Suzuki et al. Applied Cancer Research (2017) 37:14
DOI 10.1186/s41241-017-0025-9

Applied Cancer Research

RESEARCH ARTICLE Open Access

Loss of expression of the Neural Cell Adhesion Molecule 1 (NCAM1) in atypical teratoid/rhabdoid tumors: a new diagnostic marker?

Mario Suzuki[1,2,4†], Kashyap Patel[2†], Chiang-Ching Huang[5], Felipe D'Almeida Costa[6], Akihide Kondo[4], Fernando Augusto Soares[6], Tadanori Tomita[1,2,3] and Simone Treiger Sredni[1,2,3*]

Abstract

Background: Atypical teratoid/rhabdoid tumors (AT/RT) are aggressive embryonal tumors of the central nervous system. They are largely characterized by inactivating mutations of the *SMARCB1* tumor suppressor gene. AT/RT patients have a very poor prognosis and no standard therapeutic protocol has been defined yet. Recently, multimodal therapy with multiple drug combinations has slightly improved the overall survival, however drug toxicity remains high. In this scenario, a better understanding of the pathophysiology of the disease is needed.

Methods: We evaluated the gene expression profile of AT/RT samples to find new genetic factors contributing to the pathophysiology of the disease. We found target genes significantly differentially expressed between AT/RT and medulloblastoma (MB), the most common embryonal brain tumor. The mRNA expression was validated by quantitative real-time PCR and, at the protein level, expression was validated by immunohistochemistry in an independent set of tumors.

Results: The *Neural cell adhesion molecule 1* (*NCAM1*) gene was found to be consistently downregulated in AT/RT samples when compared to MB and normal brain tissue. Immunohistochemistry showed that the expression of NCAM1 in AT/RT was significantly lower than that of MB.

Conclusion: NCAM1 is an important molecule involved in neuron-to-neuron and neuron-to-muscle adhesion during development. Downregulation of NCAM1 has been implicated in several human cancers suggesting that it might have a tumor repressor role. In this study we found a significantly reduced expression of NCAM1 in AT/RT when compared to MB and we suggest that this feature can be used as a diagnostic marker, along with demonstration of SMARCB1 (INI1) or SMARCA4 (BRG1) inactivation. The roles of NCAM1 in the pathophysiology of AT/RT are still to be determined.

Keywords: AT/RT, Rhabdoid tumor, NCAM, CD56

* Correspondence: ssredni@luriechildrens.org; ssredni@northwestern.edu
†Equal contributors
[1]Division of Pediatric Neurosurgery, Ann and Robert H. Lurie Children's Hospital of Chicago, 225 E. Chicago Ave., Chicago, IL 60611, USA
[2]Cancer Biology and Epigenomics, Stanley Manne Children's Research Institute, 2430 N Halsted St., Chicago, IL 60614, USA
Full list of author information is available at the end of the article

Background

Malignant rhabdoid tumors (MRT) are highly aggressive embryonal tumors. They can arise at any anatomical location and their cell of origin is unknown. The most common site of origin is the central nervous system (CNS) where they are known as atypical teratoid/rhabdoid tumors (AT/RT) [1]. According to recent data from the German Childhood Cancer Registry, it is estimated that AT/RTs make up to 65% of all rhabdoid tumors, while 5–10% are originated in the kidneys and are called rhabdoid tumors of the kidney or RTK. The remaining 25% originate in extracranial/extrarenal locations. AT/RT is the most common malignant CNS tumor in patients under 6 months of age [2]. The current treatment protocol involves maximal surgical resection of the primary tumor, chemotherapy and radiation therapy depending on the age of the patient and location of the tumors. Maximal tumor resection is associated with increased overall survival rates [3]. Recent reports showed that high-dose chemotherapy including intrathecal chemotherapy has improved patients' outcome to 70% survival in 2 years [4] and 45% survival in 6 years [5]. Other studies showed that radiation therapy in high-doses early in the course of the disease can also increase survival rates of children diagnosed with AT/RT [6, 7]. However these intensive adjuvant therapies have significant side effects including high risk of leukoencephalopathy or radiation necrosis [8]. Thus, there is an immediate need for a better understanding of the biological basis of AT/RT in order to develop novel and more effective targeted therapeutic approaches.

