patrón de metilación en ependimomas pediátricos.
2. Conocer la expresión génica en ependimomas pediátricos.
3. Relacionar las alteraciones cromosómicas, metilación y expresión de genes asociados a los ependimomas pediátricos.
4. Conocer los cambios moleculares (alteraciones cromosómicas, cambio en el estado de metilación, expresión génica y proteica) con potencial para ser utilizados como biomarcadores de pronóstico en ependimomas pediátricos.
5. Evaluar la expresión proteica por ensayos de inmunohistoquímicas en tejido tumoral de ependimomas pediátricos.
6. Correlacionar la expresión de proteínas con la sobrevida y el tiempo libre de progresión de los pacientes diagnosticados.

6. Hipótesis

- Si conocemos las alteraciones cromosómicas, los cambios en la metilación y la expresión génica entonces se identificarán los cambios moleculares involucrados en la tumorigénesis.

- Si se realiza una revisión sistemática y un meta-análisis, entonces se conocerán los cambios moleculares ya descritos con potencial a ser utilizados como biomarcadores moleculares.

- Si evaluamos la expresión proteica correlacionándola con la reincidencia y la sobrevida del paciente, entonces se determinará si estas proteínas pueden ser utilizadas como marcadores diagnósticos.

7. Diseño Experimental

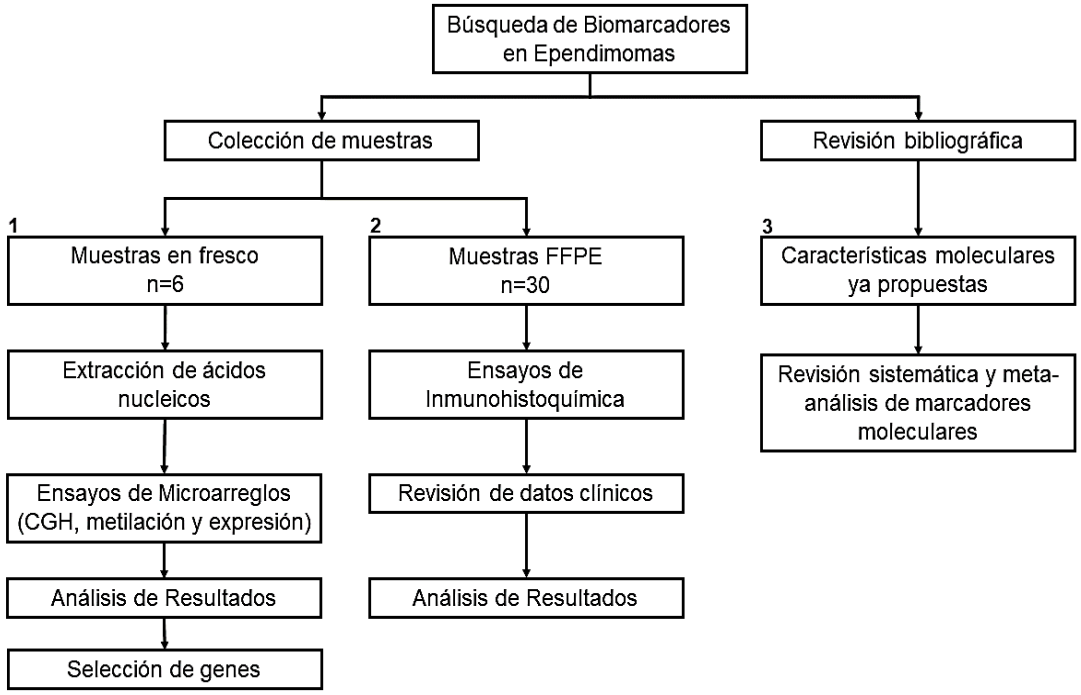

Figura 5. Diseño Experimental. Se muestra el proceso utilizado durante el desarrollo del proyecto. Los números indican el proceso para el cumplimiento de los objetivos. 1. Cumple con los objetivos de 1 al 3; 2. Hace referencia a los objetivos 5 y 6; 3. Comprende el objetivo 4.
FFPE. Muestras embebidas en parafina.

7.1 Duración del estudio

El estudio se realizó en un periodo de 4 años, de agosto de 2014 a julio de 2018, en la Unidad de Investigación Médica en Genética Humana del Hospital de Pediatría "Dr. Silvestre Frenk Freud" del Centro Médico Nacional "Siglo XXI", Instituto Mexicano del Seguro Social (IMSS), con colaboración del Servicio de Patología de Hospital de Pediatría "Dr. Silvestre Frenk Freud" del Centro Médico Nacional "Siglo XXI", IMSS y con los Servicios de Neurocirugía Pediátrica del Hospital General "Dr. Gaudencio González Garza", Centro Médico Nacional "La Raza", IMSS y del Hospital Infantil de México Federico Gómez.

El proyecto fue aprobado por la Comisión Nacional Científica con los números R-2009-785-042 y R-2014-785-094.

8. Resultados

Se identificaron las alteraciones cromosómicas, los cambios en el estado de metilación y la expresión génica en pacientes pediátricos diagnosticados con Ependimoma. Se colectaron seis muestras de tejido fresco; este número de muestras se consiguió durante dos años de colecta, debido a la baja incidencia de los tumores ependimarios. Se realizaron ensayos de microarreglos de Hibridación Genómica Comparada, Metilación y Expresión Génica en la plataforma Agilent, lográndose identificar alteraciones cromosómicas, genes hipermetilados e hipometilación, genes subexpresados o sobreexpresados, se conoció la correlación entre los diferentes resultados y se identificaron las vías de señalización involucradas en los EP pediátricos. Estos resultados se describen en Pérez-Ramírez *et al.,* 2016 “Genomics and epigenetics: A study of ependimomas in pediatric patients” (Artículo 1 y Anexo I)

Se describieron los cambios moleculares en EP pediátricos con base en la literatura internacional. Se realizó una revisión bibliográfica en la base de datos PubMed de NCBI de los artículos publicados de enero 1990 a junio de 2016. Las contribuciones internacionales se presentan cronológicamente, se hace referencia a las alteraciones cromosómicas, cambios en el estado de metilación, microRNAs, variaciones en la expresión génica y proteica. Los resultados se encuentran en Pérez-Ramírez *et al.,* 2016 “Pediatric Ependymoma: Molecular Characteristics and Useful Prognostic Markers” (Artículo 2).

Actualmente, no existen características moleculares específicas para los EP pediátricos con potencial para ser utilizados como biomarcadores de pronóstico, por lo cual fue de interés conocer las alteraciones cromosómicas, el cambio en el estado de metilación y de expresión génica que son candidatos a biomarcadores moleculares en EP pediátricos. Se realizó una revisión sistemática a partir de artículos encontrados en las bases de datos PubMed, Embase, Scopus y Medline. Se propone a la región 1q25 y al gen *hTERT,* entre otros como característicos de los EP pediátricos. La información se describe en el artículo enviado Pérez-Ramírez

et al., “Molecular characteristics of pediatric ependymoma: a systematic review” (Artículo 3).

Se evaluó la expresión proteica y se correlacionó con la reincidencia y la sobrevida de los pacientes. Se evaluaron las proteínas GNAO1, ASAH1, IMMT, IPO7, Ciclina D1, P53 y KI67 en tejido tumoral de EP pediátricos, estas proteínas fueron seleccionadas por su importancia biológica. Se colectaron 30 muestras embebidas en parafina procedente de archivos histopatológicos; se evaluó la expresión proteica por medio de ensayos de inmunohistoquímica, los resultados se correlacionaron con la edad, género, grado histopatológico, reincidencia y sobrevida. Los resultados se describen en el artículo en preparación Pérez-Ramírez *et al.,* “Pediatric ependymoma: GNAO1, ASAH1, IMMT and IPO7 protein expression and prognosis correlation” (Artículo 4 y Anexo II).

Además, se identificaron las alteraciones cromosómicas, los cambios en el estado de metilación y la expresión génica en tumores germinales intracraneales pediátricos. Se realizaron ensayos de microarreglos en la plataforma Agilent. Se observó que estos tumores comparten algunos cambios moleculares con los EP pediátricos. Los resultados se describen en Pérez-Ramírez *et al.,* 2017 “Pediatric pineal germinomas: Epigenetic and genomic approach” (Artículo 5 y Anexo III).

Artículo 1
Genomics and epigenetics: A study of ependimomas in pediatric patientes

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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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0303-8467/

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.

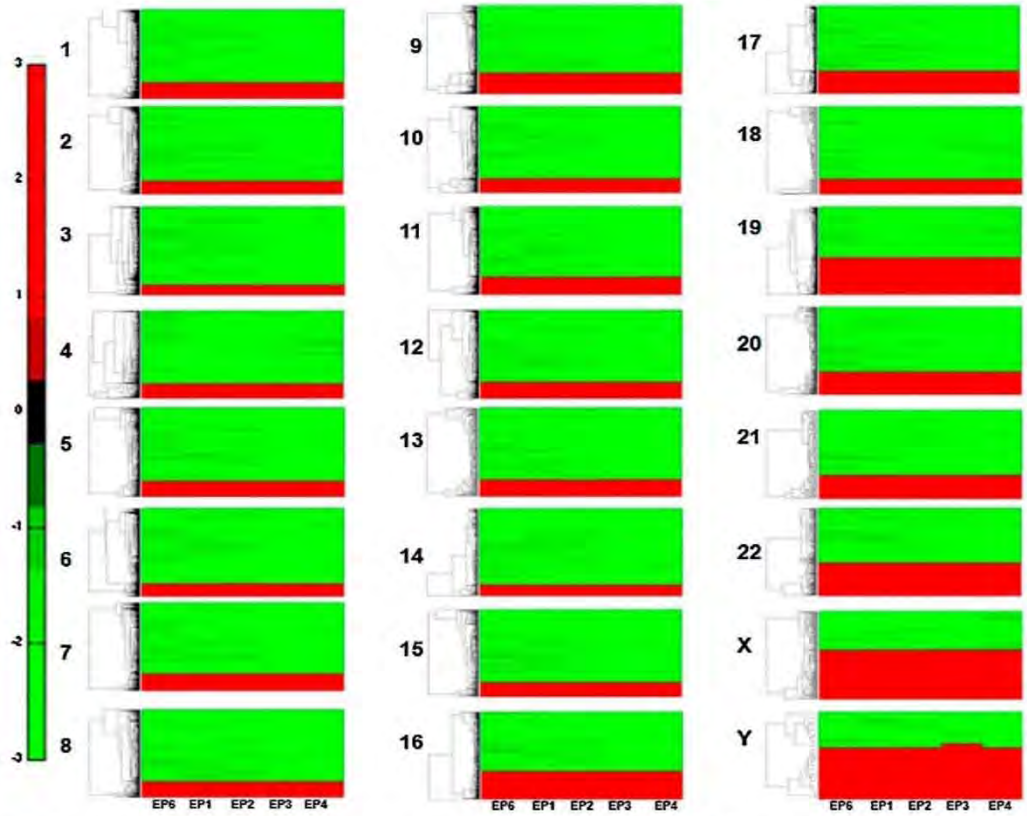

Fig. 1. Ependymoma methylation pattern. The figure shows a cluster that represents the methylation pattern in the studied ependymal tumors; data were grouped according to their normalized value and analyzed for each chromosome (Chr). Hypermethylation values range from 0.5 to 3 and hypomethylation values range from −0.5 to −3, according to the color scale.

Table 1
Sample features. The features of the patients from whom the samples were obtained are shown age (Age), gender (Sex), and tumor location (Localization). Histopathological grade classification was according to the World Health Organization (Grade) and whether the tumor was de novo or recidivism (Observations).

Sample	Age	Sex	Grade	Localization	Observations
EP1	15	M	II	Posterior fossa	*De novo*
EP2	15	M	III	Posterior fossa	*De novo*
EP3	13	F	II	Spinal cord (L2-L5)	Recidivism
EP4	9	F	II	Posterior fossa	*De novo*
EP5	2	F	II	Posterior fossa	*De novo*
EP6	15	M	II	Posterior fossa	*De novo*

3. Results

3.1. Histopathologic features

The collected tumors were located in the posterior fossa and only one in spinal cord, as seen in Table 1; all the analyzed samples had the diagnosis of ependymoma.

3.2. Chromosomal alterations

In the aCGH results, amplifications and deletions for each of the samples were found, it is worth noting the 8p11.23-p11.22 regions, which was found, deleted in four samples and amplified in sample EP4, and the region 14q32.33 was found amplified in four samples. In Table 2 are shown chromosomal alterations found in the analyzed samples.

Table 2
Chromosomal alterations. We show the chromosomal alterations found in over 50% of the samples, which include amplifications at 1q21.3, 2p22.2, 2p22.3, 3p22.2, 7p13, 7p15.2, 8p22, 10q21.1, 11p15.4 and 14q24.3. Also show deletions at 2p11.2, 2p22.3, 7q34, 11q11, 16q12.2 and 17q12. We observe that chromosome 2p had the highest number of chromosomal alterations.

CHR	Size (pb)	Deletion	Amplification
1p31.1	26625	EP1, EP2, EP6	EP3
1q21.3	25495	EP1, EP2, EP4, EP6	
2p11.2	148728		EP1, EP2, EP3, EP6
2p22.2	63459	EP1, EP2, EP3	
2p22.3	32424		EP1, EP2, EP3, EP6
2p22.3	22854	EP1, EP2, EP3	
3p22.2	5239		EP1, EP2, EP6
7p13	6741		EP1, EP2, EP6
7p15.2	48136		EP1, EP2, EP6
7q34	15385	EP1, EP2, EP6	
8p22	20332		EP1, EP2, EP6
8p11.23-p11.22	151166	EP1, EP2, EP5, EP6	EP4
10q21.1	18948		EP1, EP2, EP6
11p15.4	19765		EP2, EP3, EP6
11q11	82634	EP1, EP2, EP3	
12p13.31	81523	EP1, EP6	EP3
14q24.3	9884		EP1, EP2, EP6
14q32.33	13454		EP1, EP2, EP3, EP4, EP5, EP6
15q11.2	1338278	EP1, EP2, EP4, EP6	
16q12.2	23189	EP1, EP2, EP6	
17q12	25109	EP1, EP2, EP6	

The analysis showed that EP1 had 30 amplifications and 32 deletions; EP2 had 24 amplifications and 36 deletions; EP3 had 141 amplifications and 18 deletions; EP4 had 24 amplifications and 13 deletions; EP5 had 19 amplifications and 17 deletions and finally

EP6 had 57 amplifications and 42 deletions. Some of amplified the regions were shared in 33% of the processed samples: 1q24.2 (EP2, EP4), 3q26.1 (EP4, EP6), 4p15.1 (EP1, EP2), 7q33 (EP1, EP6), 7q34 (EP3, EP4), 17q21.31 (EP4, EP6) and deleted regions: 6p21.32 (EP1, EP6), 7q34 (EP2, EP6), 7q35 (EP1, EP3), 8p23.1 (EP1, EP2), 9p24.1 (EP2, EP6) and 19q13.42 (EP1, EP3).

3.3. Ependymal tumor methylation

We found 42.387 CpG islands modified in their methylated state. Five samples were considered, excluding EP5 because the sample quality issues. We observed a similar methylation pattern, shared among the ependymal tumors. The data were represented via hierarchical clustering (Fig. 1). EP1, EP2 and EP6 methylation showed similar patterns, and EP3 and EP4 showed similarity in almost all chromosomes. The figure shows the generated cluster for each chromosome; hypomethylation which is displayed as banding in dark hues.

We found 272 altered genes, 106 genes were hypermethylated and 166 were hypomethylated (Table S2, DOI: 10.6070/H4RN35WP). Hypermethylated genes were found involved in the following pathways: endocytosis, axon guidance, neuroactive ligand receptor interaction and the phosphatidylinositol signaling system. Hypomethylated genes were found involved in: the TGF-β signaling pathway, the Hedgehog signaling pathway, cancer pathways, Parkinson's disease, the Wnt signaling pathway, inositol phosphate metabolism, cell cycle, the MAPK signaling pathway, Fc gamma R mediated phagocytosis, glioma, RNA degradation, Alzheimer's disease, regulation of the actin cytoskeleton, the chemokine signaling pathway, the T cell receptor signaling pathway, the phosphatidylinositol signaling pathway, adherent junction and the GnRH signaling pathway (Fig. S1, DOI: 10.6070/H4W95763). According to published reports about methylation in other cancers and our results in EP, changes in the patterns and altered genes, which coincide with the literature (Table S3, DOI: 10.6070/H4RN35WP).

3.4. Gene expression

We found 481 modified genes; 219 overexpressed genes and 262 underexpressed genes (reported in Table S4, DOI: 10.6070/H4RN35WP). These values were represented with a hierarchical cluster in which we could observe an expression pattern from ependymal tumors. We observed overexpression in EP4 of *PKP3*, *KREMEN2*, *EPS8L1*, *SHOX* and *CD8B* genes; in EP2 of *AQP4*, *DPP10*, *BHLHE22* and *NETO2* genes; in EP5 *KCNC2*, *NEUROD1*, *HSD17B6*, *RGS7*, *CDK5R1*, *CNR1*, *STYK1*, *SCN3B*, *SV2B*, *PRKCB*, *KIF5A*, *VSTM2A*, *SCG5* and *SNAP25* genes (See Fig. 2).

According to the literature, we correlated gene expression reported in other important malignancies. We also considered the methylation status found in EP (Table S5, DOI: 10.6070/H4RN35WP). From these findings, we determined the biological function and participation in regulatory pathways such as cancer pathways, primary immunodeficiency, transendothelial leukocyte migration, ECM receptor interaction, the p53 signaling pathway, cell adhesion molecules (CAMs) and the VEGF signaling pathway, which are known to be important in carcinogenesis (see Table 3).

We identified eight genes that correlated among the three studies, the methylation pattern, chromosomal alterations and expression profiles (see Table 4)

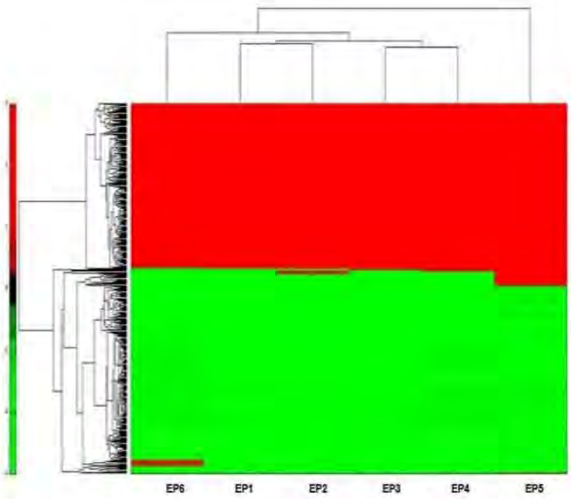

Fig. 2. Gene expression heatmap. The figure shows a cluster representing gene expression in ependymal tumors. Data were grouped according to their normalized value. Overexpression was 0.5 to 3 and underexpression was −0.5 to −3 as shown in the color scale.

Table 3
Biological function and cancer pathways. Shows genes (Gene) with expression changes (Status) involved in a carcinogenesis pathway (pathway).

Gene	Pathway	Status
BID	Pathways in cancer, p53 signaling pathway	Under
CBLB	Pathways in cancer	Over
CCNB2	P53 signaling pathway	Over
CDK1	P53 signaling pathway	Over
FGF22	Pathways in cancer	Under
HSP90AA2	Pathways in cancer	Over
HSP90B1	Pathways in cancer	Over
ITGA6	Pathways in cancer	Over
KIT	Pathways in cancer	Over
LAMA2	Pathways in cancer	Over
MAPK11	VEGF signaling pathway	Under
MDM2	Pathways in cancer, p53 signaling pathway	Over
PRKCB	Pathways in cancer, VEGF signaling pathway	Under
PRKCG	Pathways in cancer, VEGF signaling pathway	Under
PTK2	Pathways in cancer, VEGF signaling pathway	Under
RAC2	Pathways in cancer, VEGF signaling pathway	Under
RAD51	Pathways in cancer	Over
SESN1	P53 signaling pathway	Over
STAT1	Pathways in cancer	Over
WNT10B	Pathways in cancer	Under

Table 4
Correlation between studies. *Abbreviations*: A. amplification, D. deletion, Hypo. hypomethylation, Hyper. hypermethylation. Over. overexpression. Under. subexpression.

CHR	Gene	aCGH	Methylation	Expression
2p11.2	IMMT	A	Hypo	Over
7p13	FLJ35390	A	Hyper	Under
7q34	JHDM1D	D	Hypo	Over
8p22	ASAH1	A	Hypo	Over
10q21.1	ZWINT	A	Hypo	Over
11p15.4	IPO7	A	Hypo	Over
16q12.2	GNAO1	D	Hypo	Under
17q12	CISD3	D	Hypo	Under

4. Discussion

Genomic and epigenetic studies contribute to the understanding of oncogenic processes and there have been many different genomic approaches to EP tumors that have discovered that structural chromosomal rearrangements and epigenetic abnormalities that contribute to tumor development [22]; in aCGH studies, it was realized that ependymal tumors have amplification in regions 1q, 7p, 9q [9], 12 [23] and 5p [24] and deletion in chromosomes 6q

and 14q [25]. We found amplifications in 2p11.2, 2p22.3, 3p22.2, 7p15.2, 8p22, 10q21.1, 11p15.4 and 14q32.33 and deletions in 1p31.1, 1q21.3, 2p22.2, 7q34 and 8p11.23-p11.22. Olsen et al. [24] found that the 14qter region was deleted. This chromosomal region is proposed to be important in the development of ependymal tumors. It is also important to mention that chromosomal alterations and methylation vary according to the anatomical location of the tumor [26], as displayed in sample EP3 showing the unique sample located at the spinal cord. There is evidence that methylation is a major developmental mechanism more so in the EP due to tumor development in 89% of cases [27]. Because its location makes them molecularly distinct, the medullar EP has a profile for more overregulation and hypermethylation compared with those located in the posterior fossa [22]. We visualized these differences in sample EP3. However, a similar pattern of methylation was observed in the other EPs, identifying methylation as a characteristic marker.

Currently, EP has been reported to have many hypermethylated genes. Those that are considered as tumor suppressors include *CDKN2A, CDKN2B, HIC1, RASSFI1, CASP8, MGMT, TP73* [9] and *RB1* [28]; cell cycle progression *CDKN2/p16/MTS1*; DNA repair *MLH1*; cell adhesion *CDH1, E-Cadherin*; and members of the Sonic Hedgehog and Notch pathways [14]. We found methylated genes that were mainly related to apoptosis (*MYC*), cell cycle (*SESN2, MAD1L1, CUX1, E2F8*), neuronal differentiation (*EBF3*), transcriptional activation (*GATA2*) and the oncogene related genes *TFGβ, p53* and *c-myc*, as well as cellular division (Table S2, DOI: 10.6070/H4RN35WP). We found hypomethylated genes that have been reported to be overexpressed such as: *IGFBP5, WNT5A* [29] and *CCND1* [13], suggesting that this could be due to changes in the methylation pattern.

It is known that the number of abnormalities and changes in gene expression alter signaling pathways and may contribute to the development and progression of tumors. Studies have actually reported expression changes in genes such as *EGFR, CDKN2A, p16INK4A,* and *p14ARF*, while others involved in tumorigenesis included *CLU, IGF2, RAF1, MMP12, PSAP, MSX1* and *BMP4* [30]. We found overexpressed genes related to transcription (*MAPK11*), cell cycle (*MDM2, CCNB2, CDK1*), proliferation (*CBLB, KIT, SESN1*), tumor formation (HSP90B1), cellular adhesion (*ITGA6*), migration (*LAMA2*), DNA repair (*RAD51*) and underexpressed genes related to apoptosis (*BID, PRKCB*), cellular adhesion (*PTK2*) and tumor growth (*FGF22*) (Table S4, DOI: 10.6070/H4RN35WP). These changes suggest involvement with initiation, progression and establishment of the tumor and possess that capability to evade cellular processes as was found in the downregulation of genes involved in apoptosis, cell growth and cell adhesion. While genes related with proliferation, migration and tumor formation were found to be overexpressed. Based on the methylation pattern and gene expression, we observed genes already reported in glial and epithelial tumors that shared characteristics with the ependymal tumor, suggesting potential importance during tumor formation and maintenance.

In the correlation between genes with an altered methylation pattern and chromosomal abnormalities, we propose as a result of the chromosomal abnormalities that some found genes may have hypomethylation or hypermethylation (Table S6, DOI: 10.6070/H4RN35WP). We found 156 genes from which approximately 50% were suggested to show this relationship such as: *NEGR1, ILF2, RPRD2, CHRNB2, EPBH6, BAG4, KIF26A* and *JAG2*. It is known that the expression level depends on hypomethylation or hypermethylation, and this was corroborated with the comparison between expression and the altered methylation pattern; underexpressed genes were hypermethylated and overexpressed genes were hypomethylated (Table S7, DOI: 10.6070/H4RN35WP). Regarding the comparison of expression changes and chromosomal abnormalities, we observed 15 genes that correlated, underexpressed genes were deleted and overexpressed genes were amplified (Table S8, DOI: 10.6070/H4RN35WP). Eight genes correlated in the three studies; we assume that the methylation pattern and chromosomal alterations have an impact on gene expression. Thus, according to their biological function and the literature, we consider the *IMMT, FLJ35390, JHDM1D, ASAH1, ZWINT, IPO7, GNAO1* and *CISD3* genes important because they act during maintenance and tumor formation. Because they interact with other genes related to growth factors (*A2M*), tumor suppression (*APC*), neuronal plasticity development (*PRNP*), cell cycle and differentiation (*PTPN11*), neuronal adhesion (*APP*) and anti-apoptotic genes (*BAG3*) [20].

The importance of these genes has been reported in several studies. The *CISD3* gene was found underexpressed in ependymomas despite being considered necessary for tumor cell proliferation by preventing cell death [31], possibly as a result of chromosomal deletion. The *IMMT* gene correlates with an aggressive phenotype because it has been found to be overexpressed in gliomas, ependymomas and oligodendroglioma and is related with cell proliferation and the promotion of angiogenesis [32]. This implicates their importance in tumor development. The *ZWINT* gene is related with kinetochore formation and it has been reported that its low expression causes mitotic arrest and its high expression promotes cell proliferation [33].

These genes found in the EP have been reported in colorectal and prostate cancer [34], such as overexpressed IPO7, a nuclear protein that is upregulated by c-Myc and p53; when IPO7 decreases their expression triggers the activation of p53 and thus cell arrest [35]. *JHDMD1*, another overexpressed gene, has been seen mainly in neurons compared to other CNS cells such as astrocytes and microglia [36]. The histone demethylase *JHDM1D* is highly expressed in various cancer cells in response to nutrient starvation, and subsequent suppression of solid tumor growth was associated with the downregulation of several proangiogenic factors such as *VEGFβ*. It has also been shown to be involved in neural development, but its role in tumor progression has not been elucidated [29].

GNAO1 has been reported as an important signaling molecule in the CNS and peripheral nervous system and is abundantly expressed in brain tissue. It constitutes up to approximately 0.5% of membrane proteins and likely serves an important role in brain function [37]. Although *GNAO1* was identified as part of the human plasma proteome, the high abundance of the GNAO1 protein was suggested to promote cancer cell viability via pro-apoptotic protein interference [38].

Finally, *ASAH1* (AC) has emerged as important based on the roles for ceramide and sphingosine in regulating cell growth, as these sphingolipids have become important targets in cancer therapy [32]; AC in addition to enhancing cell survival and resistance to programmed cell death, stands as a critical regulator of cell progression, migration, and invasion [39]. Multiple groups involved in sphingolipid cancer research have challenged the mechanisms of action of commonly used chemotherapeutic agents. It is now widely accepted that most of these agents used in cancer treatments are also implicated in cancer cell death via activation of the ceramide pathways [40].

We suggest that the *CISD3, IMMT, JHDMD1, IPO7, GNAO1* and *ASAH1* genes play an important role in the development of EP and are involved in cell proliferation, different metabolic pathways, methylation and other cellular processes. Furthermore, these genes may be used as possible ependymal prognostic markers because it was suggested that they favor maintenance and tumor formation due to participation in cell cycle deregulation, cell migration and perhaps even metastasis. Currently, in the analyzed patients, it was not possible to know whether their genes were involved with metastases. We believe that these genes deserve further study.

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Artículo 2
Pediatric Ependymoma: Molecular Characteristics and Useful Prognostic Markers

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Pediatric Ependymoma: Molecular Characteristics and Useful Prognostic Markers

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Abstract

Ependymoma is a type of brain tumor that originates from ependymal cells in the ventricular system of the brain and the central canal of the spinal cord. Ependymoma tumors remain a public health concern. Over the last several decades, ependymoma tumors have been studied at the molecular level. In pediatric ependymomas, deletions of chromosomes 6q and 22q as well as the amplification of chromosome 1q have been observed. The MMP2, MMP14, PI3K, and MGMT genes have been proposed to be prognostic markers of ependymoma. At the epigenetic level, medullary ependymoma has a different methylation pattern from intracranial EP. The EZH2 and MGMT genes play an important role in cell cycle-related gene expression.

Keywords

Pediatric ependymoma; Characterization; Therapeutic target

Introduction

Central Nervous System Tumors (CNST) comprise between 15 and 20 percent of all neoplasms that occur during childhood and adolescence [1]. According to regional and global incidence reports of CNST in children, there is a considerable degree of variation among different regions and countries. The Central Brain Tumor Registry of the United States (CBTRUS) estimated that the average annual age-adjusted childhood CNST incidence rate was 5.26 per 100,000 individuals in the population [2]. Thus, CNST is a significant public health problem. Ependymoma (EP), a type of CNST, is a slow-growing tumor that originates from ependymal cells in the ventricular system, choroid plexus, and central canal of the spinal cord. EP tumors mainly form in the fourth ventricle but may also arise less frequently in the brain parenchyma as a result of ependymal cell migration during embryogenesis [3].

Over the last several years, EP tumors have been studied at the molecular level to try to understand their development and maintenance (Figure 1). These studies have shown that although intracranial and spinal ependymomas are histopathologically similar, their molecular biology is very heterogeneous, and they possess different DNA copy number alterations, messenger-RNA expression profiles, and genetic and epigenetic alterations as well as diverse transcriptional programs [4].

The first molecular study in EP tumors was published in 1992; the study argued that cytogenetic analysis of EP tumors was important. In fact, in most other neoplasias, cytogenetic abnormalities constitute an important prognostic factor [5]. A complex karyotype of supratentorial pediatric EP revealed a translocation t(10:11:15)(p12;q13;p12) and a breakpoint at 11q13 [5]. Another study reported karyotypes from two EPs. Predominantly well-differentiation EP contained several numerical chromosome aberrations, including monosomy 22. Anaplastic EP, however, had a loss of one chromosome 22 homologue and a breakpoint in the remaining chromosome 22 homologue. These findings suggested the location of a potential ependymoma tumor suppressor gene on chromosome 22 [6]. This report became the basis for considering the deletion of chromosome 22q as a characteristic of EP. Furthermore, the neurofibromatosis type II (NF2) gene, located at chromosome 22q12.2, is associated with a hereditary predisposition for ependymomas; EP is caused by a loss of the Merlin tumor suppressor protein encoded by the NF2 gene. In patients with NF2, EPs are the most common intramedullary spinal tumors, with a predilection for the cervical region [7,8].

Over the following decade, studies began to identify other chromosomal alterations in EP, such as gains on chromosome 9 and losses of chromosome 6q [9]. The frequent gains and high-level amplification of EGFR at 7p11.12 revealed that EGFR protein overexpression is correlated with poor prognosis [10]. A supervised tumor classification showed that a gain of 9qter was associated with tumor recurrence, age older than 3 years, and posterior fossa location. Furthermore, the study found an overexpression of two potential oncogenes at the 9qter locus, Tenascin-C (TNC) and Notch1. The relevance of this critical region led to the application of a candidate-gene strategy on 9q33-34 that includes two oncogenes previously linked to brain tumorigenesis, Notch1 and TNC [11].

Nevertheless, the search continues for which genetic factor promotes chromosomal instability in EP; telomere alterations were proposed to be the promoters of chromosomal abnormalities [12]. Genomic instability is a hallmark of cancer, and it accelerates the process of cell transformation. This knowledge may lead to clinical applications of treatment strategies in EP patients, such as therapeutic targets of specific genetic abnormalities [13,14] relative to the anatomical location of the tumor (intracranial or spinal) [15].

Among the chromosomal abnormalities, researchers began to identify genes that showed altered expression in EP. The aim of these studies was to correlate altered gene expression with tumor aggressiveness, as established for BCL-2, a well-known biomarker linked to aggressive tumor behavior. The low levels of caspase-3 expression in EP are not associated with the biological behavior or prognosis of EP [16]. Furthermore, the loss of RAC2 at 22q13 or the amplification of TPR at 1q25 was significantly associated with shorter overall survival in younger patients [17].

Additional genes have been associated with other malignancies, particularly in brain tumors, including COL4A1, IBP2, HOX7, WEE1, GAL1, WNT5A, and FN1 [18]. These genes are useful

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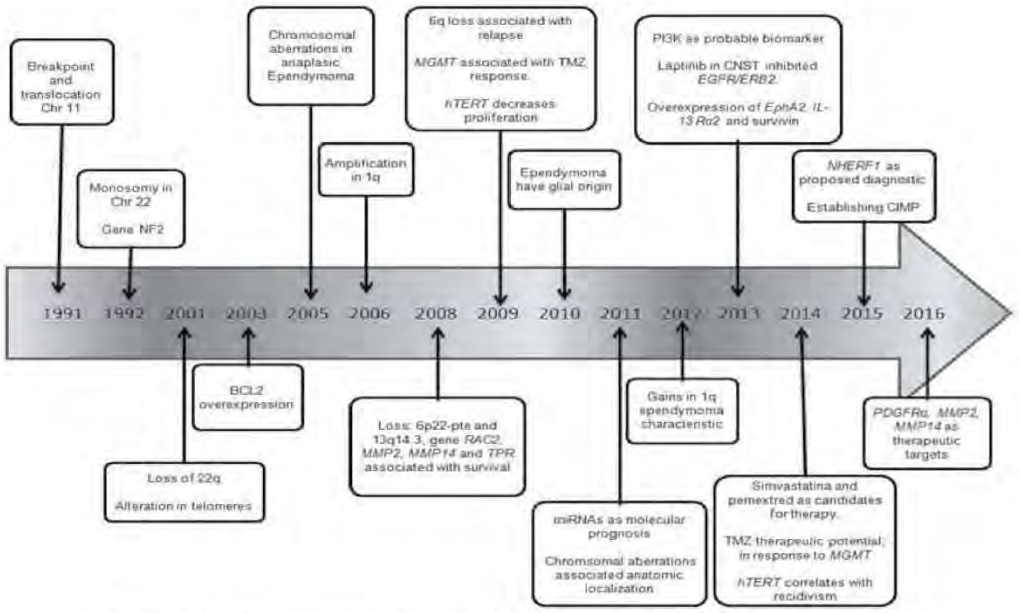

Figure 1 Timeline: The most significant discoveries in EP are shown as well as the year that they were published.

prognostic markers that may be helpful in stratifying patients for adjuvant treatments. Moreover, tumor microinvasion into adjacent brain tissues and tumor expression of MMP2 and MMP14 are significant predictors of overall and progression-free survival in pediatric ependymomas [19].

The P-AKT protein expression, indicating PI3K pathway activation, was observed in 72% of EP tumors, and P-AKT expression was found to be an independent marker of poor progression-free survival. Another association was identified between PI3K pathway activation and cell proliferation. PTEN protein loss was not associated with P-AKT staining and no mutations were identified in PIK3CA [20]. Moreover, AIF1, multiple MHC class II alleles (HLADMA, HLA-DMB, HLA-DPB1, HLA-DRB5, and CD74), and the leukocyte immunoglobulin-like receptor subfamily B member 1 (LILRB1) genes were associated with the activity of microglia/macrophages, the key cellular components of the innate immune system, and were found to correlate with a good outcome in EP [21].

Epigenetic studies of EP proposed that methylation correlated with genomic instability and may act as a driving force of tumorigenesis [22,23]. The methylation pattern of genes related to tumor development has been studied in tumor suppressor (RASSFI1, CASP8, MGMT, TP73), cell cycle regulation (CDKN2, p16, MTS1), DNA repair (MLH1), and Sonic Hedgehog and Notch pathway genes [15,24]. The insufficient response of children with a brain tumor to oral temozolomide (TMZ) may depend on the repair-action of inducible MGMT. The DNA damage caused by TMZ is repaired by MGMT, and this mechanism is likely efficient on combinations of TMZ with other antineoplastic drugs. The promoter region of the MGMT gene is unmethylated in most intracranial ependymomas and may confer chemoresistant properties in glioblastomas [25]. In addition, the let-7d, miR-596, and miR365 microRNAs at 14q32 are associated with time to relapse and life expectancy of EP patients [26].

Recently, new chromosomal alterations have been confirmed to contribute to EP epigenetics. Amplification occurs on chromosomes 1q, 1q24.2, 3q26.1, 4p15.1, 7p, 7q33, 7q34, 9q, 12, 15q, and 17q21.31, while deletions occur on 4q33-qter, 3q23-qter, 6p21.32, 6q, 7q34, 7q35, 8p23.1, 9p24.1, 14q, 18q22.2, and 19q13.42, but less frequently on 2q, 4q, 5q, 10q, 15q, 16, 17p, y 19p (7; 15; 27; 28).

Ependymal tumors may share similar methylation patterns [7] where, generally, supratentorial and spinal ependymomas have hypermethylated profiles, similar to the CpG island methylator phenotype (CIMP) described in other malignancies [27]. While posterior fossa ependymomas exhibit a very low mutation rate overall, their DNA methylation patterns separate them into 2 distinct subgroups; posterior fossa group A tumors exhibit a significantly increased methylation of CpG-islands within promoter regions compared to group B ependymomas [30]. CpG methylated genes showed a remarkable convergence on genes documented as being silenced in embryonic stem cells by the Polycomb repressive complex 2 (PRC2).

The identification of CIMP in EP led to the study of the enhancer of zeste homolog 2 (EZH2) gene, which is primarily responsible for chromatin remodeling through histone methylation. EZH2 converts dimethylated histone H3K27 (H3K27me2) to trimethylated H3K27 (H3K27m3) to form the PRC2 complex. The downregulation of EZH2 inhibits cell growth and cell cycle progression. Furthermore, EZH2 maintains cancer cells in an undifferentiated state, which mirrors the role of EZH2 in promoting tumorigenesis by maintaining the multipotent identity of stem cells. Thus, the EZH2 gene may be a potential therapeutic target in EP.

As the search continues for new therapies to treat EP patients, the mortality and relapse rates of patients treated with conventional

methods still remains high. Radiotherapy is a commonly utilized adjuvant therapy for the treatment of intracranial ependymomas, with postoperative irradiation being utilized in patients with anaplastic tumors. EPs are generally considered resistant to radiotherapy.

Conclusion

Ependymoma analysis revealed abnormal gene expression profiles and aberrant molecular pathways that lead to the development and progression of these tumors. This analysis may contribute to the identification of potential therapeutic targets [25]. Despite the recent identification of driver oncogenes and molecular subtypes of ependymoma, the treatment of children and adolescents with the disease remains challenging.

Overexpression of TNC in pediatric ependymomas is associated with increased tumor vascularity, decreased interval to relapse, and an overall poor prognosis in other brain tumors [25]. Recent data shows that tumor microinvasion into adjacent brain tissues and tumor expression of MMP2 and MMP14 are significant predictors of both overall and progression-free survival in pediatric ependymomas. These results are useful prognostic markers that may be helpful in stratifying patients for adjuvant treatments [19].

Drugs that target DNA CpG methylation, PRC2/EZH2, and/or histone deacetylase inhibitors could represent the first rational strategies for PFA-CIMP+ ependymoma therapy. Moreover, the PRC2 complex is upregulated in more aggressive ependymomas and may be a suitable target for tumors that require adjuvant therapy to treat the disease. Small molecule inhibitors of EZH2, such as 3-Deazaneplanocin A (DZNep) and decitabine (DAC), have already demonstrated promise in derepressing PRC2 target genes in several tumor models and in impairing tumorigenesis. These promising studies suggest that EZH2 is both a crucial mediator of ependymoma proliferation and an amenable therapeutic target.

The investigation of the molecular characteristics of ependymoma led to the conclusion that deletions of chromosome 22q and amplifications of chromosome 1q are the most important chromosomal alterations. At the epigenetic level, medullary ependymoma has a different methylation pattern from intracranial EP. The EZH2 and MGMT genes play important roles in cell cycle-related gene expression and in the immune system. A more detailed understanding of these various genetic aberrations may support the identification of additional specific prognostic markers as well as the development of customized targeted therapies.

Acknowledgments

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Artículo 3: Enviado
Molecular Characteristics of Pediatric Ependimomas: A Systematic Review

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Abstract

The prognosis for patients diagnosed with ependymoma is relatively poor, with a 5-year overall survival rate of 24–75%. Currently, tumors are treated by surgical resection followed by radiotherapy, as resection is the most consistent prognostic marker (up to 80%). Therefore, there is a pressing need to improve our understanding of the biology of these tumors and to develop new therapeutic targets. The present work was a systematic review of the current molecular knowledge of pediatric ependymomas. From January 2000 to December 2017, we carried out a search using "MeSH" (Medical Subject Heading), and "free-text" protocols in the databases Medline/PubMed, SCOPUS, Web of Science, and EMBASE (OVID platform), combining the terms chromosomal alterations, genetic changes, epigenetic changes and protein expression changes. We selected articles with samples from pediatric patients and chose publications with trials. Only thirty-two articles met the criteria for a meta-analysis, suggested by the state of methylation and expression of a characteristic marker of pediatric ependymomas. We found a chromosomal alteration and one gene associated with survival; these are candidates for bad prognosis biomarkers.

Keywords	Ependymoma; Molecular characteristic; Systematic Review; Pediatric patients
Taxonomy	Tumor Biology, Molecular Pathogenesis, Cancer Genetics
Manuscript category	Most pediatric cancer genomics papers

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Molecular characteristics of pediatric ependymomas: a systematic review.

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Abstract. The prognosis for patients diagnosed with ependymoma is relatively poor, with a 5-year overall survival rate of 24–75%. Currently, tumors are treated by surgical resection followed by radiotherapy, as resection is the most consistent prognostic marker (up to 80%). Therefore, there is a pressing need to improve our understanding of the biology of these tumors and to develop new therapeutic targets. The present work was a systematic review of the current molecular knowledge of pediatric ependymomas. From January 2000 to December 2017, we carried out a search using "MeSH" (Medical Subject Heading), and "free-text" protocols in the databases Medline/PubMed, SCOPUS, Web of Science, and EMBASE (OVID platform), combining the terms chromosomal alterations, genetic changes, epigenetic changes and protein expression changes. We selected articles with samples from pediatric patients and chose publications with trials. Only thirty-two articles met the criteria for a meta-analysis, suggested by the state of methylation and expression of a characteristic marker of pediatric ependymomas. We found a chromosomal alteration and one gene associated with survival; these are candidates for bad prognosis biomarkers.

Keywords. Ependymoma; Molecular characteristic; Systematic Review; Pediatric patients

Introduction

Ependymoma (EP) arises from the ependymal lining of the cerebral ventricles (infra- and supratentorial) as well as from the remnants of the central spinal canal. These tumors can develop in patients of all age groups; however, intracranial ependymomas occur more often in children, whereas spinal ependymomas are most common in adults [1].

EP is the third most common pediatric tumor of the central nervous system (CNS), accounting for 6–12% of brain tumors in children [2]. The prognosis is relatively poor for patients with this diagnosis, with a 5-year overall survival rate of 24–75%. Currently, these tumors are treated by surgical resection followed by radiotherapy, as resection is the most consistent prognostic marker (up to 80%); therefore, there is a pressing need to improve our understanding of the biology of these tumors and to develop new therapeutic targets [2,3].

To improve risk-adapted treatment strategies for EP, clinical and histopathological diagnostic and prognostic criteria are necessary. Potentially relevant factors, such as patient age, tumor location, extent of surgical resection, and tumor grade, have been studied quite extensively, but the results are inconsistent [4]. EP exhibits heterogeneous clinical courses that cannot be predicted accurately by clinical, pathologic or molecular markers, with the noticeable exception of extent of surgery. Nevertheless, several histopathology features have been investigated as prognostic markers in different case series from multiple studies, for example KI67 [5].

Several previous studies have suggested that epigenetic silencing of tumor suppressor genes is an important mechanism in EP pathogenesis in supratentorial and spinal tumors. These analyses allowed identification of candidate pathways and genes with potential methylation induced expression changes that may play a role in tumor pathogenesis [2].

Currently, we do not understand the universal established characteristics of pediatric EP. Therefore, in this systematic review, we aimed to establish the molecular characteristics of these tumors that may establish tumor markers.

Material and Methods

Search strategy and selection criteria.

An electronic search was carried out in the Medline/PubMed and SCOPUS, Web of Science, and Ovid/EMBASE databases and was restricted to articles published in English between January 2000 and December 2017. The intent was to identify chromosomal alterations and changes in the methylation status and gene expression that are part of the molecular characteristics of pediatric EP and that could be use as molecular prognostic biomarkers. These studies were examined based on their title, abstract and keywords. The strategy used a combination of the following key terms: “Ependymoma pediatric”, “Ependymoma children”, “Ependymoma molecular”, “Brain tumor molecular ependymoma”, “Ependymoma biomarker” (Figure 1). We also examined the references in the selected articles, looking for studies that were not selected in the initial query.

Included articles were based on an *a priori* selected set of criteria: articles published in English, complete data of patients, complete text, cross-sectional studies, molecular data studies, and studies carried out totally or partially in pediatric patients (under 18 years of age). If the reviewed articles were based on pediatric and adult patients, it was considered that the samples would be easily identified through the codes or numbering that different authors granted for each of the samples. The patients had complete clinical characteristics that included age, sex and diagnosis; in addition, the results described were specific for each sample.

The primary outcome was data on changes in chromosomal alterations, methylation status, gene and protein expression reported in pediatric patients, cross-sectional studies, molecular changes associated with the patient's prognosis, frequency of appearance of molecular changes and values of hazard ratios and odds ratios.

The exclusion criteria included case-controlled studies, studies without measures of association and case series. No systematic reviews or meta-analyses on this topic were found.

Data Extraction.

Three reviewers participated in the review process. Two reviewers completed the initial review, examined the papers, confirmed the inclusion and exclusion criteria, and completed the second stage, extracting all data; a third reviewer independently examined the data to identify any discrepancies between reviewers. Discrepancies in article selection were resolved by discussion among the reviewers. A similar approach was used to determine which of these studies should be included in the meta-analysis. Information about date of publication, country where the study was undertaken, sample size, data relating to participants, a specific illness, age, sex, histopathology diagnosis, and tumor location was acquired from the included articles for full review. Odds ratios (ORs), and rate ratios (RRs), with 95% confidence interval (CI) for each chromosomal alteration, genetic, epigenetic and protein expression were extracted when they were available.

Statistical analysis.

Thirty-two studies were selected and included for this systematic review. The number of studies, analyzed by condition, varied from two to five. For each study and each characteristic, an event rate and its CI were computed from the reported numbers, considering the reference group. Review Manager 5.3 was used for the analyses *[*Review Manager (RevMan) [Computer program]. Version 5.3. Copenhagen: The Nordic Cochrane Centre, The Cochrane Collaboration, 2014]. Random-effect models were conducted, separating the studies into one analysis for those reporting OR statistics and another for those reporting OR. Heterogeneity was estimated using the I^2 statistic [6]. The I^2 describes the percentage of variation across studies due to heterogeneity rather than chance alone [6,7]. As the I^2 percentage increases, so too does the proportion of effect size variability that is due to between-study heterogeneity. Finally, the frequency of chromosomal, genetic and epigenetic alterations was analyzed.

Results.

Article selection.

Initially, we identified 3564 related articles. After evaluation, 32 studies fulfilled the inclusion criteria as follows: 14 contained studies on chromosomal alterations, 4 had methylation data, 4 articles contained information related to the expression of messenger RNA and 13 referred to proteins. Table 1 shows the studies included in the analysis with sample size, study design and the alteration detected in the molecule of interest.

Chromosomal Alterations.

From the selected articles, 14 were related to chromosomal alterations to the EP. We considered a total of 545 samples from the analyzed publications. According to this analysis, the alterations oscillated up to 2% of the total data (data not shown). The most frequent chromosomal changes were gains at 1q, 9q, 9p and 17q and losses at 1p, 3p, 6q and 13q. We found chromosomal alterations (losses and gains) in several cytobands. From these data, we found that chromosomes 1q, 6q, 9p, 13q and 22q are features of pediatric ependymal tumors (Figure 2A). The alterations showed the following statistical values: to 1q (Tau2=1.53, I2=45%, P=0.02 Chi^2=9.13); to 9p (Tau2=1.27, Chi^2=6.84, I^2=42%, P=0.24) to 6q (Tau2=0.00, Chi^2=2.06, I^2=0%, P=0.58); to 13q (Tau2=0.00, Chi^2=0.08, I^2=0%, P=0.14) and to 22q indicated perfect homogeneity (Tau2=0.00, Chi^2=0.08, I^2=0%, P=0.14). Over a wide-range, our model indicated heterogeneity with the following statistical values: Tau2=1.72, Chi^2=37.8, P=0.01, I^2=0%. Therefore, chromosomal alterations in 1q, 6q, 9p, 13q and 22q are features of pediatric ependymal tumors. Additionally, HR values were considered. We found that chromosome 1q25 is the best candidate for a prognostic biomarker in pediatric EP, and this chromosome correlated with lower progression-free survival and overall survival (Figure 2B). Our model was heterogeneous with the following statistical values: Chi^2=4.76, P=0.03, I^2=79%.

Methylation analysis.

We assessed 4 related articles on methylation that included 237 patients, in which we found genes with changed methylation status in the pediatric EP: *hTERT*, *RAC2* and *CHIBBY*, with the following frequencies 27.8%, 9.2% and 8.8%, respectively. These results suggested that un-methylation of *hTERT* is associated with pediatric patients diagnosed with EP (Figure 3); however, the change in the methylation status did not correlate with the prognosis. The model indicated homogeneity with the following statistical values: Tau2=0.0, Chi^2=0.02, I^2=0%.

Gene expression.

We selected 4 articles that met the eligibility criteria with 136 samples. It was observed that genes with the most frequent expression changes were: *hTERT* (36%), *ERBB* (33%), *ERBB1* (12%), *ERBB2* (12%), *ERBB3* (9%), *STB* (4%), *SHC1* (6%) and *TPR* (9%). We observed that *hTERT* and *ERBB* genes are features of pediatric ependymal tumors; but only *hTERT* correlated with a bad prognosis

for patients with the following statistical values: Chi^2=3.31, P=0.19, I^2=40%, which indicated that our model was heterogeneous (Figure 4).

Protein expression.

We selected 14 publications that met the eligibility criteria with protein expression assays, with a total of 697 patients showing different states of protein expression. We reviewed the frequencies of the results, where it was observed that EGFR was found with 22.02% of the cases, with strong expression, and with 18.75% with absence of protein expression; for Cav-1 with 12.35% had a strong expression, and 13.54% had weak staining. With respect to YB1, the expression was weak with a prevalence of 19.94%. EZH2 showed weak or negative staining with a prevalence of 19.35%. NCL showed strong staining in 19.94% and weak staining in 10.86% of the cases (Figure 5).

Discussion

We found 3564 articles that were related to EP, but most referred to clinical aspects of this neoplasm and some others did not meet the inclusion criteria, such as articles published in English, with complete data of patients, complete text, cross-sectional studies, molecular data studies, and studies carried out totally or partially in pediatric patients (under 18 years of age). Therefore, a total of 32 studies related to EP were included in this work. These papers were related to molecular studies in EP and included complete data of patients and complied with the eligibility criteria.

The following alterations were reported: losses in 1p, 3p11, 3p12, 3p23-p13, 3p24, 3q23-qter, 4q33-qter, 18q22.2, 22, gains distributed along chromosomes 7, 12, 15, cytobands 6q14-q27, 9q13, 9q21-q32, 9q33, 9q34, 10q25.2–q26.3, 10q25.2–q26.3 and 19p13, the loss at 18q22.2. These alterations were significantly associated with patients over three years [21,25]; after data analysis, we found that the most frequent and characteristic chromosomal changes of the ependymal tumors were gains at 1q, 9q and 17q and losses at 1p, 3p, 6q, 13q and 22q. Furthermore, the regions 6q24.3 and 6q25.2-q25.3 were defined as the regions with the highest number of deletions, and preliminary analysis of genes in this region suggested that the genes *NOX3*, *ARID1B*, *ZDHHC14* and others could play a role in the pathogenesis and biology of EP [20].

Due to methodologies constantly advancing, becoming more specific and with better resolution, it is not surprising that the main differences in the chromosomal alterations reported is due to the methodologies used. Since the CGH array was used in the most recent publications and in the older works, they still use conventional CGH in analyses. Despite these methodological differences, it was possible to determine the most important chromosomal alterations, such as the chromosome 22 loss was that found in 57.5% of the studied EP cases; more specifically, it was associated with 45% of intracranial EP cases and 82% of spinal EP [1]. This is consistent with the idea that the loss or mutation of NF2 is frequently involved in their development [10,21], ranging from 30% to 71% of the patients [15]. Another important chromosomal alteration is a gain at 1q, which is one of the most

common regions with a gain in EP [21,22]. It has therefore been important to investigate the gain at 1q as a potential marker of a poor prognosis in a larger number of pediatric EPs treated in a standard manner [12]. The cytoband 1q25 is associated with a bad prognosis for patients because of this correlation with lower progression-free survival and overall survival [24]. We identified members of the *S100* family located within the commonly gained amplicon 1q21.3 and provide evidence of their differential expression in clinical subgroups of pediatric ependymoma: *S100A4* is associated with patients of a very young age at diagnosis and *S100A6* with supratentorial tumor location [22]. S100 protein possesses a wide range of intracellular and extracellular functions, such as regulation of calcium homeostasis, cell proliferation, apoptosis, cell invasion and motility, cytoskeleton interactions, protein phosphorylation, regulation of transcriptional factors, autoimmunity, chemotaxis, inflammation and pluripotency. Many lines of evidence have suggested that altered expression of S100 proteins was associated with tumor progression and prognosis [38]. Finally, it has been defined that 9q33-34 is the region with the most frequent gains and its occurrence correlated significantly with relapse [24]. We found a greater incidence at relapse compared with the initial diagnosis for a gain at 1q, 9q34, 15q22, and 18q21, and losses at 6q [24,27].

With respect to the genes reported to have changes in methylation, we found that the most frequent genes were *CHIBBY, RAC2, and hTERT*, in 8.8%, 9.2%, and 27.8% of the cases, respectively. It is possible that loss of *RAC2* function has a greater impact in younger patients in which the central nervous system is still undergoing development [19]. This gene plays an essential role in the regulation of cytoskeleton remodeling, in the gene expression of oncogenes and endothelial cell migration on specific provisional matrix proteins. *RAC2* promotes tumor growth, angiogenesis and invasion [39]. It has been reported in EP that the *CHIBBY* promoter has a high frequency of methylation [19], and it has been found to activate endoplasmic reticulum stress and apoptosis in breakpoint cluster protein/Abl kinase 1-positive human myeloid leukemia cells by inducing the cytoplasmic accumulation of β-catenin. The downregulation of *CHIBBY* in cancer cells might provide information regarding the use of *CHIBBY* as a therapeutic target and prognostic factor. It has been identified to be an antagonist of β-catenin, and it binds to β-catenin in the nucleus. It exhibits a negative regulating effect on the Wnt signaling pathway and the transcriptional activities of genes downstream of Wnt. Mutations of *CHIBBY* have been associated with tumorigenesis [40]. Furthermore, the results suggested that apart from less aggressive molecular subtypes, *hTERT* promoter hypomethylation might characterize ependymal tumors with a more favorable outcome [37]. It has even been suggested that *hTERT* and *CHIBBY* methylation changes are characteristic of pediatric ependymomas, but they are not yet associated with the patient's prognosis.

In relation to gene expression, we found the *hTERT, ERBB, ERBB1, ERBB2, ERBB3, STB, SHC1* and *TPR* genes were overexpressed. In several publications, a series of characteristic and prognostic molecular markers have been suggested without reaching any consensus, but it has been reported

that *ERBB1, ERBB2, ERBB3*, and *ERBB4* are important in the development of EP and participate in cell proliferation [13]. Currently, it has been proposed that inhibition of telomerase enzymes is associated with an increased progression resulting in the lack of proliferation, self-renewal and tumorigenicity; these findings provide novel insight into the importance of telomerase as a predictor of outcome and as a therapeutic target in pediatric EP [31]. By exploring molecular mechanisms, telomerase activity has been described as a characteristic potential biomarker, showing an association of telomerase reactivation with a chromosome 1q gain and RelA fusion [37]. According to our analysis, the *hTERT* and *ERBB* genes are characteristic in pediatric EP; but only *hTERT* can be correlated with the prognosis. The *hTERT* expression can be used to divide both totally and subtotally resected tumors into good and bad prognostic groups because the EP lacking telomerase activity are unable to maintain telomeres and proliferative indefinitely, suggesting that less aggressive therapeutic intervention may be offered for children with telomerase-negative tumors. It has been shown that *hTERT* plays an essential role in the Wnt/β-catenin signaling pathway by serving as a cofactor in a β-catenin transcriptional complex. In addition, STAT III selectively stimulates *hTERT* expression through the JAK/STAT signaling pathway, and several signaling cascades can contribute to the control of *hTERT* gene expression, promoting telomere length or immortalizing cells in distinct types of tumors [18,31].

About protein expression, several research groups have proposed different proteins as features of ependymomas and as potential prognostic biomarkers. A previous study suggested an association between the overexpression of PDGFR protein, mainly in tumor cells but also in the tumor endothelia. Regarding patient outcomes, a hypothesis has been presented that overexpression of PGDFRs could have a good prognostic role in EP [30]. It has been observed that EPs showed at least focal immunopositivity for MDM2, and only some showed immunopositivity for P53. These findings are consistent with previous reports describing ependymomas rarely have *P53* gene mutations and that neoplasms with *MDM2* amplification typically lack *P53* mutations that deregulate the cell cycle [8]. The proteins NOTCH-1, TN-C and Hes-1 showed a significantly higher expression in grade III tumors in comparison to grade II tumors. NOTCH-1 showed increased expression with tumor grade and a difference between grade I and grade III tumors [32].

The general results suggest that protein expression plays an important role in pediatric ependymomas and that it can be considered as a possible molecular biomarker of prognosis. Chromosomes 1q and 22q are features in pediatric EP, and the gain at 1q25 has a high probability of being used as a prognostic biomarker. When considering the poor prognosis and survival rate of patients, the *hTERT* gene changes in expression and methylation status may play an important role in the development of pediatric ependymal tumors.
Nevertheless, the results should be interpreted with caution. Additional research is needed to assess the true effect of protein expression to identify patients at risk.

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Conflict of Interest

The authors declare that they have no competing interest.

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Table 1. Articles included in the systematic review

Reference	Methodology	Detected Alteration	N
Suzuki et al., 2000 [8]	IHC	Protein	11
Lamszus et al., 2001 [9]	PCR	Chromosomal Alteration	10
Hirose et al., 2001 [10]	CGH	Chromosomal Alteration	14
Ward et al., 2001 [11]	CGH	Chromosomal Alteration	36
Dyer et al., 2002 [12]	CGH	Chromosomal Alteration	42
Gilbertson et al., 2002 [13]	qRT-PCR	Methylation	16
Jeuken et al., 2002 [14]	CGH	Chromosomal Alteration	5
Singh et al., 2002 [15]	IHC	Protein	10
Zamecnik et al., 2003 [16]	IHC	Protein	31
Waha et al., 2004 [17]	MS-PCR	Expression	27
Ammerlaan et al., 2005 [1]	aCGH	Chromosomal Alteration	19
Tabori et al., 2006 [18]	qRT-PCR	Methylation	65
Karakoula et al., 2008 [19]	qRT-PCR	Methylation and Expression	31
Moronaru et al., 2008 [20]	PCR	Chromosomal Alteration	29
Pezzolo et al., 2008 [21]	CGH	Chromosomal Alteration	18
Rand et al., 2008 [22]	aCGH	Chromosomal Alteration	7
Snuderl et al., 2008 [23]	IHC	Protein	41
Puget et al., 2009 [24]	aCGH	Chromosomal Alteration	59
Rousseau et al., 2010 [25]	aCGH	Chromosomal Alteration	45
Andreiuolo et al., 2010 [26]	IHC	Protein	66
Korshunov et al., 2010 [27]	aCGH	Chromosomal Alteration	190
Alexiou et al., 2011 [28]	IHC	Protein	13
Modena et al., 2012 [5]	IHC	Protein	47
Hagel et al., 2013 [29]	IHC	Protein	25
Moreno et al., 2013 [30]	IHC	Protein	12
Barszczyk et al., 2014 [31]	qRT-PCR	Expression	97
Gupta et al., 2014 [32]	FISH	Chromosomal Alteration	19
Virág et al., 2014 [33]	IHC	Protein	16
Li et al., 2015 [34]	IHC	Protein	174
Araki et al., 2016 [35]	FISH, IHC	Chromosomal Alteration, Protein	52
Chen et al., 2016 [36]	IHC	Protein	174
Gojo et al., 2017 [37]	qRT-PCR	Methylation and Expression	24

Methodologies used in the differed articles: Immunochemistry (IHC), Comparative Genomic Hybridization (CGH), Array CGH (aGCH), Fluorescence Hybridization In Situ (FISH), Real Time Polymerase Chain Reaction (qRT-PCR), Methylation Specific-PCR (MS-PCR).