Genetically, the majority of AT/RT is characterized by inactivating mutations of the *SMARCB1* gene, also known as *hSNF5*, *BAF47* and *INI1*, while less than 5% exhibit mutations in the *SMARCA4* gene, also known as *BRG1* gene [9]. Both genes are part of the SWI/SNF chromatin remodeling complex. In fact, it has been demonstrated that more than 20% of human malignancies have a mutation in at least one subunit of this complex [10]. AT/RT most frequently arises sporadically, but there are also familial cases in the so called rhabdoid tumor predisposition syndrome (RTPS) [11].

Histologically, AT/RT may display a diverse combination of cellular types consisting of undifferentiated "small round blue cells", mesenchymal and epithelial components. The classic rhabdoid phenotype, characterized by large cells with eccentrically placed nuclei, a prominent nucleolus, abundant eosinophilic cytoplasm and occasional pale cytoplasmic inclusion bodies may or may not be present [12]. Depending on the area of the tumor submitted to histologic examination it may be challenging to morphologically differentiate AT/RT from medulloblastoma (MB) and other embryonal brain tumors [13–15]. Therefore, establishing the diagnosis based solely on histopathological observations can be challenging. The immunohistochemical profiling of the tumor is also necessary for an accurate diagnosis. AT/RT frequently co-expresses mesenchymal and epithelial markers such as vimentin and epithelial membrane antigen (EMA) and does not express markers of skeletal muscle differentiation. Remarkably, about 90% of AT/RT demonstrate loss of expression of SAMRCB1 [16] by immunohistochemistry. This loss of SMARCB1 expression is currently the most reliable marker for AT/RT diagnosis. However, about 10% of the cases, which do not lose SMARCB1 expression, remain undiagnosed.

The aim of this study was to investigate new diagnostic markers and/or new genetic factors contributing to the pathophysiology of AT/RT. To achieve this goal, ee analyzed the gene expression profiles of frozen AT/RT samples. Because the AT/RT's cell of origin is unknown, we chose to compare AT/RT to MB, which is the most common CNS pediatric embryonal tumor, but has a better outcome than AT/RT, with over 90% of cure rates for WNT group and 40–60% for group 3 [17, 18]. The result of this analysis revealed the neural cell adhesion molecule 1 (*NCAM1*) among the most significantly differentially expressed genes.

Methods

Tumor samples

Fresh frozen tumor tissues were provided by the Falk Brain tumor bank (Chicago, IL, USA) and by the Center for Childhood Cancer's Biopathology Center (Columbus, OH, USA), which is a section of the Cooperative Human Tissue Network of The National Cancer Institute (Bethesda, MD, USA).

Written informed parental consents were obtained prior to sample collection. The study was approved by the Institutional review board of Ann and Robert H. Lurie Children's Hospital of Chicago (IRB#2009-13778).

Gene expression (GE) profile

Total RNA was isolated using Trizol Reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. The RNA concentration and quality were assessed by NanoDrop (ThermoFisher Scientific, Waltham, MA, USA) and Bioanalzyer 2100 (Agilent technologies Inc., Santa Clara, CA, USA) respectively. GE profiling was performed using Illumina HT-12 BeadChip whole-genome expression arrays (Illumina, San Diego, CA, USA). Each RNA sample was amplified using the Ambion Illumina RNA amplification kit with biotin UTP labeling (Enzo Biochem, New York, NY, USA). In vitro transcription was completed in order to synthesize biotin-labeled cDNA using T7 RNA polymerase. The cDNA was then column-purified and checked for size and yield using the Bio-Rad Experion system (Bio-Rad Laboratories, Hercules, CA, USA). A total of 1.5 μg of cDNA was hybridized to each array using standard Illumina protocols. Slides were scanned on an Illumina Beadstation and analyzed using BeadStudio (Illumina, San Diego, CA, USA).