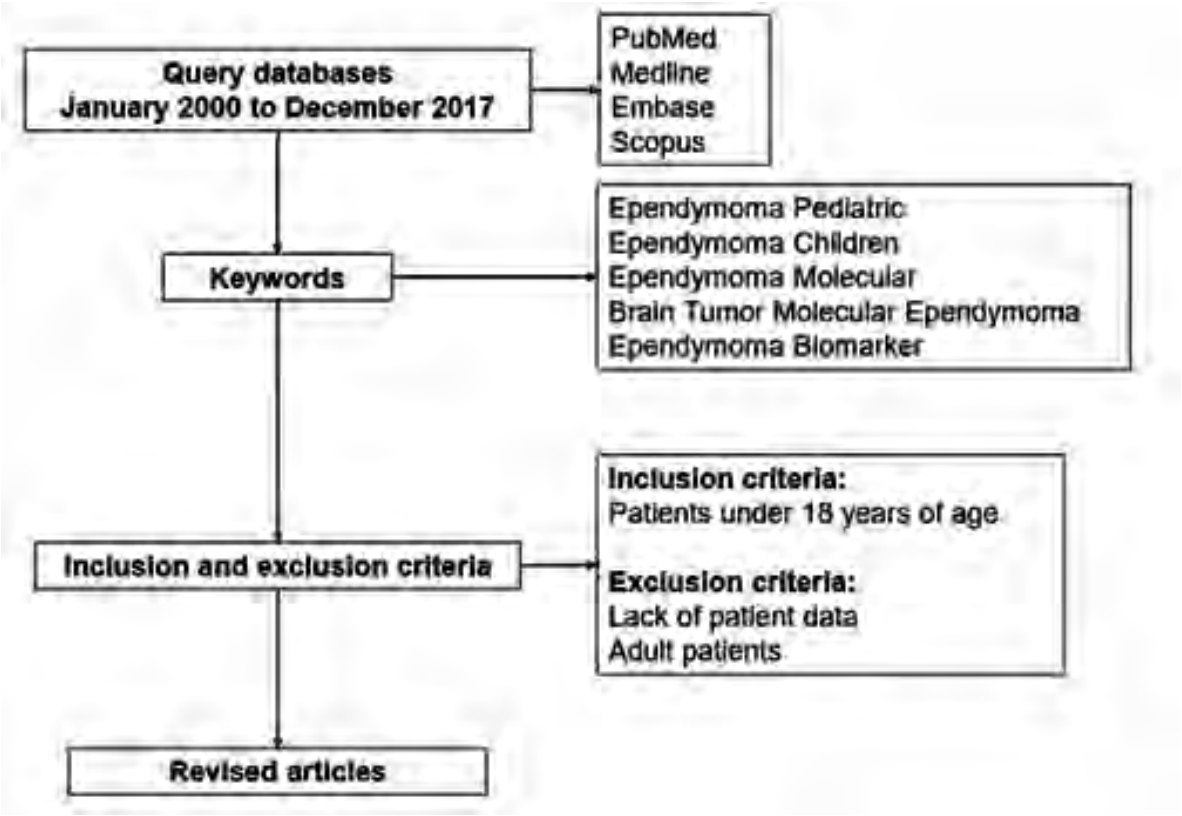

Figure 1. Flow diagram of process for identification of relevant studies.

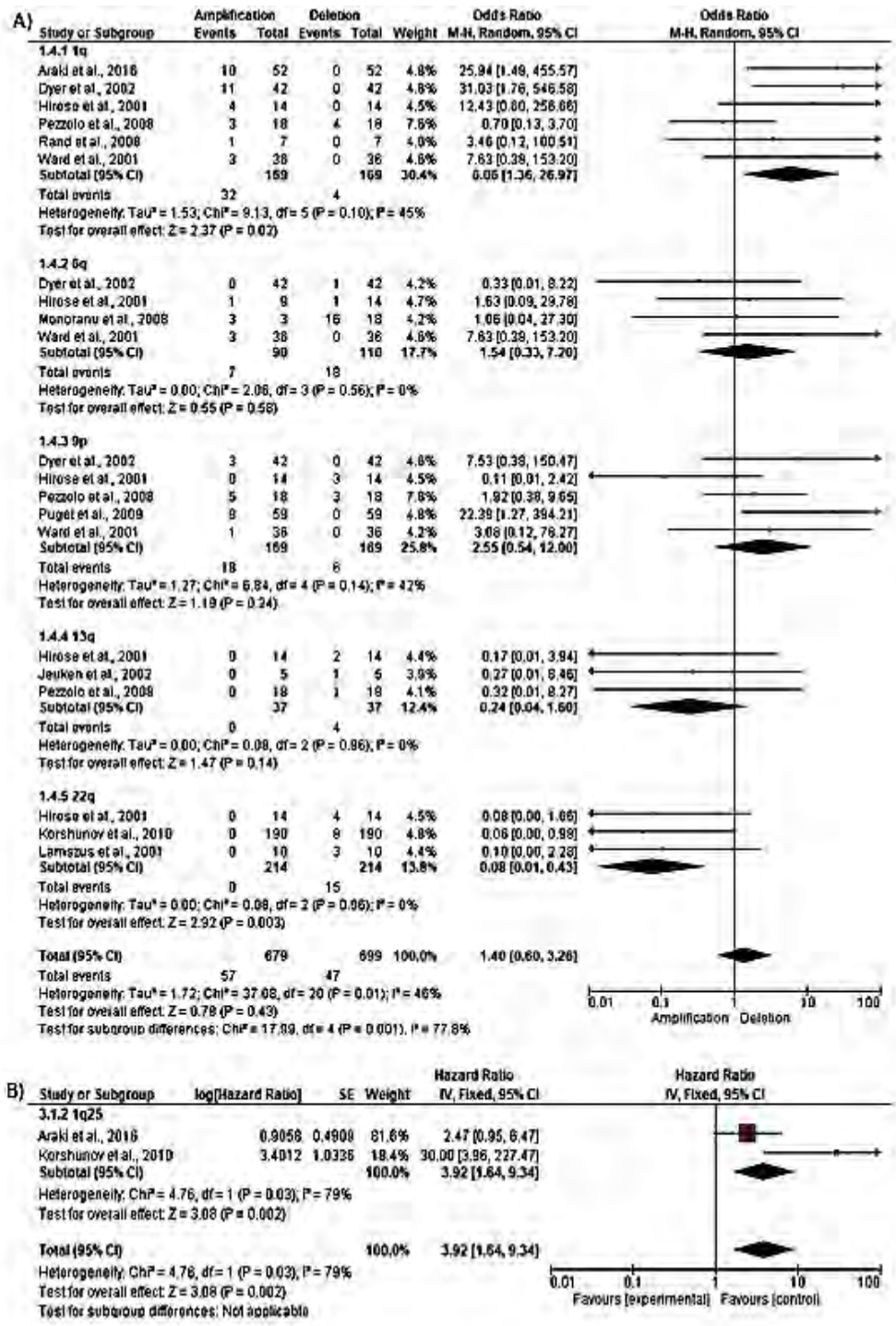

Figure 2. A) Forest plot of Chromosomal Alterations, B) Forest plot of Chromosomal Alterations and prognostic.

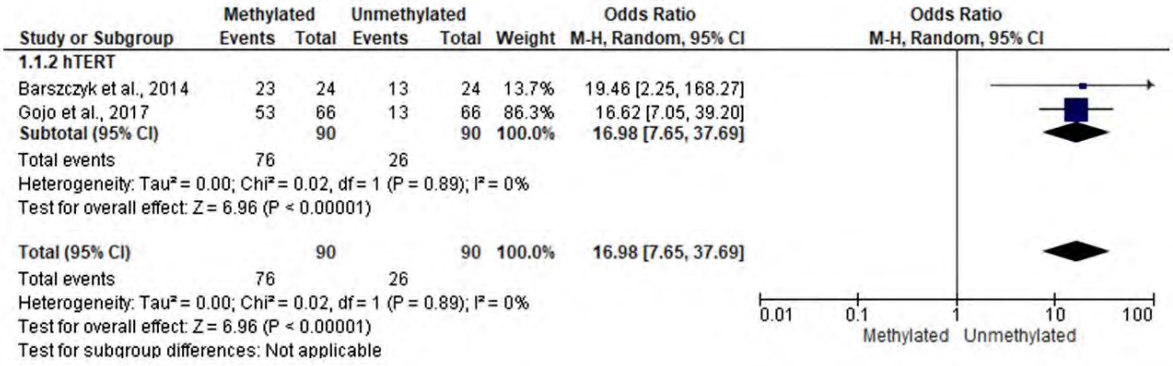

Figure 3. Forest Plot of Methylation Status.

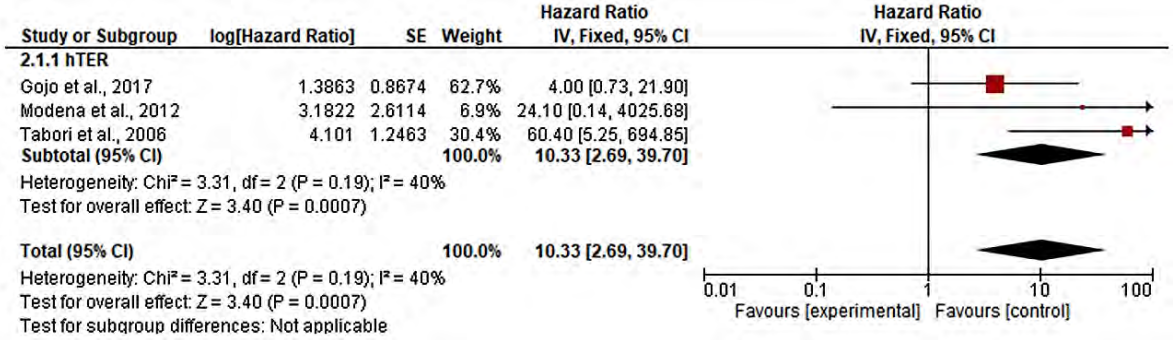

Figure 4. *hTERT* and prognostic.

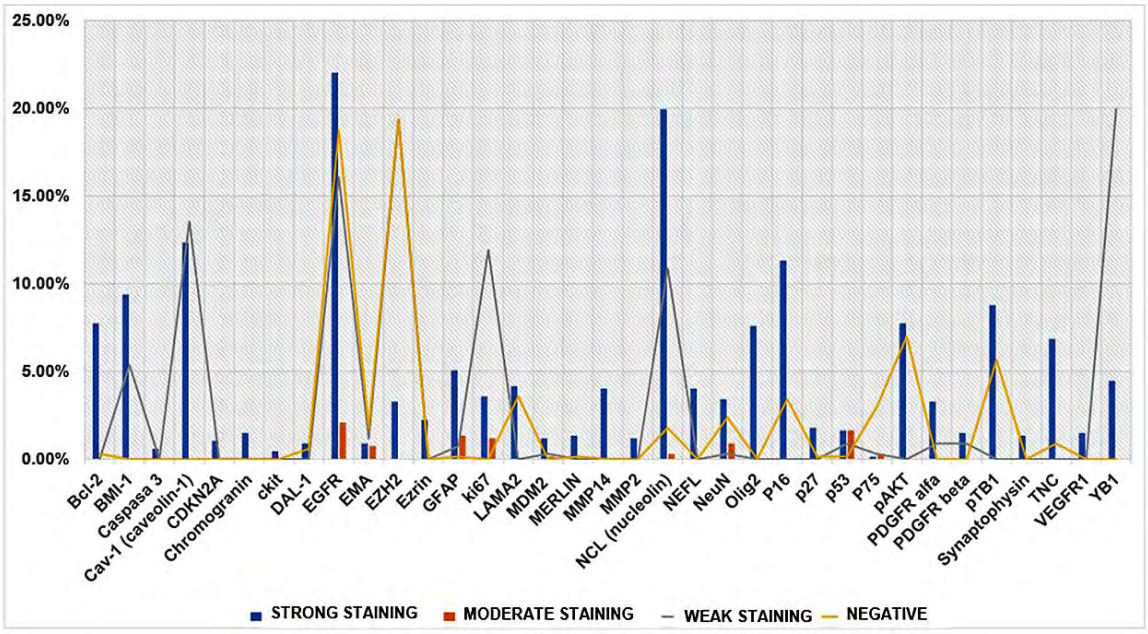

Figure 5. Frequency of protein expression.

Artículo 4. Manuscrito

Pediatric ependymoma: GNAO1, ASAH1, IMMT and IPO7 protein and expression and prognosis correlation

Pediatric ependymoma: GNAO1, ASAH1, IMMT and IPO7 protein expression and prognosis correlation.

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Abstract. Objective. Previously, we proposed *GNAO1, ASAH1, IMMT and IPO7* genes as candidates to ependymoma molecular biomarkers that may be used for prognostic. Therefore, the protein expression was evaluated, correlating with clinical features (age, sex, histopahological grade), ependymal tumor recurrence and patient survival. **Methods.** Immunohistochemistry assays were performed for: GNAO1, ASAH1, IMMT, IPO7, Cyclin D1, p53 and Ki-67 proteins. Age, sex, histopathological grade, relapse and survival correlation were made by Kaplan-Meier and Cox analysis. **Results.** We found two proteins that correlate with histopathological grade, one protein with relapse and one protein with survival. **Conclusion.** Our results suggest a protein association with survival, relapse or histopathological grade that may be used as molecular biomarkers for prognostic.

Keywords: Pediatric Ependymoma, Infratentorial ependymoma, Protein expression and survival, Protein expression and Progression-free survival.

1. Introduction

Even there has been great improvement on the treatment and prognosis of many pediatric brain tumor, which are currently the major cause of mortality, as well as long-term morbidity in oncology, the central nervous system (CNS) neoplasms remains constituting the most common solid tumors of childhood [1]. Ependymomas (EP) are the third most common pediatric CNS tumors accounting for 6–12% of all intracranial tumors [2]. Nowadays, this tumors are treated by surgical resection (one of the most consistent prognostic markers) followed by radiotherapy [3]; despite the advances in multidisciplinary care in conjunction with chemotherapy to the brain tumors, gliomas and EP are relatively resistant to chemotherapy, and patients with tumors refractory to surgery still have a poor prognosis [4]. EP exhibit heterogeneous clinical courses that cannot be predicted accurately by currently clinical, pathologic or molecular markers [5]. Consequently, new targeted therapies are urgently needed for these patients [6] and are required for studies aimed at the identification of molecular markers of clinical value [7].

The identification of genetic abnormalities responsibles for the generation and maintenance of the malignant phenotype in EP are crucial [8], since the molecular-genetic profile of these tumors remain poorly defined, in several studies has been proposed different prognostic markers for EP: the proteins Ki-67, survivin, human telomerase reverse transcriptase, ERBB family members and nucleolin [9], the high expression of: BNIP3, MRC1, TDG, EPHB3, GLIS3, CDK4, COL4A2, EBP, NRCAM, CCNA1, ICAP-1A, ABLIM, ENPP2, and PTTG1IP; and low expression of: PMS1 and PDX1 [8][10]. Actually, it has been tried to correlate clinical findings, such as patient age, sex, tumor location, tumor grade, extent of resection and treatment regimens with tumor recurrence [11].

Advances have been made in the molecular knowledge of EP, considering that the molecular classification is now critical for diagnosis, but molecularly guided trials are complicated by the apparent molecular EP diversity [12,13], thus it is necessary to determine specific molecular markers. In a previous work we have proposed the genes: *GNAO1*, *ASAH1*, *IMMT*, *IPO7* as candidate molecular biomarkers prognostic [14]. Therefore, the expression of the protein was evaluated and correlated with the Progression-free survival and Overall Survival.

2. Material and Methods

2.1 Samples collection.

The ependymal tumors were collected from Formalin-Fixed Paraffin-Embedded (FFPE) tissue from the Pathology Service from the Pediatrics Hospital "Dr. Silvestre Frenk Freud", from the Centro Médico Nacional "Siglo XXI", IMSS. The histopathological records from 2010 to 2017 were reviewed. The samples were collected from pediatric patients between 0 and 16 years with a confirmed diagnosis of grade II and grade III ependymoma and as a control group was used cerebellar tissue adjacent to the tumor.

2.2 Review of clinical records.

The clinical records of patients were consulted in the database and in the written archives of the hospital. The following clinical data were obtained: histopathological grade, anatomical location, age, sex, recidivism and survival.

2.3 Immunohistochemistry assays

Tissue sections of 5 µm were made and placed on adhesive lamellae (Santa Cruz Biotechnology). The tissue was desparaffinized in the stove at 56°C for 30 minutes and rehydrated for 5 minutes in xylol, alcohol (with the following concentrations: 100%, 90%, 70%) and distilled water. The antigen was recovered with target retrieval solution at 10% with microwave irradiation for 2 minutes. Tissue cooled (room temperatura), washed witn PBS and peroxidase bloking for 30 minutes, washed with PBS. Tissue sections were incubated in a wet chamber for 20 minutes with the primary antibodies: GNAO1 (1:100, GTX114439, GeneTex), ASAH1 (1:100, GTX114267, GeneTex), IMMT (1:100, GTX81949, GeneTex), IPO7 (1:100, GTX106408), Cyclin-D1 (sc-718, Santa Cruz Biotechnology), p53 (sc-126, Santa Cruz Biotechnology) and Ki-67 (1:50, DAKO corporation, Carpenteria Ca), following the manufacturer recommendations. Primary antibody was detected with a biotinylated secondary antibody (sc-471863, Santa Cruz Biotecnology), incubated with streptavidin conjugated (horseradish peroxidase) for 30 minutes, revealed with diamino-benzidine chromogen (ab64238, Abcam) for 5 minutes and contrasted by hematoxylin. The tissue dehydrated with alcohols of lower to higher concentration (70%, 90% and 100%) and xylol. The preparations were mounted with entellan (107961, Merck Millipore).

For each antibody was included a positive control for GNAO1 and ASAH1 pancreas tissue; for IPO7 and IMMT esophageal tissue; for Ki-67, Cyclin D1 and p53 was used glioma. The quality of the tissue was evaluated with tha expression of Ki-67, a positive marker in all EP.

2.4 Image capture

Immunohistochemistry results images were taken with 40X objective of Photo-microscope 6X31 (Olympus Life Sciences). These images were stored in TIFF format. The quantification was made out with the ImageJ software (16).

2.5 Analysis of results.

The protein expression was considered qualitatively by the percentage of cells expression. The following values were established: Negative (<1%), weak staining (1-15%), moderate staining (15-30%), strong staining (>30%). The statistical analysis (Kaplan-Meier and Cox analysis) was performed in the IBM SPSS Statistics 24 Software. We considered statistically significant P-value <0.05.

3. Results

3.1 Collected samples

The samples collected were a total of 30, all the samples analyzed had a diagnosis *De Novo* and infratentorial localization (Table 1). These samples came from pediatric patients diagnosed with EP grade II or grade III (Fig. S1). The clinical features of the patients presented that 13 patients were females and 17 patients males. The 20% of patients was 7 years old and 16% had 5 years at the diagnosis time.

3.2 Clinical data.

We analized a cohort of samples between the years 2010 to 2013, to know if in five years after diagnosis patients have relapse and survival. Twelve samples with complete clinical file were obtained. Table 1 shows seven patients with relapse and seven patients with survival.

3.3 Protein expression.

We performed IHC assays for the proteins: GNAO1, ASAH1, IMMT, IPO7, Cyclin D1, p53 and Ki-67; on the following tissue: cerebellum, ependymal tumor and as positive control tissues accord with methods (Fig. S1). Through a qualitative analysis, we found different percentages of protein expression. We observed negative expression the following proteins: GNAO1 (60%), IMMT (73.3%), IPO7 (90%), Cyclin D1 (60%) and p53 (76.6%); with positive expression the proteins: ASAH1 (73%) and Ki-67 (70%) (Fig. 1, Table 2 and Table S1).

3.4 Correlation analysis between protein expression and age, gender or histopathological grade.

The correlation analysis between age and protein expression showed the following Chi-square values: GNAO1=0.554, ASAH1=0.204, IMMT=3.033, IPO7=6.269, Cyclin D1=0.011 (Fig 2A), p53=0.037 (Fig 2B) and Ki67=1.725.

The correlation analysis between sex and protein expression exhibited the following values of Chi-square: GNAO1=0.252, ASAH1=0.587, IMMT=0.156, IPO7=0.107, Cyclin D1=0.088 (Fig. 2C), p53=0.172 and Ki-67=0.019 (Fig. 2D).

The values of Chi-square results from correlation analysis between histopathological grade and protein expression were the following: GNAO1=0.347, ASAH1=0.223, IMMT=0.118, IPO7=0.205, Cyclin D1=0.019 (Fig. 2E), p53=0.001 (Fig. 2F) and Ki67=0.085 (Fig. 2G).

3.5 Correlation analysis between protein expression and recidivism or survival.

The correlation analysis between relapse and protein expression showed the following Chi-square values: GNAO1=0.974, ASAH1=1.017, IMMT=0.389, IPO7=0.345, Cyclin D1=0.094 (Fig. 3A), p53=0.013 (Fig. 3B) and Ki67=0.021 (Fig. 3C).

The correlation analysis between survival and protein expression with the following values of Chi-square: GNAO1=0.103, ASAH1=1.220, IMMT=0.007 (Fig. 3D), IPO7=0.019 (Fig. 3E), Cyclin D1=0.371, p53=1.069 and Ki-67=1.816.

4. Discussion

In a previous work related to pediatric EP we found *ASAH1*, *IMMT* and *IPO7* genes overexpressed and *GNAO1* gene underexpressed [14] also, at worldwide papers we found expression changes in *Cyclin D1*, *P53* and *Ki-67* genes in EP [15][16]. According to the reports these genes in EP participate in the tumorigenesis process, therfore we determined through IHC assays if these changes were reflected in protein expression and how is their behavior in Mexican population, to analyze these we developed Kaplan Meier and Cox analysis to know the correlation between protein expression and clinical features or prognostic.

The 30 samples collected were from children under 16 years old; the tumors were diagnosed *De Novo* with infratentorial location, 12 samples had complete file and could be followed through 5 years after diagnosis, expected time in EP to arise the relapse (Samples were collected between the years 2010 to 2013); starting from these data we generated a correlation analysis between protein expression and relapse or survival.

We found P53 protein underexpression in 76.6% of analyzed tumors, concordant to published by Wu *et al*., [17] they reported the P53 gene inactivation in more than 50% of tumors. We found that a P53 underexpression correlate with the age, probably at early age P53 is one of the main deregulated proteins that favor cell proliferation. We did not found correlation between p53 expression and sex. We found correlation of P53 underexpression with the histopatological grade III, we observed that in the samples collected from Mexican patients the P53 protein decreases in anaplastic EP; contrariwise Alexiou *et al*., reported that in caucasian patients the high P53 expression correlated with anaplastic EP [15]; possibly these differences are due polymorphisms, mutations or epigenetic factors that influence protein expression among race. The P53 underexpression found correlates with the biological function of p53. It is know that P53 regulates several cellular functions: apoptosis, cell proliferation and promote DNA repair [15] [18]; this repair ocurrs when P53 bind to the p53-responsive elements, initiating transcription of p53 responsive genes, and ultimately triggering the p53 pathway [19]; it is suggested that the P53 underexpression favors the anaplastic development of EP. We found that p53 underexpression delay the relapse; it has been reported that P53 is related to the patients survival and the aggressive biological behavior [15]. We not found correlation between protein expression and survival. It is suggested that the P53 expression is a candidate to prognosis biomarker in pediatric EP, besides we found correlation between P53 expression and histopathological grade or relapse, therefore P53 permit us to distinguish the anaplastic grade of tumor and patient prognosis.

We found that infratentorial EP had Cyclin D1 underexpression in 83.4% and overexpression in 16.6% of the samples, noticing contrary to Rogers *et al.,* [16] they reported that the Cyclin D1 expression was significantly higher in supratentorial EP; the above shows that in Mexican patients can be found the Cyclin D1 overexpressed in infratentorial EP. It is known that post-transcriptional control dictates the overall accumulation of cyclin D1 in tumor cells and the association to neoplastic transformation through the deregulation of the cell cycle; also, the nuclear Cyclin D1 overexpression can inactivate p53 through PRMT5-dependent p53 methylation, thereby allowing tumor progression. We observed on ten samples Cyclin D1 overexpression and P53 underexpresion, we not observed correlation between Cyclin D1 and P53 expression (X^2=0.776). It has reported that Cyclin D1 deletion was noted to increase the migratory behavior [20]; posibbly Cyclin D1 underexpression plays the same role in cancer. Cyclin D1 suppresses the expression of Rho-activated kinase II (ROCKII) and thrombospondin 1 (TSP-1); Cyclin D1 loss correlates with increased phosphorylation of the ROCK II substrates, favoring the cell migration [20]; we found one sample with Cyclin D1 underexpression and metastasis, it is suggested that Cyclin D1 underexpression favors metastasis. We found a tendency to overexpression in patients less than seven years old and male patients; this may be influenced by clinical data, kind samples and amount. We found that Cyclin D1 overexpression correlated with EP grade III, the Cyclin D1 overexpression favoring the neoplastic transformation. We found that Cyclin D1 expression correlated with relapse; the Cyclin D1 overexpression in infratenrorial EP delay relapse, being important to know the treatment prognosis; it has reported that in patients with chemotherapy treatment Cyclin D1 may play a more important mediator function in the residual EP to facilitate tumoral DNA repair and promote residual re-growth [21,22]. We not found correlation between Cyclin D1 expression and survival. We not found correlation between Cyclin D1 expression and survival. Given the role of Cyclin D1 in mediating extracellular cues with cell proliferation, it is not surprising that directly contributes to neoplastic growth, nevertheless we observed possibly that Cyclin D1 underexpression does not allow a correlation between Cyclin D1 and survival, it would be interest to increase the samples number and correlate the Cyclin D1 with survival.

It has reported that the Ki67 index in intracranial EP is an independent prognostic factor and accurate predictor of outcome in EP patients [23,24]; therefore, we evaluate the Ki-67 expression in EP. We found Ki-67 overexpressed in all samples, the expression was higher in grade III compared to grade II. We not found correlation between Ki-67 expression and age. We found that Ki-67 expression correlates with the histophatological grade, the Ki-67 expression increases according with the progress of the tumor, in concordance with the reported by Suri *et al.,* [25] and Alexiou *et al.*, [15] they reported that Ki-67 were significantly higher in grade III tumors; we found that high Ki-67 expression favors early relapse, thus it is suggested that Ki-67 expression favors the anaplastic phenotype, through the deregulation of cell cycle, it has demonstrated that Ki-67 affects cell cycle progression in p21 checkpoint impact cell proliferation and cell cycle progression; the cells in early

stages of cell cycle arrest have low levels of Ki-67 [26]. We not found correlation between protein expression and survival.

In a previous work, we reported the *ASAH1* gene overexpressed in pediatric EP [14], so that, we analized the protein and we found ASAH1 is overexpressed in 86.6% of the samples; it has been shown that it is overexpressed in various human cancers, such as head and neck cancer, melanoma, prostate cancer and breast cancer [25]. It has reported that ASAH1 overexpression confers resistance to apoptosis and stimulate proliferation and invasiveness of cancer cells [30], it is suggested that ASAH1 overexpression favor the tumorigenesis. ASAH1 participates in cell survival, inflammation and angiogenesis through the sphingolipid pathway that metabolize ceramide into sphingosine and free fatty acid, the Sphingosine serves as a substrate for sphingosine kinase (SphK) mediated phosphorylation to form mitogenic S1P; this protein has been shown to act as a cofactor for E3 ubiquitin ligase TRAF2, which leads to the activation of NF-Kappa B, a direct and indirect regulator of Mcl-1, which may represent yet a interplay between sphingolipid metabolism and apoptotic pathways [27][29]. We found that ASHA1 does not correlate with age, sex, histopathological grade, relapse or survival, so we think that ASAH1 may have other clinical functionality; Lai *et al.*, [30] they reported that ASAH1 overexpression confers resistance to radiation, impacting the treatment through altering the sphingolipid metabolism pathway and Doan *et al.*, [31] reported that in Glioblastoma ASAH1 is excellent drug target; it is suggested that the protein overexpression may be important in the treatment response at EP. Besides, it has reported that the cytotoxic actions of certain drugs (e.g. dacarbazine, anthracyclines) depend on the ability of these agents to increase the intracellular levels of ASAH [31]; so it would be interesting to know the expression of other proteins involved in the sphingolipid pathway as SP1 and other ceramides that help determine the treatment response and the patients prognosis.

It has know that GNAO1 is abundantly expressed in neuronal tissue [32]; we found in a previous work that *GNAO1* gene was underexpressed in pediatric EP [14]; we thought that this mRNA underexpression could modify the protein expression. We found GNAO1 protein underexpression in 60% of the samples in accordance with Zupancic *et al.,* [33] they reported GNAO1 underexpression in GBM tissue; it is suggested that GNAO1 underexpression is characteristic in EP and other gliomas. We thought that methylation play a important role in the regulation of GNAO1 expression, we previously reported that this GNAO1 underexpression were consequence of a chromosomal deletion and/or methylation [14], according with Xu *et al.,* [35] and Pei *et al.,* [36] they informed that the change of GNAO1 expression in Hepatocelluar carcinoma cells might be regulated by its methylation status. It is known that GNAO1 controlling signal transductions and it is deregulation can promote oncogenesis, apart from the activation of second messenger molecules, Gαo subunits (GNAO1 protein gene) can modulate the activity of transcription factors and thereby regulate gene expression [35,36]. We not found correlation between GNAO1 expression and age, sex,

histopathology, relapse or survival. It is important to refer that we observed a tendency to correlate the GNAO1 underexpression with increased survival, according to Liu *et al.*, [36] they reported in gastric cancer was the overall survival curves in relation to GNAO1 expression were statistically significant; it is suggested that the GNAO1 underexpression may be important for know the prognostic patients.

We found in previous work that *IPO7* gene overexpressed in pediatric EP [14], we found underexpression of IPO7 protein on 90% of analyzed samples. We not found correlation of protein expression with age, sex, histhopathological grade and relapse. We think that IPO7 overexpression in pediatric EP favors cell proliferation according with Xue *et al.,* [37] they reported that in Glioblastoma IPO7 is part of the FOXM1/IPO7/GLI1 axis promoting the cell proliferation, migration, and invasion. We found that the IPO7 overexpression favors the survival; ee think that IPO7 is related to survival through P53, according to Golomb *et al.,* [38], they described that IPO7 transcription is repressed by P53 and IPO7 depletion triggers P53 activation; it is suggested the importance of expression change of these proteins, possibly favor cell deregulation and the patients prognosis; we not found significant correlation between IPO7 expression and p53 expression (X^2=0.640) according to Golomb *et al.,* [38] they mentions that this feedback ocurrs in non-stressed cells. It is necessary to know the signaling pathways in which IPO7 participates in tumorigenesis, through the deregulation of cell proliferation. Possibly the post-translational changes actively participate in the regulation of IPO7 protein such as the participation of miRNAs; in accordance with Szczyrba *et al.,* [39] they reported that IPO7 is target of miR-22 and the downregulation of miR-22 in cancer pancreatic corresponds to an upregulation of IPO7; also Xue *et al.,* [37] they reported that the specific knockdown of IPO7 expression resulted in reduced nuclear accumulation of GLI1, indicating the dependence of nuclear import of GLI1 on IPO7.

Mitochondria are key organelles that contain double membranes and harbor their own DNA as well as components of transcrptional and translation machinery, they are implicated in a variety of processes, incluiding energy or free radical generation, regulation of apoptosis and modulation of various signaling pathways. It is known that the structural integrity of mitochondrial cristae is crucial for mitochondrial functions as structural integrity and biogenesis of mitochondrial cristae. It has reported that IMMT to be involved in regulation of mitochondrial cristae morphology besides, IMMT is a key component of MICOS complex, it was reported altered under several different pathological conditions [40,41]. We found *IMMT* gene overexpressed in 100% samples of pediatric EP [14]; we found IMMT underexpression in 73.3%. We not found correlation between clinical characteristics (age, sex, histopathological grade) and relapse. We think that IMMT is important in mitochondrial regulation according with Madungwe *et al.*,[42] they reported that IMMT is indispensable for normal mitochondrial function and has a functional impact on cellular activity. We found that the IMMT underexpression promotes increased overall survival, in accordance with Sotgia *et al.*,[43] they

reported in gastric cancer that IMMT is associated with significantly reduced time to first progression. It is suggested that IMMT is important during tumorigenesis; however, more studies are needed for known the IMMT regulation during tumorigenesis. Possible the post-transcriptional regulations participate in protein expression, but currently there are no reports, also it has been identified that Yme1L regulates the stability of IMMT [41].

5. Conclusion

In pediatric EP ASAH1 and Ki-67 have possitive expression in 86.7% and in 94% of the samples, respectively. P53, Cyclin D1, GNAO1, IMMT and IPO7 have negative expression in 76.6%, 60%, 60%, 73.3% and 90%, respectively.

The proteins: Cyclin D1, p53 and Ki-67 correlate with grade histopathological and relapse. IPO7 underexpression correlate with lower survival and IMMT underexpression with high survival. Cyclin D1 expression is not exclusive supratentorial EP in mexican patients. ASAH1 is overexpressed in pediatric EP and can have an important at treatment response.

Highlights

- Cyclin D1, p53 and Ki-67 correlate with grade histopathological and relapse.
- Cyclin D1 expression is not exclusive supratentorial EP in mexican patients.
- ASAH1 overexpressed in pediatric EP have important role in treatment response.

Conflict of Interest:

The authors declare that they have no competing interest.

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Table 1. Samples feature. The patients feature from the samples obtained. Show the histopathological grade (Grade), age (Age), sex (M.- Male or F.- Female), tumor location (Localization), relapse and survival.

SAMPLE	GRADE	GENDER	AGE	LOCALIZATION	RELAPSE	SURVIVAL
1	II	M	13	Intracranial	Yes	Yes
2	II	F	1	Intracranial	Yes	Yes
3	III	F	2	Intracranial	Yes	No
4	III	M	5	Intracranial	No	Yes
5	II	F	14	Intracranial	Yes	Yes
6	III	M	1	Intracranial	Yes	Yes
7	II	M	7	Intracranial	Yes	Yes
8	III	M	5	Intracranial	No	No
9	III	M	5	Intracranial	Yes	Yes
10	III	F	12	Intracranial	No	No
11	II	F	4	Intracranial	No	No
12	II	F	12	Intracranial	No	No
13	II	M	15	Intracranial	No data	No data
14	II	F	2	Intracranial	No data	No data
15	III	F	4	Intracranial	No data	No data
16	III	F	7	Intracranial	No data	No data
17	II	M	7	Intracranial	No data	No data
18	II	M	3	Intracranial	No data	No data
19	II	M	8	Intracranial	No data	No data
20	II	M	5	Intracranial	No data	No data
21	III	M	14	Intracranial	No data	No data
22	III	F	3	Intracranial	No data	No data
23	II	M	7	Intracranial	No data	No data
24	II	M	7	Intracranial	No data	No data
25	II	M	1	Intracranial	No data	No data
26	II	F	8	Intracranial	No data	No data
27	III	M	5	Intracranial	No data	No data
28	III	F	14	Intracranial	No data	No data
29	III	F	5	Intracranial	No data	No data
30	II	M	0	Intracranial	No data	No data

Table 2. Staining type of proteins. Show the percentange of sample for type staining.

STAINING TYPE	GNAO1	ASAH1	IMMT	IPO7	CYCLIN D1	KI67	P53
Negative	60% (18/30)	13.3% (4/30)	73.3% (22/30)	90% (27/30)	60% (18/30)	6.6% (2/30)	76.6% (23/30)
Weak	10% (3/30)	73.3% (22/30)	3.3% (1/30)	6.6% (2/30)	13.3% (4/30)	70% (21/30)	10% (3/30)
Modearate	16.6% (5/30)	13.3% (4/30)	16.6% (5/30)	3.3% (1/30)	10% (3/30)	16.6% (5/30)	6.6% (2/30)
Strong	13.3% (4/30)	0% (0/30)	6.6% (2/30)	0% (0/30)	16.6% (5/30)	6.6% (2/30)	6.6% (2/30)

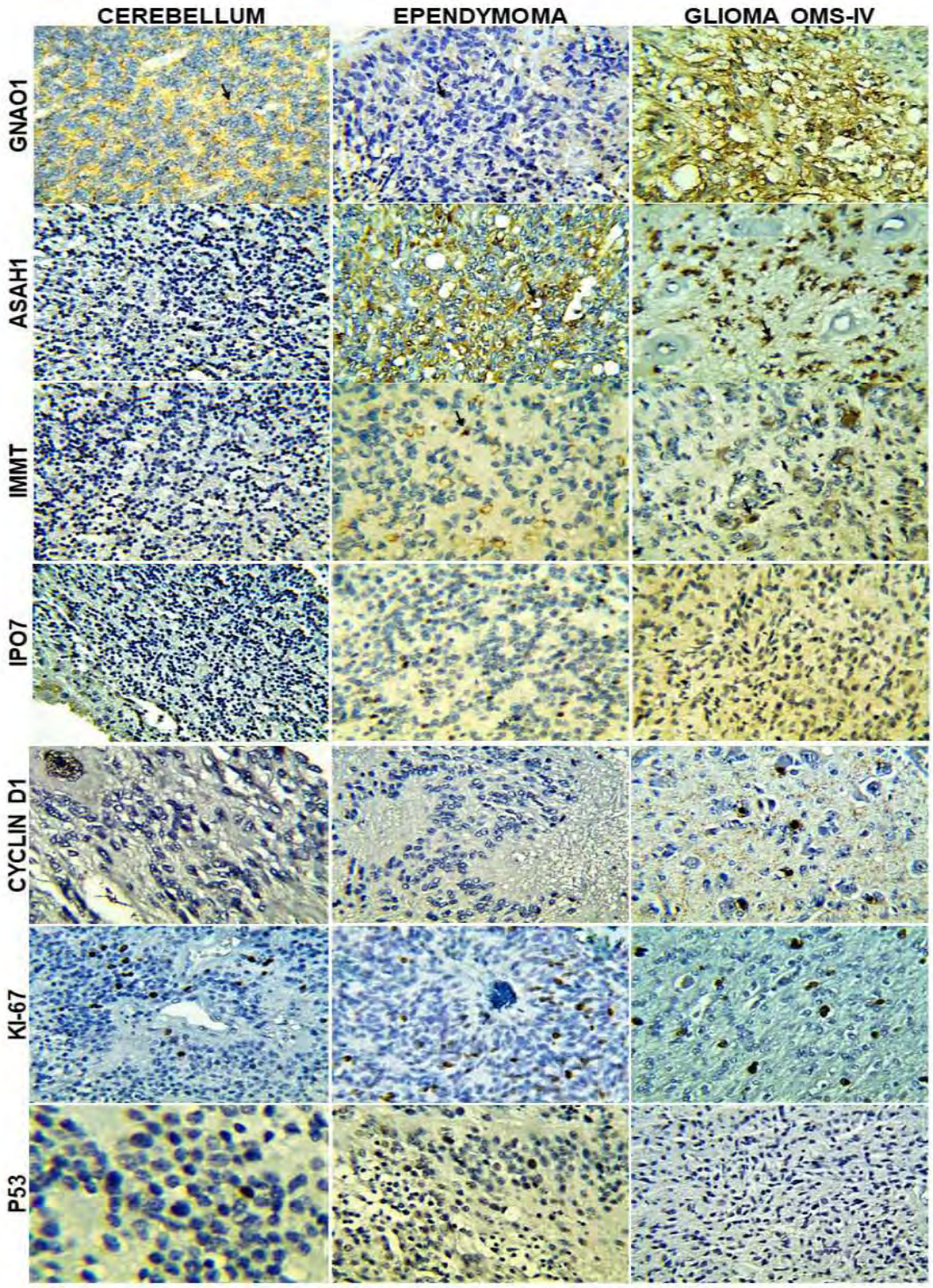

Figure 1. Proteins expression by immunochemistry assay. Show the protein staining in the tissue analyzed. The expression of: IPO7, Ki-67 and p53 were nuclear, expression of: GNAO1, ASAH1 and Cyclin D1 were cytoplasmic, IMMT expression was cytoplasm and mitochondria.

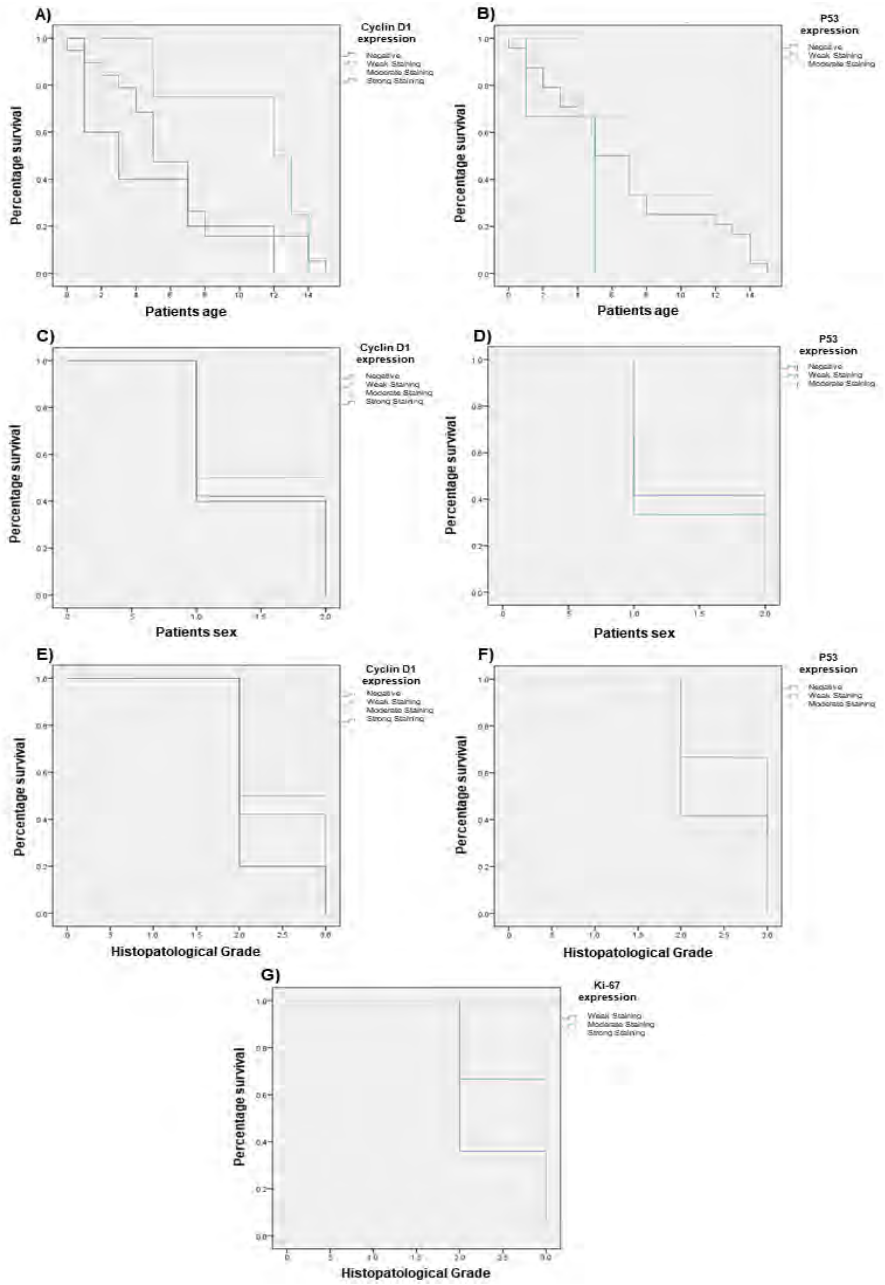

Figure 2. Clinical feature correlation for Kaplan Meier analysis. The line color refers to the staining type. On ordinates axis show the patients percentage survival. **A). Cyclin D1 expression and age.** On abscissa axis show the age patients. **B) P53 expression and age.** On abscissa axis show the age patients. **C) Cyclin D1 expression and sex.** On abscissa axis show the sex patients. 1.-Male, 2.- Female. **D) Ki-67 expression and sex.** On abscissa axis show the sex patients. 1.-Male, 2.- Female. **E) Cyclin D1 expression and histopathological grade**. On abscissa axis show the histophalogical grade. 2.-EP grade II. 3.-EP grade III. **F) p53 expression and histopathological grade.** On abscissa axis show the histophalogical grade. 2.-EP grade II. 3.-EP grade III. **G) Ki-67 expression and histopathological grade**. On abscissa axis show the histophalogical grade. 2.-EP grade II. 3.-EP grade III.

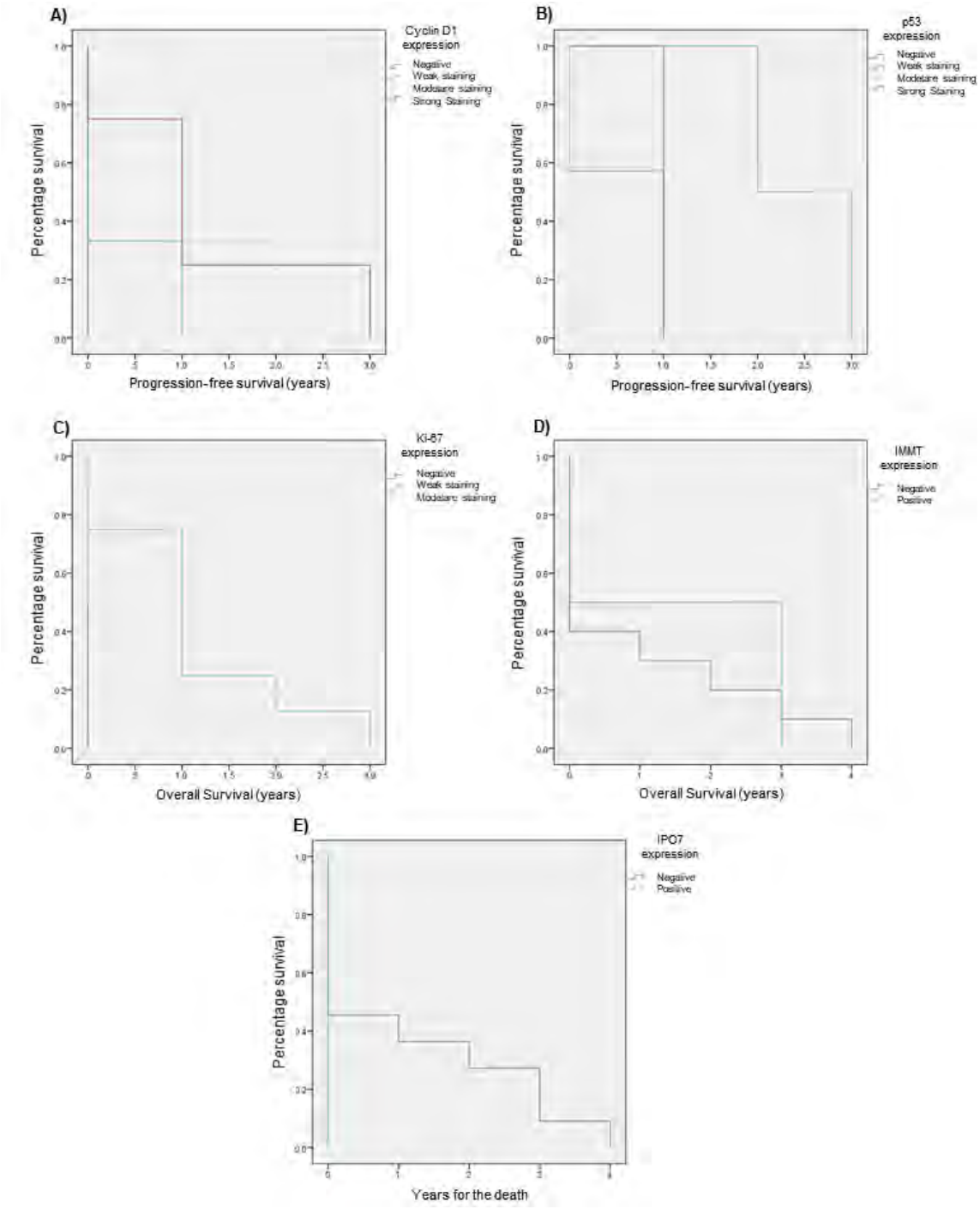

Figure 3. Pronostic correlation for Kaplan Meier analysis. The line color refers to the staining type. On ordinates axis show the patients percentage survival. **A) Cyclin D1 expression and relapse.** On abscissa axis show relapse**. B) p53 expression and relapse.** On abscissa axis show the relapse. **C) Ki-67 expression and relapse.** On abscissa axis show relapse. **D) IMMT expression and survival.** On abscissa axis show the survival. **E) IPO7 expression and survival.** On abscissa axis show survival.

Artículo 5
Pediatric pineal germinomas: Epegenetic and genomic approach

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Pediatric pineal germinomas: Epigenetic and genomic approach

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ABSTRACT

Objective: We identify and correlate chromosomal alterations, methylation patterns and gene expression in pediatric pineal germinomas.
Methods: CGH microarray, methylation and gene expression were performed through the Agilent platform. The results were analyzed with MatLab software, MapViewer, DAVID, GeneCards and Hippie.
Results: Amplifications were found in 1q24.2, 1q31.3, 2p11.2, 3p22.2, 7p13, 7p15.2, 8p22, 12p13.2, 14q24.3 y 22q12; and deletions were found in 1q21.2, 9p24.1, 10q11.22, 11q11, 15q11.2 and 17q21.31. In the methylation analysis, we observed 10,428 CpG Islands with a modified methylation status that may affect 11,726 genes. We identified 1260 overexpressed genes and 470 underexpressed genes. The genes *RUNDC3A, CDC247, CDCA7L, ASAH1, TRA2A, LPL and NPC2* were altered among the three levels.
Conclusions; We identified the 1q24.2 and 1q31.3 amplified regions and the 1q21.3 and 11q11 deleted regions as the most important aims. The genes *NPC2* and *ASAH1* may play an important role in the development, progression and tumor maintenance. The *ASAH1* gene is an ideal candidate to identify drug responses. These genomic and epigenetic studies may help to characterize the formation of pineal germ cell tumors to determine prognostic markers and also to identify shared characteristics in gonadal and extragonadal tumors.

1. Introduction

Germ cell tumors (GCTs) are a rare malignancy that are localized in gonadal or extra-gonadal sites, with an incidence of 2,4 per million and represents one percent of all cancers diagnosed in children less than the age of 15 years old [1]. These tumors arise from germ cells from embryonic residues and share histological features with gonadal germ cells [2,3]. The GCTs are histologically are divided into germinoma, teratoma, embryonal carcinoma, yolk sac tumor and malignant tumor of the germ cells [2,4]. In the central nervous system (CNS), these tumors are found in the pineal and suprasellar regions with an incidence of four percent of all brain tumors [5].

Studies have attempted to elucidate how the germ cells reach the pineal region originating a tumor in the CNS [6], and to answer this question, two dominant theories have been postulated to describe germ cell development. The first proposes that primordial cells are misplaced due to an aberrant migration during to fourth gestation week and are located in the brain midline. While, the second theory proposes a widespread distribution of germ cells during embryogenesis in the normal brain, thymus, liver and bone marrow to provide regulatory functions in different sites [3].

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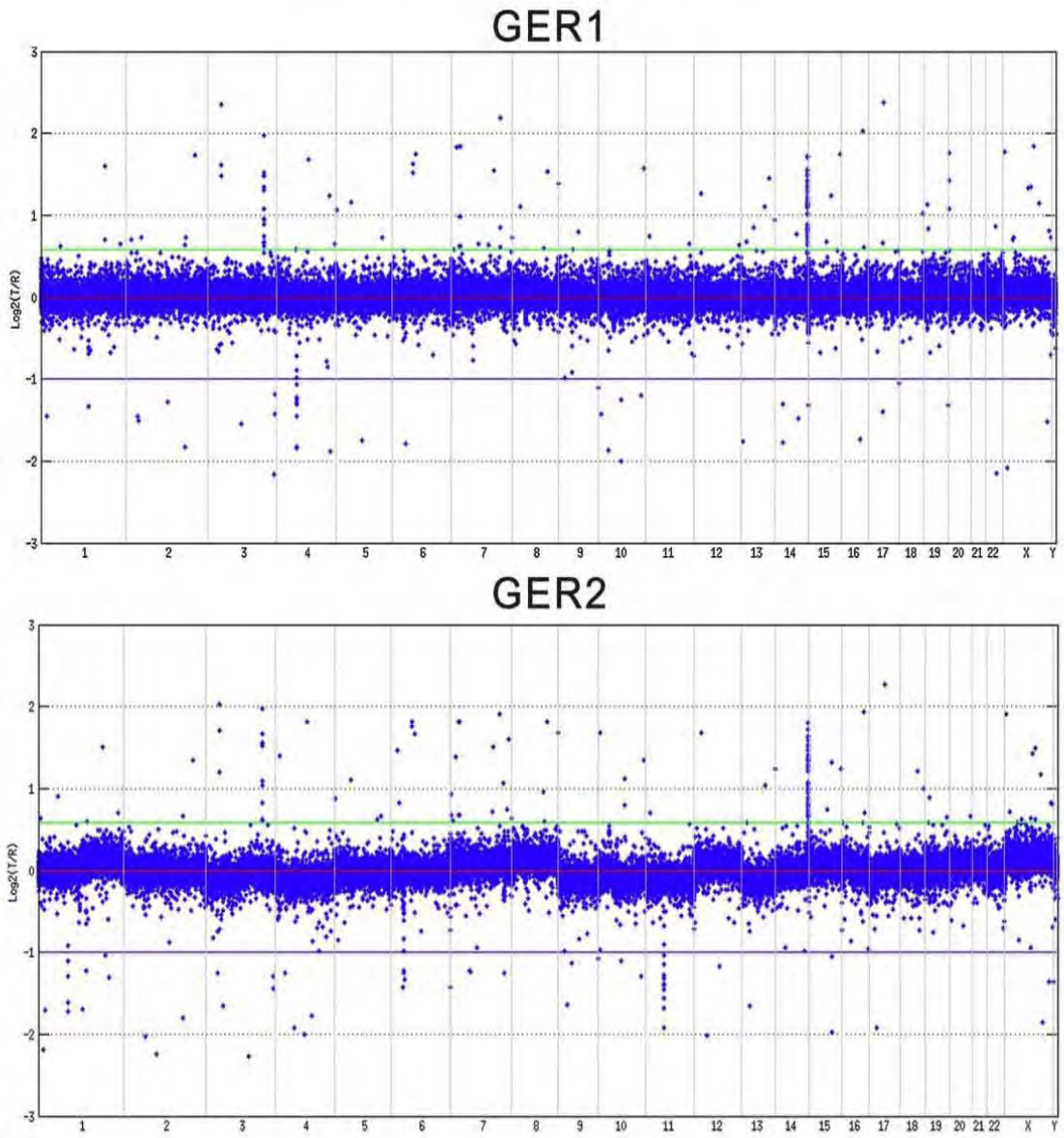

Fig. 1. Chromosomal alterations histogram. The amplification and deletions found in the germinomas are shown through the histograms generated from the RAW files. Chromosomes are indicated at the X-axis, and the fold change at the Y-axis corresponds to the chromosomal alteration. (A) Histogram of the GER1 sample. (B) Histogram of the GER2 sample.

displayed, and we observed that chromosomes 1, 4, 7, 20 and 22 have a more similar methylation pattern (Fig. 2).

The genes that showed changes in the methylation pattern are involved in signaling pathways, such as Cell adhesion molecules (CAMs), Neuroactive ligand receptor, Gap junction, axon guidance, TGFβ signaling pathway, Progesterone mediated oocyte maturation, Wnt signaling pathway, Notch signaling pathway, Nicotine and Nicotinamide metabolism, p53 signaling pathway, cell cycle, Oocyte meiosis, MAPK signaling pathway and GnRH signaling pathway.

3.3. Germinoma expression

In the expression profiles, we observed 1730 modified genes as follows: 1260 overexpressed and 470 under-expressed. From these data, we generated a cluster in which the GER1 and GER2 samples can be appreciated. Two regions are displayed with little significant expression changes, which contain the genes *USP18*, *E2F7*, *ROBO2*, *MPP1*, *MIB2* and *WNT2B*. These genes are related to the immune system, cell cycle progression, axon guidance, cell migration and proliferation. Both regions are highlighted in the dendrogram (green and blue), where the major differences between the samples are observed (Fig. 3).

From these results, we established genes that are involved in the following signaling ways: Cell adhesion molecules (CAM); Natural killer cell mediated cytotoxicity; cell cycle; T cell receptor signaling pathway; p53 signaling pathway; progesterone mediated oocyte maturation and B cell receptor signaling pathway and others.

The correlation results indicate that eight genes coincide among the chromosomal alterations, gene expression and methylation pattern analyses (Table 1), and these genes are involved in neuronal processes (*RUNDC3A*), the immune system (*CDC247*), apoptosis and

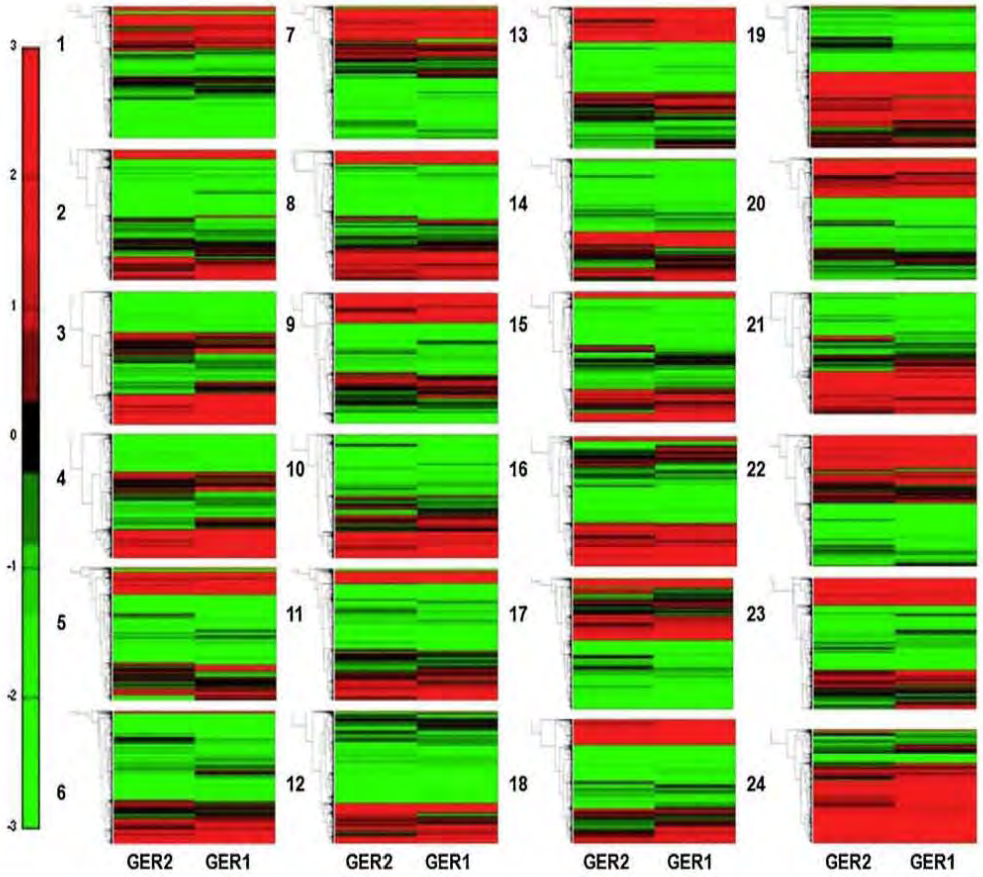

Fig. 2. Germinoma methylation pattern. The cluster shows the methylation pattern from the analyzed samples. For chromosomes 4, 5, 6, 8, 13, 15 and 18, the methylation patterns differ among the samples. The hypermethylation from 0.5 to 3 and the hypomethylation from −0.5 to −3 are indicated in the color range.

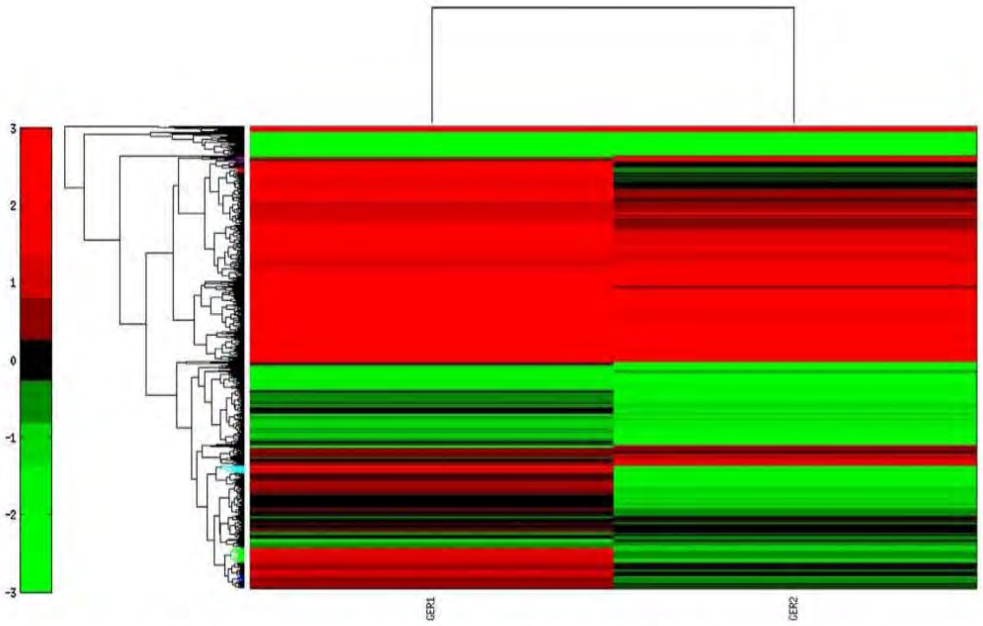

Fig. 3. Germinoma gene expression. (A) The cluster represents the gene expression profiles from the tumors. The data are grouped according to the normalized value. The overexpression from 0.5 to 3 and the under-expression from −0.5 to −3 are indicated in the color range. The most important differences are indicated in the colors by the dendrogram. (For interpretation of the references to color in the text, the reader is referred to the web version of this article.)

Table 1
Correlation between studies.