Data were normalized using the quantile normalization procedure from the bioconductor package, *affy* (www.bioconductor.org). Cluster and TreeView (www.eisenlab.org) were used for data clustering and visualization.

Quantitative real-time PCR (qRT-PCR)

A total of 1 μg of RNA was used to make cDNA using the high capacity RNA-to-cDNA Kit (ThermoFisher Scientific, Carlsbad, CA, USA), and the expression of the selected gene was validated by real-time PCR using the TaqMan gene expression assay for *NCAM1* (Hs00941830_m1) (ThermoFisher Scientific, Carlsbad, CA, USA). Reactions were performed in triplicates with adequate positive and negative controls. The normalized GE levels were calculated by the ΔΔCt method using the housekeeping gene *GAPDH* (Hs02758991_g1) as reference [19], and a pool of all samples as calibrator.

Immunohistochemistry (IHC) on Tissue microarray (TMA)

To assess the expression of genes of interest by immunohistochemistry, two TMAs were analyzed. The TMAs were designed as follows: (1) a rhabdoid tumor array with 35 unique cases in duplicates with control cores consisting of cerebellum (three cases), kidney (three cases), and tonsil (three cases) and a (2) MB array with 40 unique cases in duplicates and cores of cerebellum as control. Cores were placed randomly across the blocks.

TMA sections (5 μm thick) were stained using standard immunohistochemical procedures with the following antibodies; polyclonal NCAM1 antibody (1:100, Millipore, USA) and polyclonal hSNF5 antibody (1:200 Novus Biologicals, USA) Interpretation of the TMA slides was performed in a blinded fashion.

Results

GE profile showed significant downregulation of *NCAM1* in AT/RT

GE profiling was performed in 14 AT/RTs, 6 MBs and four normal brain tissues including three fetal and one adult. The analysis revealed 1,002 genes significantly differentially expressed with a fold change higher than 2 or lower than −2 and a *p*-value ≤−0.05 when comparing AT/RT and MB (Fig. 1a). Among the most differentially expressed genes,