CHR	Gene	aCGH	Methylation	Expression
1q24.2	*CD247*	A	Hypo	Over
7p15.3	*CDCA7L*	A	Hypo	Over
7p15.3	*TRA2A*	A	Hypo	Over
8p21.3	*LPL*	A	Hypo	Over
8p22	*ASAH1*	A	Hypo	Over
14q24.3	*NPC2*	A	Hypo	Over
17q21.31	*RUNDC3A*	D	Hypo	Under
22q12.1	*ASPHD2*	A	Hyper	Under

Abbreviations: A, amplification; D, deletion; Hypo, hypomethylation; Hyper, hypermethylation; Over, overexpression; Under, subexpression.

cell cycle (*CDCA7L*, *ASAH1*), miRNA regulation (*TRA2A*) and fatty acid metabolism (*LPL*, *NPC2*).

Through real-time qRT-PCR assays we analyzed the *ASAH1* gene expression in five pineal germinomas, we found overexpression in all of the samples compared to the control (Fig. 4A y B).

A bioinformatics analysis was performed to identify the gene interactions within the CNS and gonads in view of the hypothesis of cell migration during the embryonic stage. We observed that the genes *RUNDC3A* and *ASAH1* might interact at the CNS with the genes *RAP1A*, *RAP2A*, *TFPT*, *TSC22D1* and *SLC25A5* (Fig. 4D). While in the gonads, the genes *TRA2A and NPC2* might interact with the *SF3A1*, *PRPF6*, *SAP18*, *EIF4A3*, *HNRNPU*, *TAF9*, *PPP1CC*, *CDC5L*, *SAMRCB1*, *MAP1LC3B*, *SEPT2* and *PAXP1* (Fig. 4C).

4. Discussion

Currently, germ cell tumors of the pineal region have not been broadly studied at the molecular level, and genomic and epigenetic approaches enable us to obtain information regarding genes involved in tumor development, maintenance and possibly prognostic markers. According to our research, we reported amplifications in 1q, 12p and 21q and deletions in 5q, 11q and 13q [19]. Germinomas located in the testis show duplications in 7p15.2, 12p12.3-p11.1, 12p13.33-p12.3 and 21q22.2 and deletions in 4p16.3, 10p15.3-p15.2, 13q21.1-q21.2 and 15q11.1-q11.2 [20,21]. Germinomas located in the ovary present amplifications in chromosomes 1, 6, 16, 19, 20 and 21 [22]. We want to spotlight the amplification reports from the pineal germinoma on the 12p, 8q and 1q regions and the deletions in 13q, 18q, 9q and 11q [11].

We observed regions considered characteristic of a pineal tumor amplified in 1q24.2 and 1q31.3, as well as in 7p15.2 and 12p13.2 reported in the testis and pineal germ tumor [20,21]. We also found deletions in 1q21.3 and 11q11, which were already reported for pineal germ tumors, and 15q11.2 was reported for testicle germinoma [20,21]. Thus, we suggest that these alterations are characteristics of germ tumors regardless of their location intra or extragonadal, correlating with the histological similarity with gonadal GCTs [23].

It is know that the epigenetic process plays an important role in pluripotency, cancer formation and progression, including CGTs, which are subject to epigenetic reprogramming. Epigenetic

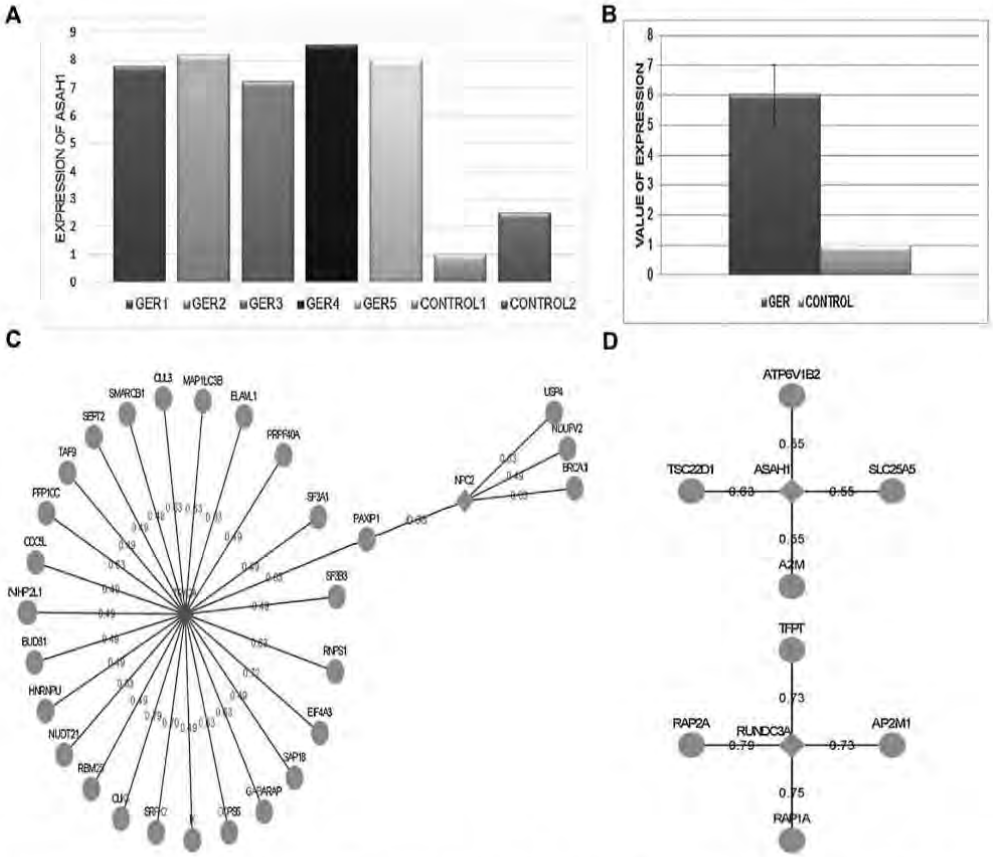

Fig. 4. *ASAH1* expression in germinomas and gene interactions. The expression and possible gene interactions are shown. (A) Graphic of the *ASAH1* gene expression among the germinomas. (B) Graphic of the *ASAH1* average gene expression compared to the control. (C) Possible interaction at the gonads includes the genes *NPC2* and *TRA2A*. (D) Possible interaction at the CNS includes the genes *ASAH1* and *RUNDC3A*. The diamond shape indicates a gene of interest, and the score signifies how likely the partnership is.

deregulation, through genetic and environmental processes, disrupts embryo physiology germ cell development [1,24]. It has been suggested the methylation pattern is associated with the anatomical location of the GCT, due to hypomethylation in the gonadal tumor and hypermethylation in the extragonadal tumors [9]. In accordance with other reports, indicating the methylation status in GCTs, we found *RUNX3* hypermethylation and *SFRP2*, *RASSF5*, *SRFP5*, *HOXB5*, *SFRP1* and *APC* hypomethylation, suggesting the methylation pattern is also modified according to the anatomical location of the tumor [1,25].

Furthermore, we found that *TCF4*, *BDNF*, *BMP3*, *WNT2*, *APC*, *SOX2*, *NTRK2*, *NTRK3*, *TGFB2* and *WNT1* were hypomethylated and *Fzd9* was hypermethylated, and these genes are highly relevant since they are described in the human stem cell path in GCTs, suggesting their involvement in tumor formation [9]. These genes c-KIT, *SALL4* and *PDPN* were also overexpressed and have been reported as biomarkers for GCTs [26–28].

Correlations among the three studies show 18 common altered genes between aCHG and expression (Table S2 DOI: 10.6070/H4NV9G96); 97 common altered genes between aCGH and methylation (Table S3 DOI: 10.6070/H4NV9G96); and 755 common altered genes between methylation and expression (Table S4 DOI: 10.6070/H4NV9G96). Only eight genes were common in the expression, methylation pattern and chromosomal alterations, and these genes were involved in neuronal processes (*RUNDC3A*), the immune system (*CDC247*), apoptosis and cell cycle (*CDCA7L*, *ASAH1*), miRNA regulation (*TRA2A*) and fatty acid metabolism (*LPL*, *NPC2*).

There is not sufficient data for all these genes to establish their relevance in to the germinoma pineal neoplasic process; nevertheless, the gene *TRA2B* is overexpressed in breast, cervical, ovarian and colon cancer, contributing to their malignancy [29], and *CD247* is an essential molecule for natural killer cell activation that is associated with the tumor microenvironment and immunosuppression [30]. In addition, *CDCA7L* is reported as a c-Myc target, and its overexpression was observed in several types of cancer, inducing proliferation and cell transformation in medulloblastoma [31].

NPC2 can be detected in neurons and is associated with neurodegenerative diseases. When it is secreted by pre-malignant lung tumors it interferes with the tumor microenvironment through paracrine mechanisms. In vitro and in vivo xenograft data demonstrates that the expression of *NPC2* influences cell proliferation, migration and tumorigenesis through the regulation of *ERK1/2* activation, and its downregulation accelerates cell proliferation and tumor growth through the activation of *ERK1/2*. The data point to a novel role for *NPC2* in the regulation of the pro-tumorigenic microenvironment [32,33].

Lastly, *ASAH1* (AC) is an enzyme in the ceramide metabolic pathway, and it plays its role by metabolizing the pro-apoptotic ceramide to a proliferative and tumor inducing metabolite. The enzyme AC is constitutively overexpressed in several human cancers. The consequence of such high AC expression is the maintenance of low ceramide levels in tumor cells. High AC activity leads to elevated levels of the downstream metabolite, *S1P*, promoting cell survival. Recent studies suggest that the upregulation of acid ceramidase plays an essential role in the development of multi drug resistance in cancer chemotherapy; and the consequences of AC inhibition could be enhanced ceramide-mediated tumor killing and/or reduced drug resistance [34,35].

Therefore, we suggest that extragonadal tumors share chromosomal alterations and similar gene expression, proposing that the 1q24.2 and 1q31.3 amplification and the 1q21.3 and 11q11 deletion are the most important in germ cell tumors. The *NPC2* and *ASAH1* genes may play an important role in tumor development, progression and maintenance. ASAH1 may be a candidate to identify a patient's response to therapeutic drugs. Finally, the gene expression pattern may help to elucidate a patient's prognosis, to understand germ cell tumor formation in the pineal region and to identify shared characteristics of gonadal and extragonadal germ cell tumors.

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9. Discusión

Es primordial conocer a través de estudios moleculares los cambios genéticos y epigenéticos que ocurren en las neoplasias al permitir dilucidar el mecanismo de la tumorigénesis y la comprensión de las características específicas de cada neoplasia. Sin embargo, hoy en día se tienen pocos estudios moleculares sobre las causas, el desarrollo y el mantenimiento tumoral de los ependimomas pediátricos; la mayor información encontrada en la literatura hace referencia a los aspectos clínicos y con respecto a los abordajes moleculares la mayoría de estos estudios han sido realizados en ependimomas de adultos; además, no existen estudios publicados en pacientes mexicanos.

En el análisis de pacientes pediátricos mexicanos con EP reveló ganancias en 2p11.2, 2p22.3, 3p22.2,7p15.2, 8p22, 10q21.1, 11p15.4, 14q32.33 y pérdidas en 1p31.1, 1q21.3, 2p22.2, 7q34 y 8p11 .23-p11.22. Se considera que la región 14q32.33 juega un papel importante en la tumorigénesis; de acuerdo con *Olsen et al.* [35] reportan a la región 14qter como perdida; cabe resaltar que en la región cromosómica 14q32.33 se localiza el miR-203, el cual exhibe expresión aberrante en múltiples tumores malignos. Muchos estudios han revelado que miR-203 juega un papel esencial durante el crecimiento celular, la migración, la invasión [36]; se sugiere a la región 14q32.33 como importante en EP pediátrico.

Además, se profundizó en la búsqueda de las alteraciones cromosómicas características de los EP pediátricos a nivel mundial, encontrando que las alteraciones en 1q, 6q, 9p, 13q y 22q son características de estas neoplasias y la alteración de la citobanda 1q25 tiene potencial para ser utilizado como marcador pronóstico al correlacionar con una corta sobrevida libre de progression [37,38].

Otro de los factores importantes durante la tumorigénesis son los epigenéticos; la metilación es un mecanismo importante durante el desarrollo del EP en el 89% de los casos [39], se conoce que las alteraciones cromosómicas, así como el patrón de metilación varían de acuerdo con la localización anatómica del tumor [40,41]. En el

análisis de resultados se encontraron genes metilados relacionados con la apoptosis: *MYC*, el ciclo celular: *SESN2*, *MAD1L1*, *CUX1*, *E2F8*, *ASAH1*, *GNAO1*, proliferación celular: *IMMT*, diferenciación neuronal: *EBF3*, activación transcripcional: *GATA2*, *IPO7* y los genes relacionados con el oncogén TFG: *p53* y *c-myc*, así como la división celular. Se encontraron hipermetilados los genes *TP73* y *RASSF1*, apoyando a lo reportado por Hamilton *et al.* [24] y Alonso *et al.* [39]. Los genes *MGMT*, *HIC-1*, *CDKN2B* y *THBS1* se observaron hipometilados, contrariamente a lo reportado por Alonso *et al.* [39], Waha *et al.* [42] y Rousseau *et al.* [43] posiblemente estos cambios se deban a que este estudio fue realizado en población mexicana, mientras que los anteriores fueron en pacientes caucásicos; además pueden influir los factores ambientales y el estilo de vida.

Se encontraron pocos estudios sobre cambios en la metilación reportada en EP, sin embargo, se conoce que los EP espinales son epigenéticamente diferentes a los intracraneales, se ha descrito la hipermetilación de *HIC-1* en el 93.8% de EP intracraneal y en el 65.0% de espinal [44]. En este estudio se observó que, en EP pediátricos, sin importar su localización anatómica, el cambio del estado de metilación del gen *hTERT* es característico de los EP y tiene potencial para ser utilizado como biomarcador molecular de pronóstico promoviendo la longitud de los telómeros o inmortalizando células en distintos tipos de tumores [45], posiblemente esta diferencia se deba al número de muestras y al análisis estadístico.

Las alteraciones cromosómicas y la metilación se verán reflejados en la expresión génica. Por lo tanto, se han reportado cambios en la expresión de los genes *EGFR*, *CDKN2A*, *p16INK4A*, y *p14ARF* [28,29]. Se encontraron genes sobreexpresados relacionados con la transcripción (*MAPK11*, *IPO7*), proliferación (*CBLB*, *KIT*, *SESN1*, *ASAH1*, *GNAO1*), adhesión celular (*ITGA6*), migración (*LAMA2*), reparación del DNA (*RAD51*) y genes subexpresados relacionados con apoptosis (*BID*, *PRKCB*, *IMMT*), adhesión celular (*PTK2*) y crecimiento tumoral (*FGF22*). Se encontraron sobreexpresados los genes: *CD44, KIT, LAMA2, MDM2* y *RAC2*, concordando con lo reportado por Hagel *et al.* [46], Zakrzeweska *et al.* [47], Zavalhia *et al.* [48] y Araki *et al.* [49]; se sugiere que la sobreexpresión de *LAMA2, KIT, CD44, y EZH2* son características de los EP pediátricos.

Existe diferenciación genética entre los EP espinales y los intracraneales, los primeros se asocian con el gen *NF2* y muestran una mayor activación de *ERBB2*, mientras que los segundos se asocian con *EPB41L3* e *HIC-1* [44]. Sin embargo, se observó que independientemente de la localización anatómica del tumor los cambios de expresión de los genes *ERBB* y *hTERT* son característicos de los EP pediátricos, pero sólo *hTERT* correlaciona con una menor sobrevida del paciente. Lamentablemente, un enfoque terapéutico dirigido a *ERBB* que incluye lapatinib, inhibidor selectivo de molécula pequeña de *ERBB1/ERBB2*, se reportó como ineficaz en niños con EP recurrente [50,51].

A partir, de los ensayos de microarreglos se propusieron los genes *CISD3*, *IPO7*, *IMMT*, *ASAH1* y *GNAO1,* los cuales no han sido reportados anteriormente en EP. De acuerdo con la literatura se sabe que *CISD3* e *IMMT* están relacionados con la proliferación celular y la promoción de la angiogénesis [35,52]. *ZWINT* participa en la formación del cinetocoro y puede promover la proliferación celular [41]. *IPO7* es una proteína nuclear sobrerregulada por *c-MYC* y *p53* [53]. *GNAO1* es importante en la señalización molecular en el sistema nervioso central y es altamente expresado en el tejido cerebral [54]. *ASAH1* tiene un rol importante en la supervivencia celular y la resistencia a la apoptosis [55].

Se realizaron ensayos de inmunohistoquímicas para conocer la expresión proteica de KI-67, P53, Ciclina D1, IPO7, IMMT, ASAH1 y GNAO1. Se observó que la expresión de P53 y KI67 correlacionan con el grado histopatológico y con la reincidencia concordando con lo reportado por Alexiou *et al.* [56]. Ciclina D1 se sobreexpresa en el 16% de los EP infratentoriales, contrariamente Rogers *et al.* [57] reportan que Ciclina D1 es mayor en tumores supratentoriales. IPO7 no ha sido asociado a cáncer, sin embargo, se encontró que IPO7 es sobreexpresado en el 10% de las muestras, correlacionando con la sobrevida. ASAH1 se sobreexpresa en más del 80% de los casos, concordando con Doan *et al*. [58], ellos mencionan que ASAH1 desempeñan un papel significativo en la progresión tumoral. IMMT se asocia al desarrollo de cáncer gástrico [59], en este estudio se encontró subexpresado en el 73.3% de las muestras, correlacionando con la sobrevida. GNAO1 se encontró subexpresado en el 60% de las muestras concordando con

Zunpancic *et al.* [60], ellos reportan que en glioblastoma GNAO1 se encuentra sobreexpresado.

Se sabe que la evolución de los datos genómicos y epigenómicos en EP pediátricos muestra una heterogeneidad significativa de estos tumores basados en la biología molecular, esto ha comenzado a arrojar luz sobre la razón de la diversidad de respuestas clínicas [51]. Se han hecho grandes esfuerzos para refinar los criterios histológicos y las categorías de riesgo clínico, no sólo con base en aspecto y localización del tumor, sino también para incluir los hallazgos genéticos subyacentes. Estos nuevos hallazgos moleculares, están volviendo evidente que, en el futuro, los criterios histológicos pueden ser insuficientes para dirigir el tratamiento y predecir resultados [61]. Tanto los aspectos genómicos y epigenómicos llevan a un cambio en la expresión proteica, por lo cual este conocimiento puede ayudar a comprender el desarrollo tumoral y aportar información para un candidato a biomarcador pronóstico; por lo tanto, se revisaron estudios donde analizan los cambios de diversas proteínas en EP pediátricos. Se encontró que EGFR, EMA, GFAP, NeuN y p53 son características de estas neoplasias y las proteínas: BCL-2, EGFR y NCL están relacionadas fuertemente con el desarrollo del EP pediátrico y tienen potencial para ser utilizadas como marcadores de pronóstico (Artículo en preparación).

10. Conclusiones

Las regiones cromosómicas 1q25, 7q34, 14q32 y 22q son importantes en los EP pediátricos, considerándose a 22q y 1q25 como características de estos tumores.

Se sugiere que la sobreexpresión génica de *hTERT, ERBB* y la expresión proteica de EGFR, EMA, GFAP, NeuN y p53 son características de los EP pediátricos.

La ganancia de 1q25 y la sobreexpresión de *hTERT* tienen potencial para ser utilizados como marcador pronóstico.

Se sugiere que Ciclina D1 se puede encontrar sobreexpresado en EP pediátricos y no es exclusivo de los ependimomas supratentoriales en pacientes mexicanos.

En EP pediátricos las proteínas ASAH1 y Ki-67 se encuentran sobreexpresadas en el 86.7% y el 94%, respectivamente. P53, Ciclina D1, GNAO1, IMMT e IPO7 son subexpresadas en el 76.6%, 60%, 60%, 73.3% y 90%, respectivamente.

Las proteínas Ki-67, p53 y Ciclina D1 correlacionan con el grado histopatológico del tumor y la reincidencia. IPO7 correlacionan con una menor sobrevida e IMMT con una mayor sobrevida.

11. Perspectivas

Evaluar la expresión del gen *hTERT* en ependimomas pediátricos en pacientes mexicanos, porque es un fuerte candidato a biomarcador molecular.

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Anexo I

Genomics and epigentics: A study of ependimomas in pediatric patients.

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

Table S1. Sample features. The features of the patients from whom the samples were obtained are shown age (Age), gender (Sex), and tumor location (Localization). Histopathological grade classification was according to the World Health Organization (Grade) and whether the tumor was de novo or recidivism (Observations). The ependymomas is highlighted in bold.

Sample	Age	Sex	Grade	Localization	Observations
Medulloblastoma	1	F	II	Posterior fossa	*De novo*
Ependymoma1	**15**	**M**	**II**	**Posterior fossa**	***De novo***
Medulloblastoma	1	F	II	Posterior fossa	*De novo*
Ependymoma2	**15**	**M**	**III**	**Posterior fossa**	***De novo***
Plexus papilloma	0	F	I	Posterior fossa	*De novo*
Ganglioglioma	13	M	I	Posterior fossa	*De novo*
Ependymoma3	**13**	**F**	**II**	**Spinal cord (L2-L5)**	**Recidivism**
Glioblastoma multiforme	11	M	IV	Posterior fossa	*De novo*
Ganglioglioma	12	F	I	Posterior fossa	*De novo*
Ganglioglioma	11	M	I	Posterior fossa	*De novo*
Medulloblastoma	1	F	II	Posterior fossa	*De novo*
Ependymoma4	**9**	**F**	**II**	**Posterior fossa**	***De novo***
Germinoma	11	M	II	Posterior fossa	*De novo*
Astrocytoma	6	F	II	Posterior fossa	*De novo*
Transitional Meningioma	15	F	I	Posterior fossa	*De novo*
Medulloblastoma	9	M	IV	Posterior fossa	*De novo*
Astrocytoma	13	F	II	Posterior fossa	*De novo*
Astrocytoma	13	F	I	Posterior fossa	*De novo*
Plexiform Neurofibroma	1	M	I	Posterior fossa	*De novo*
Medulloblastoma	15	M	IV	Posterior fossa	*De novo*
Medulloblstoma	3	M	IV	Posterior fossa	*De novo*
Medulloblastoma	13	M	IV	Posterior fossa	*De novo*
Astrocytoma	4	M	II	Posterior fossa	*De novo*
Sarcoma	6	F	IV	Posterior fossa	*De novo*
Astrocytoma	12	M	I	Posterior fossa	*De novo*
Germinoma	13	M	II	Posterior fossa	*De novo*
Ependymoma5	**2**	**F**	**II**	**Posterior fossa**	***De novo***
Germinoma	11	M	II	Posterior fossa	*De novo*
Astrocytoma	16	F	II	Posterior fossa	*De novo*
Ependymoma6	**15**	**M**	**II**	**Posterior fossa**	***De novo***
Germinoma	13	M	II	Posterior fossa	*De novo*
Astrocytoma	5	F	II	Posterior fossa	*De novo*
Astrocytoma	11	F	I	Posterior fossa	*De novo*
Cortical dysplasia	10	M		Posterior fossa	*De novo*
Astrocytoma	5	F	I	Posterior fossa	*De novo*
Germinoma	7	F	II	Posterior fossa	*De novo*
Astrocytoma	1	F	II	Posterior fossa	*De novo*
Oligodendroglioma	10	M	II	Posterior fossa	*De novo*
Germinoma	15	M	II	Posterior fossa	*De novo*
Plexus Papilloma	2	F	I	Posterior fossa	*De novo*
Germinoma	12	F	II	Posterior fossa	*De novo*
Atrocytoma	11	F	II	Posterior fossa	*De novo*
Medulloblastoma	3	M	IV	Posterior fossa	*De novo*
Chordoma	1	M	II	Posterior fossa	*De novo*
Astroblastoma	15	F	IV	Posterior fossa	*De novo*
Medulloblastoma	13	M	IV	Posterior fossa	*De novo*

Schawnnoma	15	F	I	Posterior fossa	*De novo*
Hemangioblastoma	15	M	II	Posterior fossa	*De novo*
Astrocytoma	2	F	II	Posterior fossa	*De novo*
Astrocytoma	13	M	II	Posterior fossa	*De novo*

Table S2. Methylated genes in Ependymoma. Show the genes (Gene) methylation changed pattern, in log_2 value greater than 5 hypermethylation and less than -5 hypomethylation (Status), the percentage observed in samples (%) and the chromosomal location of the genes (CHR) are indicated. Hyper. Hypermethylation, Hypo. Hypomethylation

CHR	Gene	Status	%
1p13.3	GPSM2	Hypo	100%
1p22.1	TGFBR3	Hypo	100%
1p31.3	JAK1	Hypo	100%
1p31.3	NFIA	Hypo	100%
1p32.1	FGGY	Hypo	100%
1p32.3	SSBP3	Hyper	100%
1p34.3	POU3F1	Hypo	100%
1p35.3	SESN2	Hypo	100%
1p36.11	CLIC4	Hypo	100%
1p36.13	ZBTB17	Hyper	100%
1p36.21	FLJ37453	Hypo	100%
1p36.22	SHYPER	Hypo	100%
1p36.31	KLHL21	Hypo	100%
1p36.32	HES5	Hypo	100%
1p36.32	MEGF6	Hyper	100%
1p36.33	MIB2	Hyper	100%
1q21.2	HIST2H2AB	Hypo	100%
1q21.2	OTUD7B	Hyper	100%
1q21.3	CHRNB2	Hyper	100%
1q23.3	KLHDC9	Hypo	100%
1q32.1	NAV1	Hypo	100%
1q42.13	SNAP47	Hypo	100%
1q43	GREM2	Hyper	100%
1q44	FAM36A	Hypo	100%
1q44	HNRNPU	Hypo	100%
1q44	ZNF669	Hypo	100%
2p13.3	AAK1	Hypo	100%
2p14	AFTPH	Hypo	100%
2p16.1	PAPOLG	Hypo	100%
2p21	EPAS1	Hypo	100%
2p23.2	PPP1CB	Hypo	100%
2p24.3	FAM84A	Hypo	100%
2p25.3	TSSC1	Hyper	100%
2q21.1	TUBA3E	Hyper	100%
2q24.1	GPD2	Hyper	100%
2q31.2	TTC30A	Hypo	100%
2q32.1	NCKAP1	Hypo	100%
2q32.1	NUP35	Hypo	100%
2q35	IGFBP5	Hypo	100%
3p13	PDZRN3	Hypo	100%
3p14.1	MAGI1	Hypo	100%
3p14.3	WNT5A	Hypo	100%

3p21.1	CHDH	Hypo	100%
3p21.1	NT5DC2	Hyper	100%
3p21.31	ZNF197	Hypo	100%
3p24.2	NR1D2	Hypo	100%
3q21.2	ZNF148	Hypo	100%
3q21.3	GATA2	Hypo	100%
3q21.3	ZXDC	Hypo	100%
3q24	HPS3	Hypo	100%
3q24	PLOD2	Hypo	100%
3q25.1	MBNL1	Hypo	100%
3q26.33	SOX2	Hypo	100%
3q27.1	KLHL24	Hypo	100%
3q29	HES1	Hypo	100%
4p16.3	DGKQ	Hyper	100%
4p16.3	PCGF3	Hyper	100%
4q12	EXOC1	Hypo	100%
4q13.3	PAHYPER1	Hypo	100%
4q22.1	PKD2	Hypo	100%
5p15.33	CEP72	Hyper	100%
5q11.2	SKIV2L2	Hypo	100%
5q13.2	OCLN	Hypo	100%
5q13.2	SERF1A	Hypo	100%
5q14.3	ARRDC3	Hypo	100%
5q23.2	CEP120	Hypo	100%
5q31.1	CDC42SE2	Hypo	100%
5q31.2	MATR3	Hypo	100%
5q35.1	FGF18	Hypo	100%
6p22.1	HIST1H1B	Hypo	100%
6p22.2	HIST1H3B	Hypo	100%
6p22.2	HIST1H3F	Hypo	100%
6p22.2	TRIM38	Hypo	100%
6p22.3	HDGFL1	Hyper	100%
6p25.3	EXOC2	Hyper	100%
6q14.3	SNORD50B	Hypo	100%
6q21	FOXO3	Hypo	100%
6q23.2	AKAP7	Hypo	100%
6q24.1	CITED2	Hypo	100%
6q24.2	PHACTR2	Hypo	100%
6q25.1	UST	Hyper	100%
6q26	PARK2	Hyper	100%
6q26	QKI	Hypo	100%
6q27	PHF10	Hypo	100%
7p14.3	RP9	Hypo	100%
7p15.2	TAX1BP1	Hypo	100%
7p22.1	TNRC18	Hypo	100%
7p22.3	ADAP1	Hyper	100%
7p22.3	GET4	Hyper	100%
7p22.3	MAD1L1	Hyper	100%
7p22.3	PRKAR1B	Hyper	100%

7p22.3	UNCX	Hypo	100%
7q22.1	CUX1	Hypo	100%
7q31.33	GHYPER8	Hypo	100%
7q34	UBN2	Hypo	100%
8p21.3	GFRA2	Hyper	100%
8p21.3	SLC39A14	Hypo	100%
8p22	EFHA2	Hypo	100%
8p23.3	ERICH1	Hypo	100%
8q12.1	CHD7	Hypo	100%
8q12.1	UBXN2B	Hypo	100%
8q12.3	BHLHE22	Hyper	100%
8q13.1	MYBL1	Hypo	100%
8q13.1	TCF24	Hypo	100%
8q21.13	ZNF704	Hypo	100%
8q21.2	REXO1L1	Hyper	100%
8q21.2	REXO1L2P	Hyper	100%
8q23.1	EIF3E	Hypo	100%
8q24.21	MYC	Hypo	100%
8q24.3	ARHGAP39	Hyper	100%
8q24.3	MAFA	Hypo	100%
8q24.3	ZC3H3	Hyper	100%
9q22.32	PTCH1	Hypo	100%
9q34.13	GTF3C4	Hypo	100%
9q34.2	REXO4	Hypo	100%
9q34.3	ABCA2	Hyper	100%
9q34.3	CAMSAP1	Hypo	100%
9q34.3	EHMT1	Hyper	100%
9q34.3	EXD3	Hyper	100%
10p13	SEPHS1	Hypo	100%
10p14	FLJ45983	Hypo	100%
10p14	ITIH5	Hyper	100%
10p15.3	ADARB2	Hyper	100%
10p15.3	DIP2C	Hyper	100%
10p15.3	ZMYND11	Hypo	100%
10q21.1	TFAM	Hypo	100%
10q21.3	EGR2	Hypo	100%
10q23.2	GLUD1	Hypo	100%
10q23.31	PTEN	Hypo	100%
10q24.1	TM9SF3	Hypo	100%
10q24.2	HPSE2	Hyper	100%
10q25.1	SORCS3	Hypo	100%
10q25.3	GFRA1	Hypo	100%
10q25.3	TRUB1	Hypo	100%
10q25.3	VAX1	Hypo	100%
10q26.3	EBF3	Hypo	100%
11p11.2	CHST1	Hyper	100%
11p11.2	PRDM11	Hypo	100%
11p15.1	E2F8	Hypo	100%
11p15.1	GTF2H1	Hypo	100%

11p15.5	CDHR5	Hyper	100%
11p15.5	EPS8L2	Hyper	100%
11p15.5	LRRC56	Hyper	100%
11p15.5	MUC6	Hyper	100%
11p15.5	TALDO1	Hypo	100%
11q12.2	MS4A15	Hyper	100%
11q12.3	HNRNPUL2	Hypo	100%
11q13.1	MARK2	Hyper	100%
11q13.1	RPS6KA4	Hyper	100%
11q13.2	PITPNM1	Hyper	100%
11q13.2	SUV420H1	Hypo	100%
11q13.3	CCND1	Hypo	100%
11q13.3	FGF19	Hypo	100%
11q13.4	KRTAP5-11	Hyper	100%
11q21	SESN3	Hypo	100%
11q23.3	H2AFX	Hypo	100%
11q24.3	FLI1	Hyper	100%
11q25	B3GAT1	Hyper	100%
12p11.21	YARS2	Hypo	100%
12q12	TWF1	Hypo	100%
12q21.1	RAB21	Hypo	100%
12q21.2	NAP1L1	Hypo	100%
12q23.2	ARL1	Hypo	100%
12q23.3	CHST11	Hyper	100%
12q24.13	DDX54	Hypo	100%
12q24.33	NOC4L	Hyper	100%
12q24.33	ZNF84	Hypo Hyper	80% 20%
13q12.11	GJA3	Hypo	100%
13q12.11	IFT88	Hypo	100%
13q12.12	C1QTNF9	Hyper	100%
13q14.11	NAA16	Hypo	100%
13q31.3	GPC6	Hypo	100%
13q34	F7	Hyper	100%
13q34	GAS6	Hyper	100%
13q34	MCF2L-AS1	Hypo	100%
14q13.2	BAZ1A	Hypo	100%
14q23.1	PPM1A	Hypo	100%
14q32.32	EIF5	Hypo	100%
14q32.33	JAG2	Hyper	100%
15q11.2	TUBGCP5	Hypo	100%
15q13.2	FAM7A3	Hyper	100%
15q24.3	LINGO1	Hyper	100%
15q26.3	DNM1P46	Hyper	100%
15q26.3	WASH3P	Hypo	100%
16p12.1	TNRC6A	Hypo	100%
16p12.2	POLR3E	Hypo	100%
16p13.2	USP7	Hypo	100%
16p13.3	C1QTNF8	Hyper	100%

16p13.3	CACNA1H	Hyper	100%
16p13.3	CLCN7	Hyper	100%
16p13.3	LMF1	Hyper	100%
16p13.3	MAPK8IP3	Hyper	100%
16q22.1	NFATC3	Hypo	100%
16q23.1	TERF2IP	Hypo	100%
16q23.2	MAF	Hypo	100%
16q24.2	BANP	Hyper	100%
16q24.3	ANKRD11	Hyper	100%
16q24.3	CDK10	Hypo	100%
16q24.3	CPNE7	Hyper	100%
16q24.3	FAM38A	Hyper	100%
16q24.3	SPIRE2	Hyper	100%
1725.3	BAHCC1	Hypo	100%
17q12	PIP4K2B	Hyper	100%
17q12	SLFN13	Hyper	100%
17q21.31	ASB16	Hyper	100%
17q23.1	RPS6KB1	Hypo	100%
17q23.2	MARCH10	Hypo	100%
17q23.3	SMARCD2	Hypo	100%
17q25.1	AHYPERC7	Hyper	100%
17q25.1	SRSF2	Hypo	100%
18p11.21	PSMG2	Hypo	100%
18q11.2	ZNF521	Hypo	100%
18q12.3	PIK3C3	Hypo	100%
18q21.1	CXXC1	Hypo	100%
18q21.1	SMAD7	Hypo	100%
18q21.31	ONECUT2	Hypo	100%
18q22.3	FBXO15	Hypo	100%
18q23	ATP9B	Hyper	100%
19p13.11	CRTC1	Hypo	100%
19p13.11	KLHL26	Hyper	100%
19p13.11	PLVAP	Hyper	100%
19p13.2	MAST1	Hyper	100%
19p13.2	RAB11B	Hyper	100%
19p13.3	ARID3A	Hyper	100%
19p13.3	EEF2	Hyper	100%
19p13.3	GRIN3B	Hyper	100%
19p13.3	LONP1	Hyper	100%
19p13.3	PCSK4	Hyper	100%
19p13.3	PTPRS	Hyper	100%
19p13.3	SAFB	Hypo	100%
19p13.3	SBNO2	Hyper	100%
19p13.3	UHRF1	Hyper	100%
19q13.12	CAPNS1	Hypo	100%
19q13.2	ARHGEF1	Hyper	100%
19q13.2	LTBP4	Hyper	100%
19q13.32	CD3EAP	Hypo	100%
19q13.32	PRKD2	Hyper	100%

19q13.33	NOSIP	Hyper	100%
19q13.33	PNKP	Hyper	100%
19q13.42	CCDC106	Hyper	100%
19q13.42	FAM71E2	Hypo	100%
19q13.42	TSEN34	Hyper	100%
19q13.42	U2AF2	Hyper	100%
19q13.42	ZNF865	Hyper	100%
19q13.43	ZNF787	Hyper	100%
20p11.23	INSM1	Hypo	100%
20p11.23	SLC24A3	Hyper	100%
20p12.2	MKKS	Hypo	100%
20p12.3	BMP2	Hypo	100%
20p13	NOP56	Hypo	100%
20p13	SOX12	Hypo	100%
20q11.23	ACTR5	Hypo	100%
20q13.12	NCOA5	Hypo	100%
20q13.13	KCNG1	Hyper	100%
20q13.13	MOCS3	Hypo	100%
20q13.2	ZFP64	Hypo	100%
20q13.31	SPO11	Hyper	100%
20q13.33	CDH4	Hyper	100%
20q13.33	CHRNA4	Hyper	100%
20q13.33	KCNQ2	Hyper	100%
20q13.33	LAMA5	Hyper	100%
20q13.33	PCMTD2	Hyper	100%
20q13.33	SS18L1	Hypo	100%
21p11.1	TPTE	Hyper	100%
21q22.2	HMGN1	Hypo	100%
22q11.1	CECR5	Hyper	100%
22q11.21	GP1BB	Hyper	100%
22q12.2	NEFH	Hypo	100%
22q13.33	PLXNB2	Hyper	100%
Xp22.11	EIF2S3	Hypo	100%
Xp22.2	TCEANC	Hypo	100%
Yp11.32	PPP2R3B	Hyper	100%

Table S3. Correlation of methylation in EP with reports in other cancer in NCBI. The (Gene) genes were consistent with changes in the EP methylation pattern (Status EP) and how they have been reported (Report) for other neoplasms (Cancer). The location within the chromosome was indicated (CHR). The most important status of methylation is highlighted in bold.

CHR	Gene	EP Status	%	Status Reported	Cancer
1p22.1	TGFBR3	Hypo	100%	Hyper[16]	Bladder
1p36.32	HES5	Hypo	100%	Hyper[17]	Prostate Leukemia
1p36.33	***MIB2***	***Hyper***	***100%***	***Hyper[21]***	***Melanoma***
1q32.1	NAV1	Hypo	100%	Hyper[15]	Breast
2p21	EPAS1	Hypo	100%	Hyper[20]	Colorectal
2p24.3	FAM84A	Hypo	100%	Hyper[28]	Breast
3p14.1	MAGI1	Hypo	100%	Hyper[14]	Leukemia
3p14.3	WNT5A	Hypo	100%	Hyper[1]	Colorectal
3q21.3	GATA2	Hypo	100%	Hyper[23]	Lung, Leukemia
5q13.2	OCLN	Hypo	100%	Hyper[8]	Hepatocellular
5q14.3	ARRDC3	Hypo	100%	Hyper[25]	Breast
5q31.2	***MATR3***	***Hypo***	***100%***	***Hypo[27]***	***Leukemia***
5q35.1	***FGF18***	***Hypo***	***100%***	***Hypo[11]***	***Gasrtric***
6q26	***PARK2***	***Hyper***	***100%***	***Hyper[2]***	***Leukemia***
6q26	QKI	Hypo	100%	Hyper[3]	Grastric
9q22.32	PTCH1	Hypo	100%	Hyper[29]	Gastric
10p14	FLJ45983	Hypo	100%	Hyper[18]	Breast
10p14	***ITIH5***	***Hyper***	***100%***	***Hyper[13]***	***Colon***
10q25.3	VAX1	Hypo	100%	Hyper[19]	Lung
10q26.3	EBF3	Hypo	100%	Hyper[22]	Leukemia
11p15.5	***CDHR5***	***Hyper***	***100%***	***Hyper[4]***	***Colorectal***
11p15.5	***MUC6***	***Hyper***	***100%***	***Hyper[24]***	***Colorectal***
12q23.3	CHST11	Hyper	100%	Hypo[12]	Breast
13q31.3	GPC6	Hypo	100%	Hyper[10]	Colorectal
16q22.1	NFATC3	Hypo	100%	Hyper[7]	Ovarian
16q24.3	FAM38A	Hyper	100%	Hypo[6]	Gastric
18q12.3	PIK3C3	Hypo	100%	Hyper[26]	Breast
20q13.33	***CDH4***	***Hyper***	***100%***	***Hyper[9]***	***Nasopharyngeal***
22q12.2	NEFH	Hypo	100%	Hyper[5]	Breast

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Table S4. Gene expression in Ependymoma. Show genes (Gene) with expression changes. Overxpression or underexpression (Status), the percentage in samples (%) and the chromosomal location (CHR) are indicated. Over. Overexpression and Under. Underexpression.

CHR	Gene	Status	%
1p36.22	NPPA	Under	100%
1p36.32	MORN1	Under	100%
1p36.21	AGMAT	Under	100%
1p36.13	CAPZB	Under	100%
1p36.12	ALPL	Under	100%
1p36.12	KIF17	Under	100%
1p35.2	HCRTR1	Under	100%
1p32.1	MYSM1	Over	100%
1p32.1	DAB1	Under	100%
1p22.2	ZNF644	Over	100%
1p21.3	ABCD3	Under	100%
1p13.3	GNAI3	Over	100%
1p13.3	ATXN7L2	Under	100%
1p13.2	PHTF1	Over	100%
1p13.2	DCLRE1B	Over	100%
1p13.1	CD58	Over	100%
1p12	FAM46C	Over	100%
1q21.3	S100A1	Under	100%
1q23.1	PYHIN1	Under	100%
1q23.3	LY9	Under	100%
1q32.1	CHIT1	Under	100%
1q23.1	IFI16	Over	100%
1q31.3	PTPRC	Over	100%
1q32.1	SLC45A3	Under	100%
1q32.1	TMCC2	Under	100%
1q32.3	BATF3	Under	100%
1q41	CENPF	Over	100%
1q41	FAM177B	Under	100%
1q42.12	LBR	Over	100%
1q42.12	TMEM63A	Under	100%
1q42.3	B3GALNT2	Over	100%
1q42.3	ERO1LB	Under	100%
1q43	RGS7	Under	83%
		Over	17%
2p25.1	PQLC3	Over	100%
2p25.1	FLJ33534	Under	100%
2p25.1	TAF1B	Over	100%
2p23.3	EFR3B	Under	100%
2p23.1	CAPN13	Under	100%
2p22.1	THUMPD2	Over	100%
2p21	LRPPRC	Over	100%
2p21	SIX3	Under	100%
2p13.2	DYSF	Under	100%
2p13.1	ALMS1P	Over	100%
2p13.1	MTHFD2	Over	100%
2p11.2	IMMT	Over	100%
2p11.2	CD8B	Under	83%

		Over	17%
2q11.1	MAL	Under	100%
2q12.3	LIMS1	Over	100%
2q13	ACOXL	Over	100%
2q13	BCL2L11	Over	100%
2q14.1	DPP10	Under	66%
		Over	34%
2q21.3	UBXN4	Over	100%
2q23.3	PRPF40A	Over	100%
2q24.1	ERMN	Under	100%
2q24.2	IFIH1	Over	100%
2q24.2	LY75	Over	100%
2q24.2	RBMS1	Over	100%
2q31.1	ITGA6	Over	100%
2q31.1	CDCA7	Over	100%
2q31.1	ZAK	Over	100%
2q32.1	TFPI	Over	100%
2q32.2	SLC40A1	Over	100%
2q32.2	STAT1	Over	100%
2q33.1	CFLAR	Over	100%
2q33.1	CLK1	Over	100%
2q37.1	SP100	Over	100%
2q37.2	ASB18	Under	100%
2q31.3	NEUROD1	Under	83%
		Over	17%
2q37.3	PASK	Over	100%
3p25.3	CPNE9	Under	100%
3p22.3	CMTM6	Over	100%
3p22.1	MOBP	Under	100%
3p21.31	ZNF589	Over	100%
3q12.3	SENP7	Over	100%
3q13.11	CBLB	Over	100%
3q21.1	PARP14	Over	100%
3q25.32	MFSD1	Over	100%
3q25.33	SMC4	Over	100%
3q26.2	MYNN	Over	100%
3q27.1	HSP90AA5P	Under	100%
4p15.2	PI4K2B	Over	100%
4q12	CEP135	Over	100%
4q12	KIT	Over	100%
4q21.21	ANTXR2	Over	100%
4q21.22	MOP-1	Under	100%
4q21.23	HPSE	Over	100%
4q22.1	HERC5	Over	100%
4q24	DDIT4L	Over	100%
4q25	SETP20	Over	100%
4q31.21	SMARCA5	Over	100%
4q31.3	KIAA0922	Over	100%
4q34.1	HMGB2	Over	100%
4q35.2	DUX4	Under	100%
5p13.2	SPEF2	Under	50%
		Over	50%
5q11.1	EMB	Over	100%

5q11.2	MIER3	Over	100%
5q13.2	OCLN	Over	100%
5q15	ERAP2	Over	100%
5q15	GPR150	Under	100%
5q34	HMMR	Over	100%
5q35.1	GABRP	Under	100%
5q35.1	NEURL1B	Under	100%
5q35.1	NKX2-5	Under	100%
5q35.2	CPLX2	Under	100%
5q33.3	CLINT1	Over	100%
5q31.3	DIAPH1	Over	100%
5q33.3	NIPAL4	Under	100%
5q31.3	PCDHGA7	Under	100%
5q31.3	RELL2	Under	100%
6p24.3	SSR1	Over	100%
6p24.3	DSP	Over	100%
6p22.3	RNF144B	Over	100%
6p22.2	BTN3A2	Over	100%
6p22.1	MOG	Under	100%
6p21.32	PFDN6	Under	100%
6p21.31	GGNBP1	Under	100%
6p21.31	IP6K3	Under	100%
6p21.1	CDC5L	Over	100%
6p12.1	ZNF451	Over	100%
6q13	CD109	Over	100%
6q15	CNR1	Under	83%
		Over	17%
6q21	BEND3	Under	100%
6q21	SESN1	Over	100%
6q21	SLC16A10	Under	100%
6q25.1	NUP43	Over	100%
6p22.1	UBD	Over	100%
6q25.1	ZBTB2	Over	100%
6q25.2	FBXO5	Over	100%
6q23.3	BCLAF1	Over	100%
6q14.3	SYNCRIP	Over	100%
6q22.31	MAN1A1	Over	100%
6q22.31	MCM9	Over	100%
6q22.33	LAMA2	Over	100%
6q25.3	ZDHHC14	Under	100%
7p21.1	SP8	Under	100%
7p14.3	CPVL	Over	100%
7p14.3	WIPF3	Under	100%
7p13	CCM2	Under	100%
7p13	NACAD	Under	100%
7p11.2	VSTM2A	Under	83%
		Over	17%
7p11.2	CCT6A	Over	100%
7q21.2	SAMD9L	Over	100%
7q11.21	TYW1	Over	100%
7q22.1	ACTL6B	Under	100%
7q22.1	SH2B2	Under	100%
7q34	JHDM1D	Over	100%

7q34	PRSS1	Under	100%
7q36.1	ACCN3	Under	100%
7q36.1	EZH2	Over	100%
7q36.1	GIMAP2	Over	100%
7q36.1	ZNF467	Under	100%
8p23.1	FAM66D	Under	100%
8p22	ASAH1	Over	100%
8p21.3	LOXL2	Over	100%
8p21.3	LZTS1	Under	100%
8p21.3	LPL	Over	100%
8p21.2	BNIP3L	Over	100%
8q12.3	BHLHE22	Under	100%
8q13.1	PDE7A	Over	100%
8q22.1	INTS8	Over	100%
8q22.3	DCAF13	Over	100%
8q24.12	SNTB1	Over	100%
8q24.12	TAF2	Over	100%
8q24.13	ATAD2	Over	100%
8q24.13	FAM91A1	Over	100%
8q24.21	ASAP1-IT1	Over	100%
8q24.3	FLJ43860	Under	100%
8q24.3	MAFA	Under	100%
8q22.3	PABPC1	Under	100%
8q24.3	PTK2	Under	100%
9p24.1	KIAA1432	Over	100%
9p22.3	MGC24103	Over	100%
9p13.3	ATP8B5P	Over	100%
9p13.3	ENHO	Under	100%
9p13.3	PRSS3	Under	100%
9p11.2	KGFLP1	Under	100%
9q21.11	PGM5P2	Over	100%
9q22.2	SEMA4D	Under	100%
9q22.33	TRIM14	Under	100%
9q32	KIF12	Under	100%
9q34.2	SARDH	Under	100%
9q34.3	LCN15	Under	100%
10p15.3	TUBB8	Under	100%
10p14	UPF2	Over	100%
10p12.1	MYO3A	Under	100%
10q21.1	ZWINT	Over	100%
10q21.2	CDK1	Over	100%
10q21.3	JMJD1C	Over	100%
10q22.1	NPFFR1	Under	100%
10q22.2	DUSP13	Under	100%
10q22.2	NDST2	Under	100%
10q23.33	KIF11	Over	100%
10q24.1	RRP12	Under	100%
10q24.2	ANKRD2	Under	100%
10q26.13	NKX1-2	Under	100%
10q26.3	NKX6-2	Under	100%
11p15.5	BET1L	Under	100%
11p15.4	HBB	Under	100%
11p15.4	HBD	Under	100%

11p15.4	HBG1	Under	100%
11p15.4	IPO7	Over	100%
11p14.1	HSP90AA2	Over	100%
11p13	CD44	Over	100%
11p11.2	PEX16	Under	100%
11q13.1	NEAT1	Over	100%
11q13.2	CNIH2	Under	100%
11q13.2	SUV420H1	Over	100%
11q13.2	SYT12	Under	100%
11q13.3	GAL	Under	100%
11q13.3	GAL3ST1	Under	100%
11q21	MRE11A	Over	100%
11q23.1	IL18	Over	100%
11q24.1	SCN3B	Under	100%
12p13.32	PARP11	Over	100%
12p13.31	NANOG	Over	100%
12p13.2	ETV6	Under	100%
12p13.1	ATF7IP	Over	100%
12p13.1	GSG1	Under	100%
12p13.1	PLBD1	Over	100%
12q13.12	WNT10B	Under	100%
12p11.21	FAM60A	Over	100%
12q15	MDM2	Over	100%
12q21.1	KCNC2	Under	100%
12q21.33	BTG1	Over	100%
12q23.1	TMPO	Over	100%
12q23.2	DRAM1	Over	100%
12q24.13	NAA25	Over	100%
12q24.22	RNFT2	Under	100%
12q23.3	HSP90B1	Over	100%
12q24.31	B3GNT4	Under	100%
12q24.31	KNTC1	Over	100%
13q12.11	SKA3	Over	100%
13q13.2	RFC3	Over	100%
13q14.11	MTRF1	Over	100%
13q22.1	KLF12	Over	100%
14q11.2	RNASE13	Under	100%
14q13.2	SRP54	Over	100%
14q13.3	PAX9	Under	100%
14q22.2	WDHD1	Over	100%
14q23.2	SYNE2	Over	100%
14q24.1	DCAF5	Over	100%
14q24.1	RDH12	Under	100%
14q24.2	PAPLN	Under	100%
15q13.3	SCG5	Under	83%
		Over	17%
15q15.1	CHAC1	Under	100%
15q15.1	NUSAP1	Over	100%
15q15.1	OIP5	Over	100%
15q15.1	RAD51	Over	100%
15q21.1	B2M	Over	100%
15q22.2	ANXA2	Over	100%
15q22.2	CCNB2	Over	100%

15q22.31	KIAA0101	Over	100%
15q22.31	PLEKHO2	Under	100%
15q22.31	SNX22	Under	100%
15q22.31	ZWILCH	Over	100%
15q23	FEM1B	Over	100%
15q24.1	CPLX3	Under	100%
15q24.1	SEMA7A	Under	100%
15q26.1	IQGAP1	Over	100%
15q26.1	SV2B	Under	100%
15q26.3	TARSL2	Over	100%
16p13.3	HBA2	Under	100%
16p13.3	HBM	Under	100%
16p13.3	HBQ1	Under	100%
16p13.3	ARHGDIG	Under	100%
16p12.3	ACSM5	Under	100%
16p12.2	PRKCB	Under	100%
16p11.2	ZNF267	Over	100%
16q12.1	NETO2	Under	66%
		Over	34%
16q12.2	GNAO1	Under	100%
16q22.1	RLTPR	Under	100%
16q23.1	FA2H	Under	100%
16q24.1	ADAD2	Under	100%
16q24.2	KLHDC4	Under	100%
16q24.3	DBNDD1	Under	100%
17p13.1	ALOX15B	Under	100%
17p11.2	TRPV2	Under	100%
17q11.2	CDK5R1	Under	83%
		Over	17%
17q11.2	EVI2A	Under	100%
17q12	CACNB1	Under	100%
17q12	CISD3	Under	100%
17q21.2	HSPB9	Under	100%
17q21.31	ACBD4	Under	100%
17q21.31	BRCA1	Over	100%
17q21.32	ITGB3	Over	100%
17q21.32	NPEPPS	Over	100%
17q21.32	RPRML	Under	100%
17q21.32	SNX11	Under	100%
17q21.33	LUC7L3	Over	100%
17q22	FLJ44342	Over	100%
17q25.1	AANAT	Under	100%
17q25.1	GALK1	Under	100%
17q25.3	DNAH17	Under	100%
17q25.3	FN3K	Under	100%
17q25.3	TMC6	Under	100%
17q25.3	TSPAN10	Under	100%
18p11.32	NDC80	Over	100%
18p11.32	SMCHD1	Over	100%
18p11.32	THOC1	Over	100%
18q11.2	AQP4	Under	50%
		Over	50%
18q22.3	CNDP1	Under	100%

18q23	MBP	Under	100%
19p13.3	FGF22	Under	100%
19p13.3	HCN2	Under	100%
19p13.3	MADCAM1	Under	100%
19p13.3	MATK	Under	100%
19p13.3	MIR7-3HG	Under	100%
19p13.3	NFIC	Under	100%
19p13.3	SEMA6B	Under	100%
19p13.3	TMIGD2	Under	100%
19p13.2	CLEC4G	Under	100%
19q13.2	PPP1R14A	Under	100%
19q13.2	SARS2	Under	100%
19p13.2	ZNF823	Over	100%
19p13.2	FLJ22184	Under	100%
19p13.11	DDA1	Under	100%
19p13.11	GTPBP3	Under	100%
19p13.11	KCNN1	Under	100%
19p13.11	YJEFN3	Under	100%
19p12	ZNF208	Over	100%
19p12	ZNF254	Over	100%
19p12	ZNF429	Over	100%
19p12	ZNF714	Over	100%
19p12	ZNF738	Over	100%
19q13.12	FFAR1	Under	100%
19q13.12	MAG	Under	100%
19q13.12	ZBTB32	Under	100%
19q13.31	ZNF224	Over	100%
19q13.32	BLOC1S3	Under	100%
19q13.32	DHX34	Under	100%
19q13.32	RTN2	Under	100%
19q13.32	SNAR-C4	Under	100%
19q13.33	GLTSCR1	Under	100%
19q13.33	MYBPC2	Under	100%
19q13.33	SYNGR4	Under	100%
19q13.41	ZNF137P	Over	100%
19q13.41	ZNF200	Over	100%
19q13.41	ZNF432	Over	100%
19q13.41	ZNF468	Over	100%
19q13.41	ZNF600	Over	100%
19q13.42	LENG1	Under	100%
19q13.42	PRKCG	Under	100%
19q13.42	TNNI3	Under	100%
19q13.42	TNNT1	Under	100%
19q13.42	ZNF525	Over	100%
19q13.43	A1BG	Under	100%
20p12.2	SNAP25	Under	83%
		Over	17%
20p11.23	SNX5	Over	100%
20q11.23	DSN1	Over	100%
20q12	PRO0628	Over	100%
20q13.12	ADA	Under	100%
20q13.2	ZNF217	Over	100%
20q13.33	HAR1A	Under	100%

21q22.11	GART	Over	100%
21q22.11	GCFC1	Over	100%
21q22.11	OLIG2	Under	100%
21q22.3	AIRE	Under	100%
21q22.3	KRTAP10-3	Under	100%
21q22.3	MCM3AP-AS1	Over	100%
22q11.21	BID	Under	100%
22q11.21	GSC2	Under	100%
22q11.21	SLC7A4	Under	100%
22q12.2	SEC14L3	Under	100%
22q13.1	GALR3	Under	100%
22q13.1	RAC2	Under	100%
22q13.1	SLC16A8	Under	100%
22q13.1	SUN2	Under	100%
22q13.2	MEI1	Under	100%
22q12.2	MTFP1	Under	100%
22q13.2	POLDIP3	Under	100%
22q13.2	RRP7A	Under	100%
22q13.2	SCUBE1	Under	100%
22q13.2	TNFRSF13C	Under	100%
22q13.31	KIAA0930	Under	100%
22q13.31	PRR5	Under	100%
22q13.33	MAPK11	Under	100%
22q13.33	PANX2	Under	100%
Xp11.21	ALAS2	Under	100%
Xp11.4	CYBB	Over	100%
Xp11.4	MED14	Over	100%
Xp22.2	CA5B	Over	100%
Xp22.2	TXLNG	Over	100%
Xp22.33	CRLF2	Under	100%
Xp22.33	SHOX	Over	100%
Xq13.2	XIST	Under	50%
		Over	50%
Xq21.1	ATP7A	Over	100%
Xq22.1	RPL36A-HNRNPH2	Over	100%
Xq22.1	ZMAT1	Over	100%
Xq22.3	RBM41	Over	100%
Xq23	TMEM164	Under	100%
Xq24	DOCK11	Over	100%
Xq26.3	FAM122C	Over	100%
Xq28	RENBP	Under	100%
Yp11.2	PRKY	Over	100%
Yp11.2	TGIF2LY	Under	100%

Table S5. Correlation between expression status and the literature. Genes with expression change (Gene) in EP (EP Status) that were consistent with those reported in other neoplasms (cancer) and how they have been reported (Literature). The locations of genes within the chromosome are indicated (CHR).

CHR	Gene	EP Status	%	Status	Cancer
1p21.3	ABCD3	Under	100%	Over[32]	Prostate
1p13.3	ATXN7L2	Under	100%	Over[46]	Lung
1p13.3	GNAI3	Over	100%	Under[49]	Hepatocellular
1p13.1	CD58	Over	100%	Over[47]	Colorectal
1q42.3	B3GALNT2	Over	100%	Over[25]	Breast
2p21	LRPPRC	Over	100%	Under[53] Over[17]	Prostate Gastric
2p21	SIX3	Under	100%	Under[27]	Lung
2p13.1	MTHFD2	Over	100%	Over[20]	Breast
2q21.3	UBXN4	Over	100%	Over[33]	Colon
2q24.2	LY75	Over	100%	Over[6]	Ovarian
3q13.11	CBLB	Over	100%	Under[16]	Gastric
4q31.21	SMARCA5	Over	100%	Over[13]	Breast
5q15	ERAP2	Over	100%	Over[43]	Gbm
5q31.3	DIAPH1	Over	100%	Over[19]	Colorectal
5q35.2	CPLX2	Under	100%	Over[14]	Neuroendocrine
6p22.2	BTN3A2	Over	100%	Over[15]	Ovarian
6p21.1	CDC5L	Over	100%	Over[24]	Osteosarcoma
6q13	CD109	Over	100%	Over[11]	Pancreatic
6q25.1	ZBTB2	Over	100%	Under[45]	Gastric
6q25.3	ZDHHC14	Under	100%	Over[29]	Gastric
6q23.3	BCLAF1	Over	100%	Over[54]	Colon
8p22	ASAH1	Over	100%	Over[26] Over[34] Over[28] Over[10]	Prostate Breast Colorectal Ovarian
8q13.1	PDE7A	Over	100%	Over[48]	Endometrial
8q24.13	ATAD2	Over	100%	Over[52] Over[12] Over[44]	Cervical Hepatocellular Ovarian
9p13.3	PRSS3	Under	100%	Over[23]	Ovarian
12p13.2	ETV6	Under	100%	Under[18]	Lung
12q21.33	BTG1	Over	100%	Over[51] [22]	Gastric Thyroid
12q23.2	DRAM1	Over	100%	Over[4]	Gbm
13q13.2	RFC3	Over	100%	Over[36]	Ovarian
15q15.1	CHAC1	Under	100%	Over[7]	Breast Ovarian
15q15.1	NUSAP1	Over	100%	Over[9]	Prostate
15q15.1	OIP5	Over	100%	Over[8]	Renal
15q22.31	KIAA0101	Over	100%	Over[39]	Hepatic
15q23	FEM1B	Over	100%	Over[40]	Colon

16p11.2	ZNF267	Over	100%	Over[35]	Hepatocellular
16q12.1	NETO2	Under Over	66% 34%	Over[30]	Lung
16q12.2	GNAO1	Under	100%	Under[31] Over[21]	Hepatocellular Gastric
17q21.31	BRCA1	Over	100%	Over[38]	Breast
18p11.32	THOC1	Over	100%	Over[2]	Prostate
18q11.2	AQP4	Under Over	50% 50%	Over[3]	GBM
18q22.3	CNDP1	Under	100%	Under[5]	GBM
19q13.43	A1BG	Under	100%	Over[1] [41]	Cervical Pancreatic
20q13.2	ZNF217	Over	100%	Over[50] [37]	Colorectal Gastric
21q22.11	OLIG2	Under	100%	Over[42]	GBM

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Table S6. Correlation between chromosomal abnormalities and methylation in Ependymoma. Genes (Gene) with methylation changes in EP (Status), correlated with the chromosomal alterations (CHR), by region, are show for each of the samples (EP1, EP2, EP3, EP4, EP5, EP6) the percentage of samples is indicated (%). Hyper. Hypermethylation; Hypo. Hypomethylation; A. Amplification; D. Deletion.

aCGH						Methylation		
CHR	EP1	EP2	EP3	EP4	EP6	Gene	Status	%
1p31.1	D	D	A		D	ST6GALNAC3	Hypo	100%
1p31.1	D	D	A		D	SRSF11	Hypo	100%
1p31.1	D	D	A		D	NEGR1	Hypo	100%
1q21.3	D	D		D	D	TCHH	Hypo	100%
1q21.3	D	D		D	D	RPRD2	Hypo	100%
1q21.3	D	D		D	D	ILF2	Hypo	100%
1q21.3	D	D		D	D	CHRNB2	Hyper	100%
2p11.2	A	A	A		A	RPIA	Hyper	100%
2p11.2	A	A	A		A	RGPD1	Hyper	100%
3p22.2	A	A			A	GOLGA4	Hypo	100%
7p13	A	A			A	STK17A	Hypo	100%
7p13	A	A			A	POLD2	Hypo	100%
7p15.2	A	A			A	HIBADH	Hypo	100%
7q34	D	D			D	ZC3HAV1	Hypo	100%
7q34	D	D			D	UBN2	Hypo	100%
7q34	D	D			D	EPHB6	Hypo	100%
8p11.23 - p11.22	D	D		A	D	BAG4	Hypo	100%
10q21.1	A	A			A	TFAM	Hypo	100%
11p15.4		A	A		A	MRPL17	Hypo	100%
12p13.31	D		A		D	TPI1	Hypo	100%
12p13.31	D		A		D	LRRC23	Hyper	100%
14q24.3	A	A			A	ENTPD5	Hypo	100%
14q32.33	A	A	A		A	KIF26A	Hypo	100%
14q32.33	A		A		A	JAG2	Hyper	100%
14q32.33	A		A		A	CRIP2	Hyper	100%
15q11.2	D	D		D	D	TUBGCP5	Hypo	100%
17q12	D	D			D	SNORA21	Hypo	100%
17q12	D	D			D	SLFN13	Hyper	100%
17q12	D	D			D	RASL10B	Hyper	100%
17q12	D	D			D	PIP4K2B	Hyper	100%

Table S7. Correlation between methylation and expression. This shows the genes (Gene) with change in the methylation pattern (Status) that correlate with gene expression (Status); is indicated the chromosomal location (CHR) and the samples percentage (%).