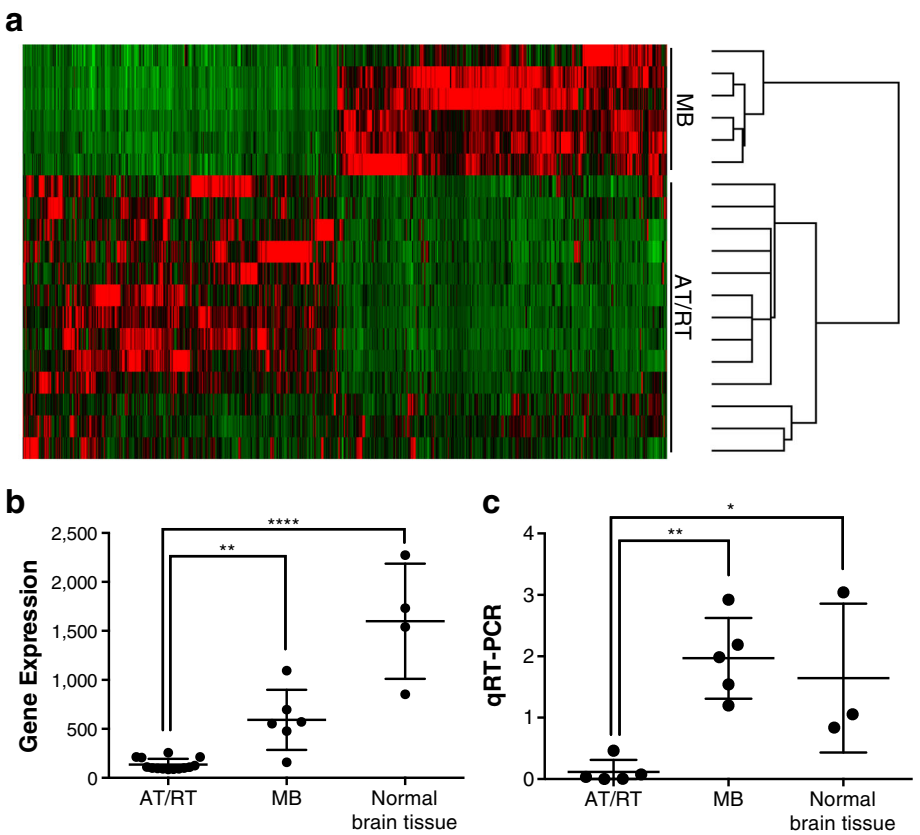

Fig. 1 GE of AT/RT, MB and normal brain tissue. **a** Hierarchical clustering of GE profiling showed 1,002 genes significantly differentially expressed with ≥2 or ≤−2 fold-change, and *p*-value <0.05. **b** Analysis of GE data showed that *NCAM1* was significantly downregulated in AT/RT when compared to MB and normal brain tissues (**$P < 0.01$, ****$P < 0.0001$, one-way ANOVA). **c** Validation of GE data by qRT-PCR. *NCAM1* expression level was significantly lower in AT/RT when compared to MB and normal brain tissues (*$P < 0.05$, **$P < 0.01$, one-way ANOVA)

NCAM1 was significantly downregulated in AT/RT when compared to MB and normal brain tissue ($P = 0.0046$ and 0.0001 respectively, one-way ANOVA) (Fig. 1b). Data was validated by qRT-PCR in 5 AT/RTs, 5 MBs and three normal brain tissues. The results confirmed the GE findings that expression of *NCAM1* in AT/RT is significantly lower than in MB and normal brain tissue ($P = 0.0321$ and 0.0047 respectively, one-way ANOVA) (Fig. 1c).

IHC showed loss of NCAM1 expression in AT/RT

Hematoxylin and eosin staining (H&E) of TMA revealed AT/RT with various amounts of rhabdoid cells within undifferentiated small round blue cells. MB cores showed typical small round blue cell tumors composed of sheets of undifferentiated cells with minimal cytoplasm, hyperchromatic and anaplastic nuclei. High mitotic activity was observed in both AT/RT and MB cores. IHC for SMARCB1 showed loss of expression in tumor cells of all AT/RT samples with retention of expression in endothelial cells, fibroblasts and inflammatory cells within the tumors. NCAM1 expression was observed in cell membranes and extracellular space of MB and controls but not in AT/RT (Fig. 2a). It was observed that 67.3 and 10.5% of the samples were immunohistochemically negative for NCAM1 in the AT/RT and the MB set of tumors, respectively ($P < 0.01$, Fisher exact test) (Fig. 2b).

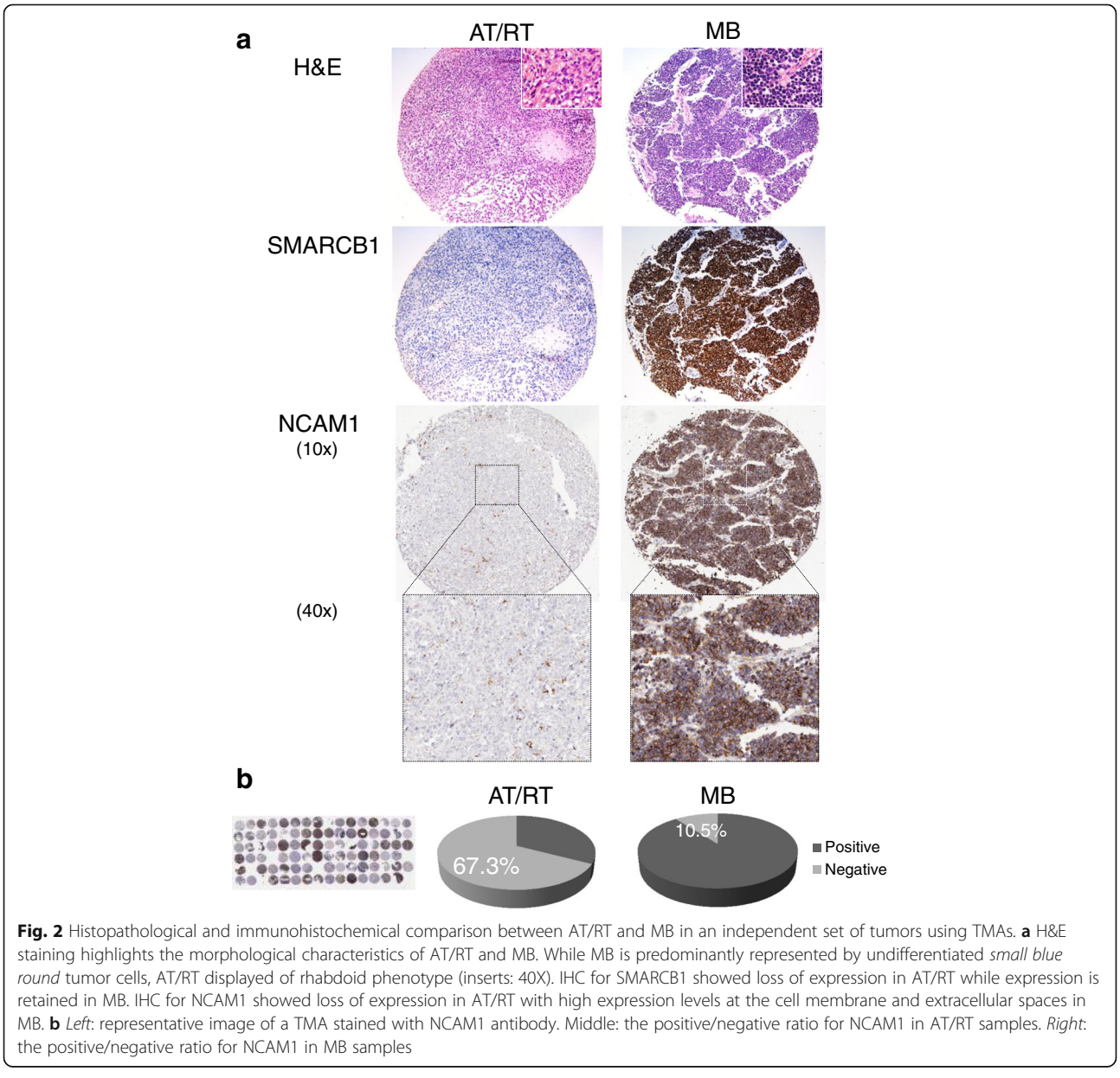

Fig. 2 Histopathological and immunohistochemical comparison between AT/RT and MB in an independent set of tumors using TMAs. **a** H&E staining highlights the morphological characteristics of AT/RT and MB. While MB is predominantly represented by undifferentiated *small blue round* tumor cells, AT/RT displayed of rhabdoid phenotype (inserts: 40X). IHC for SMARCB1 showed loss of expression in AT/RT while expression is retained in MB. IHC for NCAM1 showed loss of expression in AT/RT with high expression levels at the cell membrane and extracellular spaces in MB. **b** *Left*: representative image of a TMA stained with NCAM1 antibody. Middle: the positive/negative ratio for NCAM1 in AT/RT samples. *Right*: the positive/negative ratio for NCAM1 in MB samples

Discussion

Since the cell of origin of AT/RT is unknown [20], we chose to compare the GE profile of AT/RT with MB as both entities are malignant, embryonal brain tumors that arise in young children and display overlapping histological features. It is important to note however, that MB commonly responds to current available modalities of therapy while AT/RT does not. This fact is reflected in 5-year overall survival rates of 73.0% for MB and 2-year overall survival of 35.5% for AT/RT [21].