		Methylation		Expression	
CHR	Gene	Status	%	Status	%
1p13.1	CD58	Hyper	100%	Over	100%
1p13.2	DCLRE1B	Hypo	100%	Over	100%
1p13.2	PHTF1	Hypo	100%	Over	100%
1p21.3	ABCD3	Hypo	100%	Under	100%
1p22.2	ZNF644	Hypo	100%	Over	100%
1p32.2	DAB1	Hypo	100%	Under	100%
1p32.3	TMEM48	Hypo	100%	Over	100%
1p36.12	KIF17	Hyper	100%	Under	100%
1p36.21	AGMAT	Hypo	100%	Under	100%
1p36.33	MORN1	Hyper	100%	Under	100%
1q32.1	SLC45A3	Hypo	100%	Under	100%
1q32.1	TMCC2	Hyper	100%	Under	100%
1q32.3	BATF3	Hypo	100%	Under	100%
1q41	CENPF	Hypo	100%	Over	100%
1q42.12	LBR	Hypo	100%	Over	100%
1q42.3	ERO1LB	Hypo	100%	Under	100%
1q43	RGS7	Hypo	100%	Under	100%
2p11.2	IMMT	Hypo	100%	Over	100%
2p13.1	MTHFD2	Hypo	100%	Over	100%
2p13.2	DYSF	Hypo	100%	Under	100%
2p21	LRPPRC	Hypo	100%	Over	100%
2p21	SIX3	Hypo	100%	Under	100%
2p23.3	EFR3B	Hypo	100%	Under	100%
2p25.1	PQLC3	Hypo	100%	Over	100%
2p25.1	TAF1B	Hypo	100%	Over	100%
2q11.1	MAL	Hyper	100%	Under	100%
2q11.2	LIPT1	Hypo	100%	Over	100%
2q13	ACOXL	Hyper	100%	Over	100%
2q13	BCL2L11	Hypo	100%	Over	100%
2q14.1	DPP10	Hypo	100%	Under	100%
2q21.3	UBXN4	Hypo	100%	Over	100%
2q24.2	IFIH1	Hypo	100%	Over	100%
2q24.2	RBMS1	Hypo	100%	Over	100%
2q31.1	ITGA6	Hypo	100%	Over	100%
2q31.1	ZAK	Hyper	100%	Over	100%
2q31.3	NEUROD1	Hypo	100%	Under	100%
2q32.2	SLC40A1	Hypo	100%	Over	100%
2q32.2	STAT1	Hypo	100%	Over	100%
2q33.1	CFLAR	Hypo	100%	Over	100%
2q33.1	CLK1	Hypo	100%	Over	100%
2q36.3	COL4A4	Hypo	100%	Over	100%
2q37.3	PASK	Hypo	100%	Over	100%
3p25.3	CPNE9	Hypo	100%	Under	100%

Anexo II

Pediatric ependymoma: GNAO1, ASAH1, IMMT and IPO7 protein expression and prognosis correlation

Table S1. Culitative expression in Pediatric Ependymoma.

SAMPLE	GRADE	KI67	P53	GNAO1	CYCLIN D1	ASAH1	IPO 7	IMMT
1	II	2%	Negative	Negative	20.00%	Positive	Negative	Negative
2	II	2%	15.00%	Negative	50.00%	Positive	Negative	Negative
3	III	10%	< 1%	Negative	30.00%	Positive	Negative	Negative
4	III	8%	Negative	Negative	negativo	Positive	5%	Negative
5	II	5%	<1%	Negative	< 5%	Positive	Negative	Positive
6	III	20%	<1%	Positive	70.00%	Positive	<1%	Negative
7	II	2%	40.00%	Weak	70.00%	Positive	Positive	Positive
8	III	5%	Negative	Negative	10%	Positive	Negative	Positive
9	III	5%	5.00%	Negative	30.00%	Positive	<1%	Positive
10	III	15%	30.00%	Positive	<5%	Positive	Negative	Negative
11	II	1%	20.00%	Negative	Negative	Positive	<1%	Negative
12	II	1%	Negative	Positive	50.00%	Positive	Negative	Negative
13	II	1%	Negative	Negative	Negative	Positive	Negative	Positive
14	II	3%	Negative	Negative	Negative	Positive	Negative	Negative
15	III	1%	Negative	Positive	Negative	Positive	Negative	Positive
16	III	1%	Negative	Negative	Negative	Positive	Negative	Positive
17	II	1%	<1%	Negative	Negative	Positive	Negative	Negative
18	II	<5%	Negative	Negative	90%	Positive	Negative	Negative
19	II	5%	Negative	Negative	Negative	Positive	Negative	Positive
20	II	2%	Negative	Negative	Negative	Positive	Negative	Negative
21	III	20%	Negative	Positive	Negative	Negative	Negative	Negative
22	III	50%	Negative	Negative	Negative	Positive	Negative	Negative
23	II	20%	Negative	Negative	Negative	Negative	Negativo	Negative
24	II	10%	Negative	Positive	Negative	Negative	Negative	Negative
25	II	1%	Negative	Positive	Negative	Positive	Negative	Negative
26	II	10%	Negative	Positive	Negative	Positive	Negative	Negative
27	III	50%	<5%	Positive	<1%	Positive	Negative	Negative
28	III	1%	Negative	Positive	Negative	Positive	Negative	Negative
29	II	3%	Negative	Positive	Negative	Positive	Negative	Negative
30	II	3 %	Negative	Negative	Negative	Negative	Negative	Negative

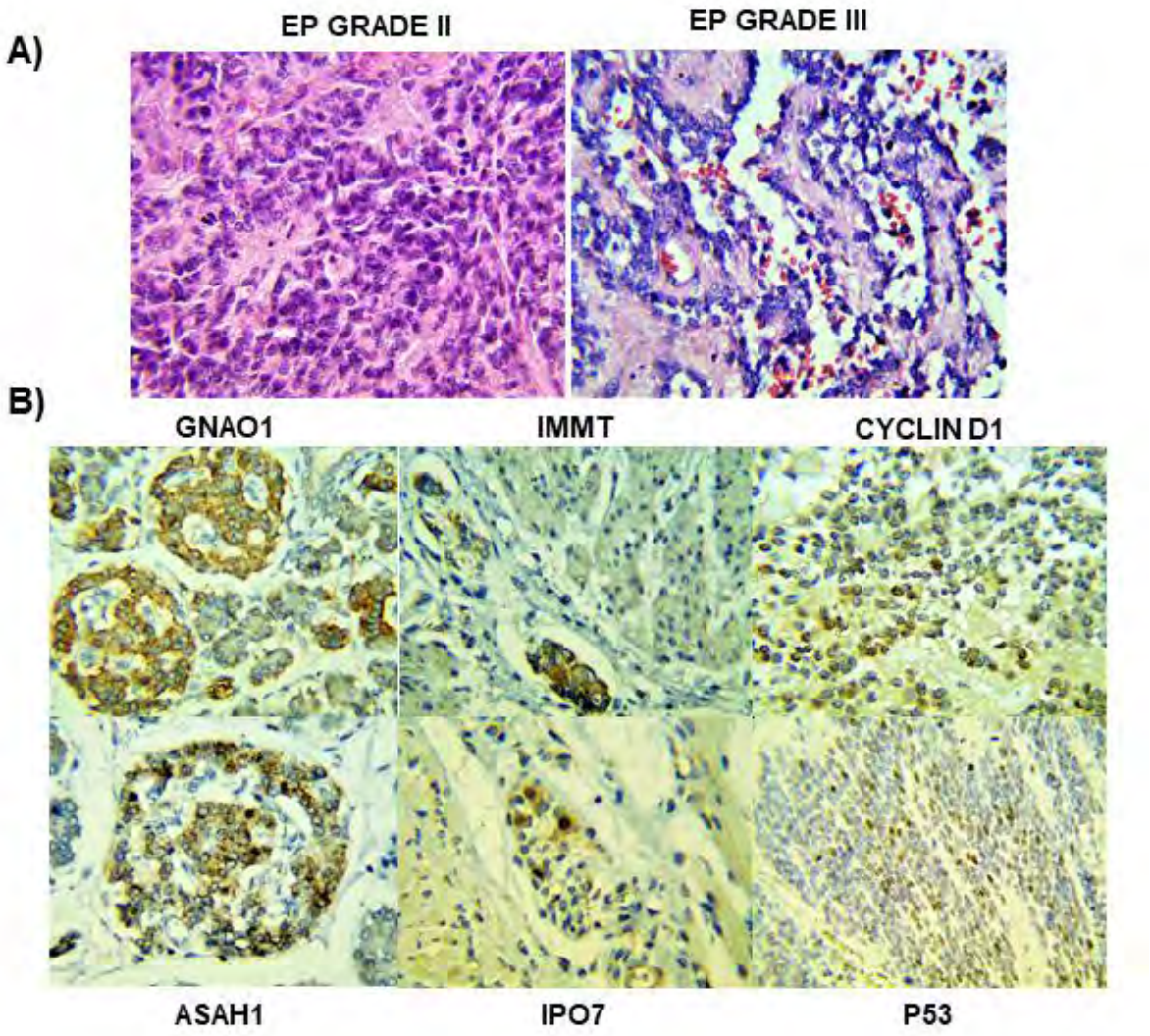

Figure S1. Histopathology and Positive Control. A) It show the H & E for the histopathological diagnosis of the respective tumor grades. B) It show the positive controls of the antibodies used.

Anexo III
Pediatric pineal germinomas: Epigenetic and genomic approach

Pediatric pineal germinomas: Epigenetic and genomic approach.

Table S1. Sample features. The features of the patients from whom the samples were obtained are shown age (Age), gender (Sex), and tumor location (Localization). Histopathological grade classification was according to the World Health Organization (Grade) and whether the tumor was de novo or recidivism (Observations). The ependymomas is highlighted in bold.

Sample	Age	Sex	Grade	Localization	Observations
Medulloblastoma	1	F	II	Posterior fossa	*De novo*
Ependymoma	15	M	II	Posterior fossa	*De novo*
Medulloblastoma	1	F	II	Posterior fossa	*De novo*
Ependymoma	15	M	III	Posterior fossa	*De novo*
Plexus papilloma	0	F	I	Posterior fossa	*De novo*
Ganglioglioma	13	M	I	Posterior fossa	*De novo*
Ependymoma	13	F	II	Spinal cord (L2-L5)	Recidivism
Glioblastoma multiforme	11	M	IV	Posterior fossa	*De novo*
Ganglioglioma	12	F	I	Posterior fossa	*De novo*
Ganglioglioma	11	M	I	Posterior fossa	*De novo*
Medulloblastoma	1	F	II	Posterior fossa	*De novo*
Ependymoma	9	F	II	Posterior fossa	*De novo*
Germinoma	**11**	**M**	**II**	**Posterior fossa**	***De novo***
Astrocytoma	6	F	II	Posterior fossa	*De novo*
Transitional Meningioma	15	F	I	Posterior fossa	*De novo*
Medulloblastoma	9	M	IV	Posterior fossa	*De novo*
Astrocytoma	13	F	II	Posterior fossa	*De novo*
Astrocytoma	13	F	I	Posterior fossa	*De novo*
Plexiform Neurofibroma	1	M	I	Posterior fossa	*De novo*
Medulloblastoma	15	M	IV	Posterior fossa	*De novo*
Medulloblstoma	3	M	IV	Posterior fossa	*De novo*
Medulloblastoma	13	M	IV	Posterior fossa	*De novo*
Astrocytoma	4	M	II	Posterior fossa	*De novo*
Sarcoma	6	F	IV	Posterior fossa	*De novo*
Astrocytoma	12	M	I	Posterior fossa	*De novo*
Germinoma	**13**	**M**	**II**	**Posterior fossa**	***De novo***
Ependymoma	2	F	II	Posterior fossa	*De novo*
Astrocytoma	16	F	II	Posterior fossa	*De novo*
Ependymoma	15	M	II	Posterior fossa	*De novo*

Pediatric pineal germinomas: Epigenetic and genomic approach.

Germinoma	**13**	**M**	**II**	**Posterior fossa**	***De novo***
Astrocytoma	5	F	II	Posterior fossa	*De novo*
Astrocytoma	11	F	I	Posterior fossa	*De novo*
Cortical dysplasia	10	M		Posterior fossa	*De novo*
Astrocytoma	5	F	I	Posterior fossa	*De novo*
Astrocytoma	1	F	II	Posterior fossa	*De novo*
Oligodendroglioma	10	M	II	Posterior fossa	*De novo*
Germinoma	**15**	**M**	**II**	**Posterior fossa**	***De novo***
Plexus Papilloma	2	F	I	Posterior fossa	*De novo*
Germinoma	**12**	**F**	**II**	**Posterior fossa**	***De novo***
Atrocytoma	11	F	II	Posterior fossa	*De novo*
Medulloblastoma	3	M	IV	Posterior fossa	*De novo*
Chordoma	1	M	II	Posterior fossa	*De novo*
Astroblastoma	15	F	IV	Posterior fossa	*De novo*
Medulloblastoma	13	M	IV	Posterior fossa	*De novo*
Schawnnoma	15	F	I	Posterior fossa	*De novo*
Ependymoma	6	F	II	Posterior fossa	*De novo*
Hemangioblastoma	15	M	II	Posterior fossa	*De novo*
Ependymoma	15	M	II	Posterior fossa	*De novo*
Rabdoide teratoide	2	F	II	Posterior fossa	*De novo*
Ependymoma	5	F	II	Posterior fossa	*De novo*
Dysembryoplastic neuroepithelial tumour	13	F	I	Posterior fossa	*De novo*
Astrocytoma	13	M	II	Posterior fossa	*De novo*
Medulloblastoma	0	M	IV	Posterior fossa	*De novo*
Astrocytoma	15	M	I	Posterior fossa	*De novo*
Ependymoma	9	M	II	Posterior fossa	*De novo*
Astrocytoma	15	M	II	Posterior fossa	*De novo*
Ependymoma	1	F	III	Posterior fossa	*De novo*
Plexus papilloma	10	F	I	Posterior fossa	*De novo*
Medulloblastoma	9	M	IV	Posterior fossa	*De novo*
PTEN	5	M	I	Posterior fossa	*De novo*
Medulloblastoma	9	F	IV	Posterior fossa	*De novo*
Medulloblastoma	2	F	IV	Posterior fossa	*De novo*
Ependymoma	13	M	II	Spinal cord	*De novo*

Tabla S2. Correlation between chromosomal abnormalities and gene expression. In table are show the genes (Gene) that correlated between gene expression and chromosomal abnormalities, chromosomal region (CHR) and the gene expression (Expression) are indicated. A. Amplification; D. Deletion; Under. Underexpression; Over. Overexpression.

CHR	Gene	aCGH	Expression
1q21.3	*ATP8B2*	D	Under
1q21.3	*CHRNB2*	D	Under
1q21.3	*S100A14*	D	Under
1q24.2	*CD247*	A	Over
1q24.2	*RCSD1*	A	Over
1q24.2	*SELL*	A	Over
2p11.2	*GNLY*	A	Over
7p15.2	*CDCA7L*	A	Over
7p15.2	*TRA2A*	A	Over
8p22	*ASAH1*	A	Over
8p22	*LPL*	A	Over
10q11.22	*FRMPD2*	D	Under
14q24.3	*BATF*	A	Over
14q24.3	*NPC2*	A	Over
14q24.3	*VRTN*	A	Over
17q21.31	*RUNDC3A*	D	Under
22q12.1	*CCDC117*	A	Over
22q12.1	*CHEK2*	A	Over

Table S3. Correlation between chromosomal abnormalities and methylation. It show genes (Gene) with methylation changes in pineal germinoma (Methylation), correlated with the chromosomal alterations (aCGH), by chromosomal region (CHR), Hyper. Hypermethylation; Hypo. Hypomethylation; A. Amplification; D. Deletion

CHR	Gene	Methylation	aCGH
1q21.3	*ADAR*	Hypo	D
1q21.3	*ATP8B2*	Hypo	D
1q21.3	*FLAD1*	Hypo	D
1q21.3	*GATAD2B*	Hypo	D
1q21.3	*HAX1*	Hypo	D
1q21.3	*ILF2*	Hypo	D
1q21.3	*INTS3*	Hypo	D
1q21.3	*PBXIP1*	Hypo	D
1q21.3	*PYGO2*	Hypo	D
1q21.3	*SLC27A3*	Hypo	D
1q21.3	*SNAPIN*	Hypo	D
1q21.3	*UBAP2L*	Hypo	D
1q21.3	*UBE2Q1*	Hypo	D
1q21.3	*ZBTB7B*	Hypo	D
1q24.2	*KIFAP3*	Hyper	A
1q24.2	*SCYL3*	Hyper	A
2p11.2	*CAPG*	Hyper	A
7p13	*AEBP1*	Hyper	A
7p13	*BLVRA*	Hypo	A
7p13	*CCM2*	Hypo	A
7p13	*DDX56*	Hypo	A
7p13	*MYO1G*	Hyper	A
7p13	*TBRG4*	Hyper	A
7p15.3	*RPS2P32*	Hyper	A
8p11.22	*ADAM9*	Hypo	D
8p11.22	*FGFR1*	Hypo	D
8p11.22	*PLEKHA2*	Hypo	D
8p11.22	*TACC1*	Hypo	D
8p11.22	*TM2D2*	Hypo	D
8p11.23	*BAG4*	Hypo	D
8p11.23	*BRF2*	Hypo	D
8p11.23	*DDHD2*	Hypo	D
8p11.23	*PPAPDC1B*	Hypo	D
8p11.23	*RAB11FIP1*	Hypo	D
8p11.23	*WHSC1L1*	Hypo	D

8p11.23	*ZNF703*	Hypo	D
8p22	*NAT2*	Hyper	A
9p22.3	*NFIB*	Hypo	D
9p24.1	*AK3*	Hypo	D
9p24.1	*CDC37L1*	Hypo	D
9p24.1	*KDM4C*	Hypo	D
9p24.1	*KIAA2026*	Hypo	D
9p24.1	*RANBP6*	Hypo	D
9p24.1	*RLN1*	Hypo	D
9p24.1	*RLN2*	Hypo	D
9p24.1	*UHRF2*	Hypo	D
10q11.22	*ANTXRL*	Hypo	D
10q11.22	*ZNF488*	Hypo	D
12p13.2	*CLEC7A*	Hyper	A
12p13.31	*TNFRSF1A*	Hyper	A
14q24.3	*BATF*	Hyper	A
14q24.3	*LIN52*	Hyper	A
14q24.3	*RPS6KL1*	Hyper	A
14q24.3	*VRTN*	Hyper	A
14q32.33	*ASPG*	Hyper	A
14q32.33	*PPP1R13B*	Hyper	A
14q32.33	*TDRD9*	Hyper	A
14q32.33	*ZFYVE21*	Hyper	A
15q11.2	*NIPA1*	Hypo	D
15q11.2	*NIPA2*	Hypo	D
15q11.2	*TUBGCP5*	Hypo	D
15q11.2	*UBE3A*	Hypo	D
15q13.1	*HERC2P2*	Hypo	D
17q21.2	*STAT3*	Hypo	D
17q21.31	*AARSD1*	Hypo	D
17q21.31	*ARL17A*	Hypo	D
17q21.31	*ARL17B*	Hypo	D
17q21.31	*ARL4D*	Hypo	D
17q21.31	*ASB16*	Hyper	D
17q21.31	*ATXN7L3*	Hypo	D
17q21.31	*CCDC103*	Hypo	D
17q21.31	*DCAKD*	Hypo	D
17q21.31	*DHX8*	Hypo	D
17q21.31	*EFTUD2*	Hypo	D
17q21.31	*ETV4*	Hypo	D
17q21.31	*FZD2*	Hypo	D

17q21.31	*G6PC3*	Hypo	D
17q21.31	*GJC1*	Hypo	D
17q21.31	*GPATCH8*	Hypo	D
17q21.31	*HEXIM1*	Hypo	D
17q21.31	*HEXIM2*	Hypo	D
17q21.31	*KIF18B*	Hypo	D
17q21.31	*LSM12*	Hypo	D
17q21.31	*MGC57346*	Hypo	D
17q21.31	*MPP3*	Hypo	D
17q21.31	*NAGS*	Hypo	D
17q21.31	*NBR1*	Hypo	D
17q21.31	*NMT1*	Hypo	D
17q21.31	*RUNDC1*	Hypo	D
17q21.31	*RUNDC3A*	Hypo	D
17q21.31	*TMEM101*	Hypo	D
17q21.31	*TMEM106A*	Hypo	D
17q21.31	*TMUB2*	Hypo	D
17q21.31	*UBTF*	Hypo	D
17q21.31	*VPS25*	Hypo	D
22q12.1	*ASPHD2*	Hyper	A
22q12.1	*PITPNB*	Hyper	A

Table S4. Correlation between methylation and expression. This shows the genes (Gene) with change in the methylation pattern (Methylation) that correlate with gene expression (Expression); is indicated the chromosomal location (CHR). Hyper. Hypermethylarion, Hypo. Hypomethylation, Over. Overexpression, Under. Underexpression

CHR	Gene	Methylation	Expression
1p11.2	*FCGR1B*	Hypo	Over
1p12	*FAM46C*	Hypo	Over
1p12	*MAN1A2*	Hypo	Over
1p13.1	*CD58*	Hypo	Over
1p13.2	*DCLRE1B*	Hypo	Over
1p13.2	*DENND2C*	Hypo	Over
1p13.2	*NRAS*	Hypo	Over
1p13.2	*PHTF1*	Hypo	Over
1p13.3	*GNAI3*	Hypo	Over
1p13.3	*VAV3*	Hypo	Over
1p22.1	*GFI1*	Hypo	Over
1p22.1	*MTF2*	Hypo	Over
1p22.1	*TMED5*	Hypo	Over
1p22.2	*ZNF644*	Hypo	Over
1p22.3	*MCOLN2*	Hypo	Over
1p22.3	*MCOLN3*	Hypo	Over
1p31.1	*SRSF11*	Hypo	Over
1p31.2	*DEPDC1*	Hypo	Over
1p31.3	*LEPROT*	Hypo	Over
1p32.1	*OMA1*	Hypo	Over
1p32.3	*TMEM48*	Hypo	Over
1p32.3	*ZYG11A*	Hypo	Over
1p33	*STIL*	Hypo	Over
1p34.1	*KIF2C*	Hypo	Over
1p34.1	*NASP*	Hypo	Over
1p34.2	*GUCA2B*	Hyper	Under
1p34.2	*MYCL1*	Hypo	Over
1p34.2	*PPT1*	Hypo	Over
1p34.2	*YBX1*	Hypo	Over
1p34.2	*ZNF643*	Hypo	Over
1p34.3	*CDCA8*	Hypo	Over
1p34.3	*CLSPN*	Hypo	Over
1p34.3	*DLGAP3*	Hyper	Under
1p35.3	*SNHG12*	Hypo	Over

1p36.11	*FUCA1*	Hypo	Over
1p36.11	*LIN28A*	Hypo	Over
1p36.11	*RCAN3*	Hypo	Over
1p36.12	*E2F2*	Hypo	Over
1p36.12	*LUZP1*	Hypo	Over
1p36.21	*PDPN*	Hypo	Over
1p36.21	*TMEM51*	Hypo	Over
1p36.32	*TP73*	Hyper	Under
1p36.33	*GABRD*	Hyper	Under
1p36.33	*PRKCZ*	Hyper	Under
1q21.1	*NOTCH2NL*	Hypo	Over
1q21.1	*POLR3C*	Hypo	Over
1q21.2	*ANP32E*	Hypo	Over
1q21.2	*HIST2H2BF*	Hypo	Over
1q21.2	*HIST2H3A*	Hypo	Over
1q21.3	*CHRNB2*	Hyper	Under
1q23.1	*GPATCH4*	Hypo	Over
1q23.1	*IQGAP3*	Hypo	Over
1q23.2	*CADM3*	Hyper	Under
1q23.3	*NUF2*	Hypo	Over
1q23.3	*PCP4L1*	Hyper	Under
1q24.2	*CD247*	Hypo	Over
1q24.2	*CREG1*	Hypo	Over
1q25.1	*KIAA0040*	Hypo	Over
1q25.2	*CEP350*	Hypo	Over
1q25.2	*RALGPS2*	Hypo	Over
1q25.3	*EDEM3*	Hypo	Over
1q25.3	*NPL*	Hypo	Over
1q31.3	*ASPM*	Hypo	Over
1q32.1	*ELK4*	Hypo	Over
1q32.1	*FAM72D*	Hypo	Over
1q32.1	*KIF14*	Hypo	Over
1q32.1	*MDM4*	Hypo	Over
1q32.1	*NFASC*	Hyper	Under
1q32.1	*ZC3H11A*	Hypo	Over
1q32.2	*CR2*	Hypo	Over
1q32.2	*HHAT*	Hypo	Over
1q32.2	*TRAF3IP3*	Hypo	Over
1q32.3	*ANGEL2*	Hypo	Over
1q32.3	*DTL*	Hypo	Over
1q32.3	*NEK2*	Hypo	Over

1q41	*GPATCH2*	Hypo	Over
1q41	*KCTD3*	Hypo	Over
1q41	*MIA3*	Hypo	Over
1q41	*TAF1A*	Hypo	Over
1q42.12	*LBR*	Hypo	Over
1q42.2	*TSNAX*	Hypo	Over
1q43	*HEATR1*	Hypo	Over
1q43	*MTR*	Hypo	Over
1q44	*HNRNPU*	Hypo	Over
1q44	*PGBD2*	Hypo	Over
2p11.2	*CD8A*	Hypo	Over
2p12	*HK2*	Hypo	Over
2p13.1	*MTHFD2*	Hypo	Over
2p13.2	*ZNF638*	Hypo	Over
2p13.3	*MPHOSPH10*	Hypo	Over
2p14	*ACTR2*	Hypo	Over
2p16.1	*PNPT1*	Hypo	Over
2p16.2	*EML6*	Hypo	Over
2p16.3	*FBXO11*	Hypo	Over
2p16.3	*FOXN2*	Hypo	Over
2p21	*LRPPRC*	Hypo	Over
2p22.1	*THUMPD2*	Hypo	Over
2p22.2	*CCDC75*	Hypo	Over
2p23.3	*CENPA*	Hypo	Over
2p24.1	*SDC1*	Hypo	Over
2p25.1	*KCNF1*	Hyper	Under
2p25.1	*PQLC3*	Hypo	Over
2p25.1	*TAF1B*	Hypo	Over
2q11.2	*LIPT1*	Hypo	Over
2q11.2	*MGAT4A*	Hypo	Over
2q11.2	*PDCL3*	Hypo	Over
2q12.1	*IL18R1*	Hypo	Over
2q12.3	*LIMS1*	Hypo	Over
2q12.3	*RANBP2*	Hypo	Over
2q13	*ANAPC1*	Hypo	Over
2q13	*BCL2L11*	Hypo	Over
2q13	*BUB1*	Hypo	Over
2q13	*CKAP2L*	Hypo	Over
2q13	*TMEM87B*	Hypo	Over
2q14.2	*TFCP2L1*	Hypo	Over
2q14.3	*POLR2D*	Hypo	Over

2q21.3	*UBXN4*	Hypo	Over
2q22.1	*CXCR4*	Hypo	Over
2q23.3	*PRPF40A*	Hypo	Over
2q24.2	*IFIH1*	Hypo	Over
2q24.2	*RBMS1*	Hypo	Over
2q24.2	*TANK*	Hypo	Over
2q31.1	*CDCA7*	Hypo	Over
2q31.1	*CIR1*	Hypo	Over
2q31.1	*ITGA6*	Hypo	Over
2q31.1	*SPC25*	Hypo	Over
2q31.1	*ZAK*	Hypo	Over
2q31.2	*HNRNPA3*	Hypo	Over
2q31.3	*ITGA4*	Hypo	Over
2q32.2	*STAT1*	Hypo	Over
2q32.3	*OBFC2A*	Hypo	Over
2q32.3	*STAT4*	Hypo	Over
2q32.3	*STK17B*	Hypo	Over
2q33.1	*CCDC150*	Hypo	Over
2q33.1	*CFLAR*	Hypo	Over
2q33.1	*CLK1*	Hypo	Over
2q33.1	*NOP58*	Hypo	Over
2q33.2	*RAPH1*	Hypo	Over
2q33.3	*CCNYL1*	Hypo	Over
2q34	*IKZF2*	Hypo	Over
2q34	*UNC80*	Hyper	Under
2q35	*BARD1*	Hypo	Over
2q35	*MREG*	Hypo	Over
2q36.1	*MRPL44*	Hypo	Over
2q36.2	*DOCK10*	Hypo	Over
2q36.3	*COL4A4*	Hypo	Over
2q36.3	*RHBDD1*	Hypo	Over
2q37.1	*CAB39*	Hypo	Over
2q37.1	*HJURP*	Hypo	Over
2q37.1	*PTMA*	Hypo	Over
2q37.3	*KIF1A*	Hyper	Under
2q37.3	*PASK*	Hypo	Over
3p12.3	*ROBO1*	Hypo	Over
3p14.1	*MITF*	Hypo	Over
3p21.1	*GLYCTK*	Hypo	Over
3p21.31	*CDC25A*	Hypo	Over
3p21.31	*KIF15*	Hypo	Over