Here we report loss of NCAM1 expression in AT/RT. While we understand that the small number of samples included in this study represents a limitation on data interpretation, the downregulation of *NCAM1* detected by microarray GE profiling, was verified by qRT-PCR and validated at the protein level, by IHC in an independent set of tumor samples. This validation in multiple levels strengthens the significance of our data. Furthermore, our group previously reported *NCAM1* to be sharply downregulated in rhabdoid tumors from the kidney (RTK) [22], what also reassures the significance of our results.

NCAM1, also known as CD56 is part of the immunoglobulin superfamily and is expressed on the surface of neural cells [23] and certain cells of the immune system [24]. NCAM1 is classically known as a cell surface molecule and cell adhesion in neuronal cells. However it has many other known functions including exon guidance and repair, migration, apoptosis and cell proliferation [25, 26]. Its multiple functions depend mainly on alternative splicing, glycosylation/polysialylation status and pattern of expression at different developmental stages. Alternative splicing generate multiple isoforms of NCAM1 which play major roles in prognosis of malignancies [26]. NCAM1 glycosylation and polysialylation have also been associated with cancer [27]. Although the literature in the field is still controversial there is a tendency to correlate low NCAM1 expression to a poorer prognosis. As an example, NCAM1 immnostaining was found to be inversely correlated with the histological grade in gliomas [28]; its levels of expression were reported to be markedly reduced by the malignant transformation in thyroid follicular carcinoma and papillary carcinoma [29], and with worst prognosis in colon cancer [30].

Conclusion

Our data show that loss of NCAM1 expression might function as a potential new diagnostic marker for AT/RT, when considered in association with SMARCB1 (INI1) or SMARCA4 (BRG1) inactivation. Further studies are needed to explore the functional significance of NCAM1 loss of expression in the biology of AT/RT.

Abbreviations

AT/RT: Atypical teratoid/rhabdoid tumor; CNS: Central nervous system; EMA: Epithelial membrane antigen; GE: Gene expression; H&E: Hematoxylin and eosin; IHC: Immunohistochemistry; MB: Medulloblstoma; MRT: Malignant rhabdoid tuomor; NCAM1: Neural cell adhesion molecule 1; qRT-PCR: Quantitative real-time polymerase chain reaction; RTK: Rhabdoid tumor of the kidney; RTPS: Rhabdoid tumor predisposition syndrome; SMARCB1: SWI/SNF-related matrix-associated actin-dependent regulator of chromatin subfamily B member 1; SWI/SNF: Switch/sucrose non-fermentable; TMA: Tissue microarray

Acknowledgements

The authors would like to thank Carlos Ferreira Nascimento for building the TMA and José Ivanildo Neves as well as Marina França de Resende for performing the immunostains.

Funding

This study was supported by the Rally Foundation for Childhood Cancer Research in memory of Hailey Trainer and the Lurie Children's Hospital Faculty Practice Plan Development Fund.

Availability of data and materials

The datasets analyzed during the current study are available upon request.

Authors' contributions

MS: drafted the manuscript, carried out qRT-PCR experiments, participated in the analysis and interpretation of the GE and qRT-PCR data. KP: drafted the manuscript, revised the IHC slides and participated in the analysis of the GE data. CCH: Analyzed the GE data. FDC: Designed the TMAs and optimized the IHC reactions. AK: Participated in the data analysis and results' interpretation. FAS: Designed the TMAs and optimized the IHC reactions. TT: Participated in the data analysis and results' interpretation. STS: Participated in the design, concept, data acquisition and analysis, and results' interpretation. All authors read and approved the final manuscript.