3p21.31	*SMARCC1*	Hypo	Over
3p21.31	*ZNF589*	Hypo	Over
3p22.3	*CMTM6*	Hypo	Over
3p22.3	*CMTM7*	Hypo	Over
3p22.3	*TRIM71*	Hypo	Over
3p24.1	*EOMES*	Hypo	Over
3p24.3	*SGOL1*	Hypo	Over
3p25.1	*OXNAD1*	Hypo	Over
3p25.3	*ATP2B2*	Hyper	Under
3q11.1	*DHFRL1*	Hypo	Over
3q12.3	*SENP7*	Hypo	Over
3q13.2	*NAA50*	Hypo	Over
3q13.33	*GOLGB1*	Hypo	Over
3q13.33	*POLQ*	Hypo	Over
3q13.33	*TMEM39A*	Hypo	Over
3q21.1	*PARP14*	Hypo	Over
3q21.1	*PDIA5*	Hypo	Over
3q22.1	*CDV3*	Hypo	Over
3q22.1	*TOPBP1*	Hypo	Over
3q25.1	*MBNL1*	Hypo	Over
3q25.2	*MME*	Hypo	Over
3q25.2	*P2RY1*	Hypo	Over
3q25.32	*MFSD1*	Hypo	Over
3q25.33	*SMC4*	Hypo	Over
3q26.2	*GPR160*	Hypo	Over
3q26.2	*MYNN*	Hypo	Over
3q26.31	*FNDC3B*	Hypo	Over
3q27.2	*IGF2BP2*	Hypo	Over
3q27.3	*DNAJB11*	Hypo	Over
3q27.3	*ST6GAL1*	Hypo	Over
3q29	*TMEM44*	Hyper	Under
3q29	*WDR53*	Hypo	Over
4p13	*BEND4*	Hypo	Over
4p15.2	*SEL1L3*	Hypo	Over
4p15.31	*NCAPG*	Hypo	Over
4p15.32	*CD38*	Hypo	Over
4p15.32	*LAP3*	Hypo	Over
4p16.3	*CPLX1*	Hyper	Under
4p16.3	*LYAR*	Hypo	Over
4p16.3	*TACC3*	Hypo	Over
4p16.3	*ZNF141*	Hypo	Over

4q12	*CEP135*	Hypo	Over
4q12	*CLOCK*	Hypo	Over
4q12	*KIT*	Hypo	Over
4q12	*REST*	Hypo	Over
4q21.1	*CCNG2*	Hypo	Over
4q21.1	*NAAA*	Hypo	Over
4q21.1	*SDAD1*	Hypo	Over
4q21.21	*PRDM8*	Hyper	Under
4q21.22	*PLAC8*	Hypo	Over
4q21.23	*COQ2*	Hypo	Over
4q22.1	*HERC5*	Hypo	Over
4q22.1	*HERC6*	Hypo	Over
4q24	*BANK1*	Hypo	Over
4q24	*CENPE*	Hypo	Over
4q25	*CCDC109B*	Hypo	Over
4q25	*ELOVL6*	Hypo	Over
4q27	*CCNA2*	Hypo	Over
4q28.2	*PLK4*	Hypo	Over
4q31.3	*KIAA0922*	Hypo	Over
4q31.3	*MND1*	Hypo	Over
4q32.3	*DDX60*	Hypo	Over
4q32.3	*PALLD*	Hypo	Over
4q34.1	*HMGB2*	Hypo	Over
4q34.2	*SPCS3*	Hypo	Over
4q35.1	*ACSL1*	Hypo	Over
4q35.1	*CASP3*	Hypo	Over
5p13.3	*SUB1*	Hypo	Over
5p15.2	*FAM105B*	Hypo	Over
5p15.33	*IRX4*	Hypo	Over
5p15.33	*ZDHHC11*	Hyper	Under
5q11.1	*EMB*	Hypo	Over
5q11.2	*MIER3*	Hypo	Over
5q12.1	*PDE4D*	Hypo	Over
5q12.3	*CENPK*	Hypo	Over
5q13.2	*CCNB1*	Hypo	Over
5q13.2	*MCCC2*	Hypo	Over
5q13.3	*F2RL1*	Hypo	Over
5q14.1	*LHFPL2*	Hypo	Over
5q15	*GLRX*	Hypo	Over
5q21.1	*SLCO4C1*	Hypo	Over
5q21.3	*MAN2A1*	Hypo	Over

5q23.1	*TNFAIP8*	Hypo	Over
5q23.2	*LMNB1*	Hypo	Over
5q31.1	*HSPA4*	Hypo	Over
5q31.2	*KIF20A*	Hypo	Over
5q31.3	*ANKHD1*	Hypo	Over
5q31.3	*WDR55*	Hypo	Over
5q33.1	*CCDC69*	Hypo	Over
5q33.1	*GM2A*	Hypo	Over
5q33.1	*RPS14*	Hypo	Over
5q33.2	*FAM114A2*	Hypo	Over
5q33.3	*CLINT1*	Hypo	Over
5q33.3	*PTTG1*	Hypo	Over
6p12.2	*MCM3*	Hypo	Over
6p12.3	*PLA2G7*	Hypo	Over
6p21.1	*CDC5L*	Hypo	Over
6p21.1	*NFYA*	Hypo	Over
6p21.1	*TMEM151B*	Hyper	Under
6p21.1	*TTBK1*	Hyper	Under
6p21.2	*KCNK5*	Hypo	Over
6p21.2	*PIM1*	Hypo	Over
6p21.31	*FKBP5*	Hypo	Over
6p21.31	*GRM4*	Hyper	Under
6p21.31	*PACSIN1*	Hyper	Under
6p21.31	*SRSF3*	Hypo	Over
6p21.32	*KIFC1*	Hypo	Over
6p21.32	*PSMB9*	Hypo	Over
6p21.32	*TAP1*	Hypo	Over
6p21.32	*TAP2*	Hypo	Over
6p21.33	*HLA-B*	Hypo	Over
6p21.33	*HLA-E*	Hypo	Over
6p21.33	*LTB*	Hypo	Over
6p21.33	*MSH5*	Hypo	Over
6p22.1	*HIST1H1B*	Hypo	Over
6p22.1	*HIST1H2AH*	Hypo	Over
6p22.1	*HIST1H2AI*	Hypo	Over
6p22.1	*HIST1H2AJ*	Hypo	Over
6p22.1	*HIST1H2AK*	Hypo	Over
6p22.1	*HIST1H2AL*	Hypo	Over
6p22.1	*HIST1H2AM*	Hypo	Over
6p22.1	*HIST1H2BK*	Hypo	Over
6p22.1	*HIST1H2BL*	Hypo	Over

6p22.1	*HIST1H2BO*	Hypo	Over
6p22.1	*HIST1H3H*	Hypo	Over
6p22.1	*HIST1H3I*	Hypo	Over
6p22.1	*HLA-J*	Hypo	Over
6p22.1	*UBD*	Hypo	Over
6p22.1	*ZSCAN16*	Hypo	Over
6p22.2	*HIST1H1D*	Hypo	Over
6p22.2	*HIST1H2BD*	Hypo	Over
6p22.2	*HIST1H2BH*	Hypo	Over
6p22.2	*HIST1H3B*	Hypo	Over
6p22.2	*HIST1H3D*	Hypo	Over
6p22.2	*HIST1H3G*	Hypo	Over
6p22.2	*HIST1H4A*	Hypo	Over
6p22.3	*DCDC2*	Hypo	Over
6p22.3	*RNF144B*	Hypo	Over
6p24.3	*DSP*	Hypo	Over
6p24.3	*TXNDC5*	Hypo	Over
6p25.3	*IRF4*	Hypo	Over
6q13	*CD109*	Hypo	Over
6q13	*MB21D1*	Hypo	Over
6q14.1	*FAM46A*	Hypo	Over
6q14.1	*TTK*	Hypo	Over
6q14.2	*CYB5R4*	Hypo	Over
6q14.3	*SYNCRIP*	Hypo	Over
6q16.3	*ASCC3*	Hypo	Over
6q16.3	*LIN28B*	Hypo	Over
6q21	*AIM1*	Hypo	Over
6q21	*PRDM1*	Hypo	Over
6q21	*SNX3*	Hypo	Over
6q22.1	*FAM26F*	Hypo	Over
6q22.31	*MAN1A1*	Hypo	Over
6q23.2	*MED23*	Hypo	Over
6q23.3	*BCLAF1*	Hypo	Over
6q23.3	*MYB*	Hypo	Over
6q24.2	*UTRN*	Hypo	Over
6q25.1	*NUP43*	Hypo	Over
6q25.1	*RMND1*	Hypo	Over
6q25.1	*TAB2*	Hypo	Over
6q25.1	*ZBTB2*	Hypo	Over
6q25.2	*FBXO5*	Hypo	Over
6q25.3	*SOD2*	Hypo	Over

6q25.3	*WTAP*	Hypo	Over
7p13	*FLJ35390*	Hyper	Under
7p15.2	*CBX3*	Hypo	Over
7p15.2	*HNRNPA2B1*	Hypo	Over
7p15.2	*NFE2L3*	Hypo	Over
7p15.3	*CDCA7L*	Hypo	Over
7p15.3	*IGF2BP3*	Hypo	Over
7p15.3	*TRA2A*	Hypo	Over
7p21.1	*BZW2*	Hypo	Over
7p22.3	*AMZ1*	Hyper	Under
7p22.3	*PRKAR1B*	Hyper	Under
7q11.21	*TYW1*	Hypo	Over
7q11.23	*PHTF2*	Hypo	Over
7q21.12	*DBF4*	Hypo	Over
7q21.12	*DMTF1*	Hypo	Over
7q21.2	*AKAP9*	Hypo	Over
7q21.2	*FAM133B*	Hypo	Over
7q21.3	*BET1*	Hypo	Over
7q21.3	*CASD1*	Hypo	Over
7q21.3	*CCDC132*	Hypo	Over
7q22.1	*EPHB4*	Hypo	Over
7q22.1	*MCM7*	Hypo	Over
7q22.1	*TRIM4*	Hypo	Over
7q22.1	*ZNF394*	Hypo	Over
7q33	*TRIM24*	Hypo	Over
7q34	*JHDM1D*	Hypo	Over
7q34	*KLRG2*	Hypo	Over
7q34	*MKRN1*	Hypo	Over
7q34	*PARP12*	Hypo	Over
7q36.1	*AGAP3*	Hyper	Under
7q36.1	*ZNF467*	Hyper	Under
7q36.2	*DPP6*	Hyper	Under
7q36.3	*MNX1*	Hypo	Over
7q36.3	*PTPRN2*	Hyper	Under
8p11.23	*PPAPDC1B*	Hypo	Over
8p12	*UBXN8*	Hypo	Over
8p21.1	*ESCO2*	Hypo	Over
8p21.2	*BNIP3L*	Hypo	Over
8p21.3	*LOXL2*	Hypo	Over
8p21.3	*LPL*	Hypo	Over
8p21.3	*PHYHIP*	Hyper	Under

8p22	*ASAH1*	Hypo	Over
8p23.1	*BLK*	Hypo	Over
8q11.21	*MCM4*	Hypo	Over
8q11.23	*SOX17*	Hypo	Over
8q11.23	*TCEA1*	Hypo	Over
8q12.1	*LYN*	Hypo	Over
8q12.1	*PLAG1*	Hypo	Over
8q13.1	*MTFR1*	Hypo	Over
8q13.1	*PDE7A*	Hypo	Over
8q13.2	*ARFGEF1*	Hypo	Over
8q21.12	*IL7*	Hypo	Over
8q21.13	*IMPA1*	Hypo	Over
8q21.13	*PAG1*	Hypo	Over
8q21.2	*REXO1L1*	Hyper	Under
8q22.1	*CCNE2*	Hypo	Over
8q22.1	*ESRP1*	Hypo	Over
8q22.1	*INTS8*	Hypo	Over
8q22.1	*KIAA1429*	Hypo	Over
8q22.3	*DCAF13*	Hypo	Over
8q22.3	*PABPC1*	Hypo	Over
8q22.3	*UBR5*	Hypo	Over
8q24.12	*SNTB1*	Hypo	Over
8q24.12	*TAF2*	Hypo	Over
8q24.13	*ATAD2*	Hypo	Over
8q24.13	*FAM91A1*	Hypo	Over
8q24.13	*WDR67*	Hypo	Over
8q24.13	*ZHX1-C8ORF76*	Hypo	Over
8q24.3	*ADCK5*	Hyper	Under
8q24.3	*BAI1*	Hyper	Under
8q24.3	*MAFA*	Hypo	Under
8q24.3	*PTK2*	Hypo	Under
8q24.3	*SCRT1*	Hypo	Under
9p13.2	*MELK*	Hypo	Over
9p13.3	*GLIPR2*	Hypo	Over
9p13.3	*RMRP*	Hypo	Over
9p13.3	*SIT1*	Hypo	Over
9p21.2	*PLAA*	Hypo	Over
9p22.1	*PLIN2*	Hypo	Over
9p24.1	*CD274*	Hypo	Over
9p24.3	*DOCK8*	Hypo	Over
9q21.13	*GCNT1*	Hypo	Over

9q21.13	*OSTF1*	Hypo	Over
9q21.13	*TMEM2*	Hypo	Over
9q22.2	*CKS2*	Hypo	Over
9q22.2	*SEMA4D*	Hyper	Under
9q22.2	*SYK*	Hypo	Over
9q22.31	*NOL8*	Hypo	Over
9q22.32	*BARX1*	Hypo	Over
9q22.32	*ZNF367*	Hypo	Over
9q22.33	*CTSL2*	Hypo	Over
9q31.3	*TXN*	Hypo	Over
9q32	*COL27A1*	Hypo	Over
9q33.3	*NR6A1*	Hypo	Over
9q34.3	*LCN15*	Hyper	Under
9q34.3	*PNPLA7*	Hyper	Under
10p11.1	*ZNF33A*	Hypo	Over
10p12.31	*DNAJC1*	Hypo	Over
10p13	*DCLRE1C*	Hypo	Over
10p13	*FAM107B*	Hypo	Over
10p13	*MCM10*	Hypo	Over
10p14	*PRKCQ*	Hypo	Over
10p15.1	*IL15RA*	Hypo	Over
10p15.3	*ADARB2*	Hyper	Under
10p15.3	*TUBB8*	Hyper	Under
10q11.21	*BMS1*	Hypo	Over
10q11.21	*CSGALNACT2*	Hypo	Over
10q21.1	*TFAM*	Hypo	Over
10q21.1	*ZWINT*	Hypo	Over
10q21.2	*CDK1*	Hypo	Over
10q22.1	*SAR1A*	Hypo	Over
10q22.2	*PLAU*	Hypo	Over
10q23.2	*FAM35A*	Hypo	Over
10q23.31	*KIF20B*	Hypo	Over
10q23.31	*LIPA*	Hypo	Over
10q23.33	*CEP55*	Hypo	Over
10q23.33	*HELLS*	Hypo	Over
10q23.33	*KIF11*	Hypo	Over
10q23.33	*MYOF*	Hypo	Over
10q24.2	*ABCC2*	Hypo	Over
10q24.33	*TAF5*	Hypo	Over
10q25.2	*ZDHHC6*	Hypo	Over
10q26.11	*RGS10*	Hypo	Over

10q26.2	*MKI67*	Hypo	Over
10q26.3	*GPR123*	Hyper	Under
10q26.3	*KNDC1*	Hyper	Under
10q26.3	*SPRN*	Hyper	Under
10q26.3	*VENTX*	Hypo	Over
11p13	*CD44*	Hypo	Over
11p15.1	*E2F8*	Hypo	Over
11p15.2	*PDE3B*	Hypo	Over
11p15.4	*IPO7*	Hypo	Over
11p15.4	*NAP1L4*	Hypo	Over
11p15.4	*ZNF143*	Hypo	Over
11p15.5	*BRSK2*	Hyper	Under
11p15.5	*IFITM1*	Hypo	Over
11p15.5	*IRF7*	Hypo	Over
11q12.1	*SLC43A1*	Hypo	Over
11q13.1	*CDCA5*	Hypo	Over
11q13.2	*RIN1*	Hyper	Under
11q13.2	*TBC1D10C*	Hypo	Over
11q13.4	*SNORD15A*	Hypo	Over
11q14.2	*CTSC*	Hypo	Over
11q14.2	*RAB38*	Hypo	Over
11q21	*FUT4*	Hypo	Over
11q21	*MRE11A*	Hypo	Over
11q22.1	*ARHGAP42*	Hypo	Over
11q23.1	*PPP2R1B*	Hypo	Over
11q23.2	*USP28*	Hypo	Over
11q23.3	*IL10RA*	Hypo	Over
11q24.1	*HSPA8*	Hypo	Over
11q24.1	*UBASH3B*	Hypo	Over
11q24.2	*CCDC15*	Hypo	Over
11q24.2	*NRGN*	Hyper	Under
11q25	*B3GAT1*	Hyper	Under
12p11.21	*FAM60A*	Hypo	Over
12p13.1	*ATF7IP*	Hypo	Over
12p13.1	*PLBD1*	Hypo	Over
12p13.31	*NCAPD2*	Hypo	Over
12p13.32	*PARP11*	Hypo	Over
12p13.33	*FOXM1*	Hypo	Over
12p13.33	*SLC6A12*	Hyper	Under
12p13.33	*TEAD4*	Hypo	Over
12q13.11	*VDR*	Hypo	Over

12q13.12	*CCNT1*	Hypo	Over
12q13.12	*FKBP11*	Hypo	Over
12q13.12	*RACGAP1*	Hypo	Over
12q13.12	*TMBIM6*	Hypo	Over
12q13.12	*TROAP*	Hypo	Over
12q13.12	*TUBA1C*	Hypo	Over
12q13.13	*CBX5*	Hypo	Over
12q13.13	*HNRNPA1*	Hypo	Over
12q13.3	*KIF5A*	Hyper	Under
12q13.3	*PTGES3*	Hypo	Over
12q14.1	*CYP27B1*	Hypo	Over
12q14.1	*USP15*	Hypo	Over
12q14.2	*RASSF3*	Hypo	Over
12q15	*MDM1*	Hypo	Over
12q15	*MDM2*	Hypo	Over
12q21.2	*E2F7*	Hypo	Over
12q21.2	*PHLDA1*	Hypo	Over
12q21.33	*BTG1*	Hypo	Over
12q22	*PLXNC1*	Hypo	Over
12q23.1	*GAS2L3*	Hypo	Over
12q23.1	*TMPO*	Hypo	Over
12q23.3	*HSP90B1*	Hypo	Over
12q23.3	*POLR3B*	Hypo	Over
12q24.11	*GIT2*	Hypo	Over
12q24.11	*PPTC7*	Hypo	Over
12q24.11	*TCHP*	Hypo	Over
12q24.11	*UNG*	Hypo	Over
12q24.12	*BRAP*	Hypo	Over
12q24.13	*DTX1*	Hyper	Under
12q24.13	*NAA25*	Hypo	Over
12q24.13	*OAS3*	Hypo	Over
12q24.13	*RASAL1*	Hyper	Under
12q24.23	*VSIG10*	Hypo	Over
12q24.33	*STX2*	Hypo	Over
13q12.11	*SKA3*	Hypo	Over
13q12.12	*BASP1P1*	Hyper	Under
13q12.12	*CENPJ*	Hypo	Over
13q12.13	*ATP8A2*	Hyper	Under
13q12.13	*USP12*	Hypo	Over
13q12.3	*HMGB1*	Hypo	Over
13q13.2	*RFC3*	Hypo	Over

13q14.11	*EPSTI1*	Hypo	Over
13q14.11	*MTRF1*	Hypo	Over
13q14.11	*NAA16*	Hypo	Over
13q14.2	*PHF11*	Hypo	Over
13q14.2	*SETDB2*	Hypo	Over
13q14.3	*HNRNPA1L2*	Hypo	Over
13q21.2	*DIAPH3*	Hypo	Over
13q22.1	*KLF12*	Hypo	Over
13q33.3	*MYO16*	Hyper	Under
14q11.2	*TEP1*	Hypo	Over
14q22.1	*ERO1L*	Hypo	Over
14q22.1	*NIN*	Hypo	Over
14q22.2	*CDKN3*	Hypo	Over
14q22.2	*WDHD1*	Hypo	Over
14q23.1	*PRKCH*	Hypo	Over
14q23.1	*RTN1*	Hypo	Under
14q23.2	*FLJ43390*	Hypo	Under
14q23.2	*SYNE2*	Hypo	Over
14q23.3	*ZBTB25*	Hypo	Over
14q24.1	*DCAF5*	Hypo	Over
14q24.3	*NPC2*	Hypo	Over
14q32.11	*RPS6KA5*	Hypo	Over
14q32.11	*TDP1*	Hypo	Over
14q32.2	*CCNK*	Hypo	Over
14q32.2	*VRK1*	Hypo	Over
14q32.31	*HSP90AA1*	Hypo	Over
15q13.2	*ARHGAP11B*	Hypo	Over
15q13.3	*ARHGAP11A*	Hypo	Over
15q13.3	*CHRFAM7A*	Hyper	Under
15q15.1	*BMF*	Hypo	Over
15q15.1	*BUB1B*	Hypo	Over
15q15.1	*CASC5*	Hypo	Over
15q15.1	*NUSAP1*	Hypo	Over
15q15.1	*OIP5*	Hypo	Over
15q15.1	*RAD51*	Hypo	Over
15q15.1	*SNAP23*	Hypo	Over
15q21.1	*B2M*	Hypo	Over
15q21.1	*SQRDL*	Hypo	Over
15q21.2	*GABPB1*	Hypo	Over
15q22.2	*ANXA2*	Hypo	Over
15q22.2	*CCNB2*	Hypo	Over

15q22.31	*CLPX*	Hypo	Over
15q22.31	*KIAA0101*	Hypo	Over
15q22.31	*ZWILCH*	Hypo	Over
15q23	*FEM1B*	Hypo	Over
15q23	*KIF23*	Hypo	Over
15q23	*SENP8*	Hypo	Over
15q24.1	*ISLR2*	Hyper	Under
15q25.1	*CHRNA5*	Hypo	Over
15q26.1	*BLM*	Hypo	Over
15q26.1	*FANCI*	Hypo	Over
15q26.1	*IQGAP1*	Hypo	Over
15q26.1	*PRC1*	Hypo	Over
15q26.3	*TARSL2*	Hypo	Over
16p11.2	*ASPHD1*	Hyper	Under
16p11.2	*SEPHS2*	Hypo	Over
16p11.2	*ZNF267*	Hypo	Over
16p12.1	*IL4R*	Hypo	Over
16p12.3	*SMG1*	Hypo	Over
16p13.13	*GSPT1*	Hypo	Over
16p13.13	*LITAF*	Hypo	Over
16p13.13	*RSL1D1*	Hypo	Over
16p13.3	*BAIAP3*	Hyper	Under
16p13.3	*LMF1*	Hyper	Under
16p13.3	*PKMYT1*	Hypo	Over
16p13.3	*RPS2*	Hypo	Over
16p13.3	*SOX8*	Hyper	Under
16p13.3	*SYNGR3*	Hyper	Under
16p13.3	*ZNF200*	Hypo	Over
16q21	*CNOT1*	Hypo	Over
16q21	*GINS3*	Hypo	Over
16q22.1	*PDPR*	Hypo	Over
16q22.1	*RLTPR*	Hyper	Under
16q22.1	*SLC7A6*	Hypo	Over
16q22.1	*SMPD3*	Hyper	Under
16q23.1	*CHST6*	Hyper	Under
16q23.1	*MLKL*	Hypo	Over
16q23.3	*PLCG2*	Hypo	Over
16q23.3	*SDR42E1*	Hypo	Over
16q24.1	*ADAD2*	Hyper	Under
16q24.1	*GINS2*	Hypo	Over
16q24.1	*IRF8*	Hypo	Over

16q24.2	*JPH3*	Hyper	Under
16q24.3	*AFG3L1P*	Hypo	Over
16q24.3	*CDT1*	Hypo	Over
16q24.3	*CPNE7*	Hyper	Under
16q24.3	*CYBA*	Hypo	Over
16q24.3	*FANCA*	Hypo	Over
17p11.2	*SLC47A1*	Hypo	Over
17p13.1	*ACAP1*	Hypo	Over
17p13.2	*GSG2*	Hypo	Over
17p13.3	*ABR*	Hyper	Under
17q11.2	*ATAD5*	Hypo	Over
17q11.2	*MYO1D*	Hypo	Over
17q11.2	*SPAG5*	Hypo	Over
17q12	*IKZF3*	Hypo	Over
17q12	*SYNRG*	Hypo	Over
17q21.2	*TOP2A*	Hypo	Over
17q21.32	*GOSR2*	Hypo	Over
17q21.32	*IGF2BP1*	Hypo	Over
17q21.32	*ITGB3*	Hypo	Over
17q21.32	*TBX21*	Hypo	Over
17q21.33	*DLX4*	Hypo	Over
17q21.33	*EME1*	Hypo	Over
17q21.33	*LUC7L3*	Hypo	Over
17q22	*SCPEP1*	Hypo	Over
17q23.1	*VMP1*	Hypo	Over
17q23.2	*BRIP1*	Hypo	Over
17q25.3	*B3GNTL1*	Hyper	Under
17q25.3	*BAIAP2*	Hyper	Under
17q25.3	*BIRC5*	Hypo	Over
17q25.3	*DNAH17*	Hyper	Under
17q25.3	*RBFOX3*	Hyper	Under
17q25.3	*TK1*	Hypo	Over
18p11.32	*EMILIN2*	Hypo	Over
18p11.32	*NDC80*	Hypo	Over
18p11.32	*SMCHD1*	Hypo	Over
18p11.32	*THOC1*	Hypo	Over
18q11.2	*PSMA8*	Hyper	Over
18q11.2	*RBBP8*	Hypo	Over
18q11.2	*TAF4B*	Hypo	Over
18q21.1	*SKA1*	Hypo	Over
18q21.2	*MBD2*	Hypo	Over

18q21.32	*PMAIP1*	Hypo	Over
18q21.32	*SEC11C*	Hypo	Over
18q22.1	*CCDC102B*	Hypo	Over
18q23	*MBP*	Hyper	Under
19p12	*ZNF682*	Hypo	Over
19p13.11	*HAUS8*	Hypo	Over
19p13.11	*IFI30*	Hypo	Over
19p13.11	*YJEFN3*	Hyper	Under
19p13.2	*CLEC4G*	Hyper	Under
19p13.2	*MAST1*	Hyper	Under
19p13.2	*SPC24*	Hypo	Over
19p13.2	*ZNF266*	Hypo	Over
19p13.2	*ZNF443*	Hypo	Over
19p13.2	*ZNF823*	Hypo	Over
19p13.3	*CD70*	Hypo	Over
19p13.3	*DIRAS1*	Hyper	Under
19p13.3	*HCN2*	Hyper	Under
19p13.3	*S1PR4*	Hypo	Over
19p13.3	*SEMA6B*	Hyper	Under
19p13.3	*THOP1*	Hyper	Under
19p13.3	*TUBB2A*	Hyper	Under
19q13.11	*ZNF792*	Hypo	Over
19q13.12	*ZBTB32*	Hypo	Over
19q13.2	*ZNF780A*	Hypo	Over
19q13.31	*ZNF224*	Hypo	Over
19q13.32	*VASP*	Hypo	Over
19q13.33	*CPT1C*	Hyper	Under
19q13.33	*FAM71E1*	Hyper	Under
19q13.33	*LMTK3*	Hyper	Under
19q13.33	*SLC17A7*	Hyper	Under
19q13.33	*SYT3*	Hyper	Under
19q13.41	*ZNF137P*	Hypo	Over
19q13.41	*ZNF432*	Hypo	Over
19q13.42	*ZNF331*	Hypo	Over
19q13.43	*ZNF154*	Hypo	Over
20p11.23	*SNX5*	Hypo	Over
20p12.1	*ESF1*	Hypo	Over
20p13	*NOP56*	Hypo	Over
20q11.21	*DNMT3B*	Hypo	Over
20q11.21	*HM13*	Hypo	Over
20q11.21	*TPX2*	Hypo	Over

20q11.22	*SPAG4*	Hypo	Over
20q11.23	*DSN1*	Hypo	Over
20q11.23	*FAM83D*	Hypo	Over
20q11.23	*RPN2*	Hypo	Over
20q12	*CHD6*	Hypo	Over
20q13.12	*MKRN7P*	Hypo	Over
20q13.12	*MMP9*	Hypo	Over
20q13.12	*UBE2C*	Hypo	Over
20q13.13	*KCNG1*	Hyper	Under
20q13.2	*AURKA*	Hipo	Over
20q13.33	*EEF1A2*	Hyper	Under
21q21.3	*BACH1*	Hypo	Over
21q22.11	*GART*	Hypo	Over
21q22.11	*IFNAR2*	Hypo	Over
21q22.11	*MIS18A*	Hypo	Over
21q22.11	*SON*	Hypo	Over
21q22.12	*CLIC6*	Hypo	Over
21q22.2	*BRWD1*	Hypo	Over
22q11.21	*CDC45*	Hypo	Over
22q11.21	*GP1BB*	Hyper	Under
22q11.21	*TUBA8*	Hyper	Under
22q11.22	*RTDR1*	Hyper	Under
22q11.22	*ZNF280B*	Hypo	Over
22q11.23	*IGLL1*	Hypo	Over
22q12.1	*ASPHD2*	Hyper	Under
22q12.2	*EWSR1*	Hypo	Over
22q12.2	*GAL3ST1*	Hyper	Under
22q12.3	*EIF3D*	Hypo	Over
22q13.1	*BAIAP2L2*	Hyper	Under
22q13.1	*CACNA1I*	Hyper	Under
22q13.2	*ARFGAP3*	Hypo	Over
22q13.2	*CENPM*	Hypo	Over
22q13.2	*MPPED1*	Hyper	Under
22q13.2	*SEPT3*	Hyper	Under
22q13.2	*ST13*	Hypo	Over
22q13.31	*GTSE1*	Hypo	Over
22q13.31	*NUP50*	Hypo	Over
22q13.33	*MAPK11*	Hypo	Under
22q13.33	*MLC1*	Hyper	Under
22q13.33	*ODF3B*	Hypo	Over
22q13.33	*PANX2*	Hyper	Under

Xp11.22	*SMC1A*	Hypo	Over
Xp11.23	*CLCN5*	Hypo	Over
Xp11.23	*PIM2*	Hypo	Over
Xp11.4	*MED14*	Hypo	Over
Xp22.11	*PRDX4*	Hypo	Over
Xp22.12	*RPS6KA3*	Hypo	Over
Xp22.2	*TMSB4X*	Hypo	Over
Xp22.2	*TXLNG*	Hypo	Over
Xp22.33	*PRKX*	Hypo	Over
Xq12	*LAS1L*	Hypo	Over
Xq13.1	*ERCC6L*	Hypo	Over
Xq21.1	*ATP7A*	Hypo	Over
Xq21.1	*MAGT1*	Hypo	Over
Xq22.1	*SYTL4*	Hypo	Over
Xq22.1	*TIMM8A*	Hypo	Over
Xq22.3	*RBM41*	Hypo	Over
Xq23	*TMEM164*	Hyper	Under
Xq24	*CUL4B*	Hypo	Over
Xq24	*DOCK11*	Hypo	Over
Xq24	*WDR44*	Hypo	Over
Xq28	*HMGB3*	Hypo	Over
Xq28	*MTM1*	Hypo	Over
Xq28	*PNCK*	Hyper	Under
Yp11.31	*RPS4Y1*	Hypo	Over