Competing interests

The authors declare that they have no competing interests.

Consent for publication

Not applicable.

Ethics approval and consent to participate

This study was approved by the Institutional review board of Ann and Robert H. Lurie Children's Hospital of Chicago (IRB#2009-13778).

Publisher's Note

Author details

[1]Division of Pediatric Neurosurgery, Ann and Robert H. Lurie Children's Hospital of Chicago, 225 E. Chicago Ave., Chicago, IL 60611, USA. [2]Cancer Biology and Epigenomics, Stanley Manne Children's Research Institute, 2430 N Halsted St., Chicago, IL 60614, USA. [3]Department of Surgery, Northwestern University, Feinberg School of Medicine, 303 E Chicago Ave., Chicago, IL 60611, USA. [4]Department of Neurosurgery, Juntendo University School of Medicine, 2-1-1 Hongo, Bunkyo-ku, Tokyo 113-8421, Japan. [5]Joseph J. Zilber School of Public Health, University of Wisconsin, 1240 N 10th St., Milwaukee, WI 53205, USA. [6]Department of Pathology, A.C.Camargo Cancer Center, R. Taguá, 440 - Liberdade, São Paulo, SP 01508-010, Brazil.

Received: 29 December 2016 Accepted: 5 May 2017
Published online: 09 June 2017

References

1. Grupenmacher AT, Halpern AL, Bonaldo MD, Huang CC, Hamm CA, de Andrade A, et al. Study of the gene expression and microRNA expression profiles of malignant rhabdoid tumors originated in the brain (AT/RT) and in the kidney (RTK). Child Nerv Syst. 2013;29:1977–83.
2. Fruhwald MC, Biegel JA, Bourdeaut F, Roberts CW, Chi SN. Atypical teratoid/rhabdoid tumors-current concepts, advances in biology, and potential future therapies. Neuro-Oncology. 2016;18:764–78.
3. Buscariollo DL, Park HS, Roberts KB, Yu JB. Survival outcomes in atypical teratoid rhabdoid tumor for patients undergoing radiotherapy in a surveillance, epidemiology, and end results analysis. Cancer. 2012;118:4212–9.

4. Chi SN, Zimmerman MA, Yao X, Cohen KJ, Burger P, Biegel JA, et al. Intensive multimodality treatment for children with newly diagnosed CNS atypical teratoid rhabdoid tumor. J Clin Oncol. 2009;27:385–9.
5. Bartelheim K, Nemes K, Seeringer A, Kerl K, Buechner J, Boos J, et al. Improved 6-year overall survival in AT/RT - results of the registry study Rhabdoid 2007. Cancer Med. 2016;5:1765–75.
6. Chen YW, Wong TT, Ho DM, Huang PI, Chang KP, Shiau CY, Yen SH. Impact of radiotherapy for pediatric CNS atypical teratoid/rhabdoid tumor (single institute experience). Int J Radiat Oncol Biol Phys. 2006;64:1038–43.
7. von Hoff K, Hinkes B, Dannenmann-Stern E, von Bueren AO, Warmuth-Metz M, Soerensen N, et al. Frequency, risk-factors and survival of children with atypical teratoid rhabdoid tumors (AT/RT) of the CNS diagnosed between 1988 and 2004, and registered to the German HIT database. Pediatr Blood Cancer. 2011;57:978–85.
8. Kralik SF, Ho CY, Finke W, Buchsbaum JC, Haskins CP, Shih CS. Radiation necrosis in pediatric patients with brain tumors treated with proton radiotherapy. AJNR Am J Neuroradiol. 2015;36:1572–8.
9. Schneppenheim R, Frühwald MC, Gesk S, Hasselblatt M, Jeibmann A, Kordes U, Kreuz M, Leuschner I, Martin Subero JI, Obser T, Oyen F, Vater I, Siebert R. Germline nonsense mutation and somatic inactivation of SMARCA4/BRG1 in a family with rhabdoid tumor predisposition syndrome. Am J Hum Genet. 2010;86:279–84.
10. Kadoch C, Crabtree GR. Mammalian SWI/SNF chromatin remodeling complexes and cancer: mechanistic insights gained from human genomics. Sci Adv. 2015;1:e1500447.
11. Sredni ST, Tomita T. Rhabdoid tumor predisposition syndrome. Pediatr Dev Pathol. 2015;18:49–58.
12. Hollmann TJ, Hornick JL. INI1-deficient tumors: diagnostic features and molecular genetics. Am J Surg Pathol. 2011;35:e47–63.
13. Parwani AV, Stelow EB, Pambuccian SE, Burger PC, Ali SZ. Atypical teratoid/rhabdoid tumor of the brain: cytopathologic characteristics and differential diagnosis. Cancer. 2005;105:65–70.
14. Rorke LB, Packer RJ, Biegel JA. Central nervous system atypical teratoid/rhabdoid tumors of infancy and childhood: definition of an entity. J Neurosurg. 1996;85:56–65.
15. Oka H, Scheithauer BW. Clinicopathological characteristics of atypical teratoid/rhabdoid tumor. Neurol Med Chir (Tokyo). 1999;39:510–7.
16. Biegel JA. Molecular genetics of atypical teratoid/rhabdoid tumors. Neurosurg Focus. 2006;20:E11.
17. Massimino M, Biassoni V, Gandola L, Garre ML, Gatta G, Giangaspero F, et al. Childhood medulloblastoma. Crit Rev Oncol Hematol. 2016;105:35–51.
18. Coluccia D, Figuereido C, Isik S, Smith C, Rutka JT. Medulloblastoma: Tumor Biology and Relevance to Treatment and Prognosis Paradigm. Curr Neurol Neurosci Rep. 2016;16:43.
19. Barber RD, Harmer DW, Coleman RA, Clark BJ. GAPDH as a housekeeping gene: analysis of GAPDH mRNA expression in a panel of 72 human tissues. Physiol Genomics. 2005;21:389–95.
20. Okuno K, Ohta S, Kato H, Taga T, Sugita K, Takeuchi Y. Expression of neural stem cell markers in malignant rhabdoid tumor cell lines. Oncol Rep. 2010;23:485–92.
21. Ostrom QT, Gittleman H, Xu J, Kromer C, Wolinsky Y, Kruchko C, et al. CBTRUS statistical report: primary brain and other central nervous system tumors diagnosed in the United States in 2009-2013. Neuro-Oncology. 2016;18:v1–75.
22. Gadd S, Sredni ST, Huang CC, Perlman EJ, Renal Tumor Committee of the Children's Oncology G. Rhabdoid tumor: gene expression clues to pathogenesis and potential therapeutic targets. Lab Invest. 2010;90:724–38.
23. Ditlevsen DK, Povlsen GK, Berezin V, Bock E. NCAM-induced intracellular signaling revisited. J Neurosci Res. 2008;86:727–43.
24. Rujkijyanont P, Chan WK, Eldridge PW, Lockey T, Holladay M, Rooney B, et al. Ex vivo activation of CD56(+) immune cells that eradicate neuroblastoma. Cancer Res. 2013;73:2608–18.
25. Zhang Y, Yeh J, Richardson PM, Bo X. Cell adhesion molecules of the immunoglobulin superfamily in axonal regeneration and neural repair. Restor Neurol Neurosci. 2008;26:81–96.
26. Stefan Gattenlo¨ hner TSh, †. Specific detection of CD56 (NCAM) isoforms for the identification of agressive malignant neoplasms with progressive development. Am J Pathol. 2009;174:1160-71.
27. Guan F, Wang X, He F. Promotion of cell migration by Neural Cell Adhesion Molecule (NCAM) is enhanced by PSA in a polysialyltransferase-specific manner. Plos one. 2015;10:e0124237.
28. Todaro L, Christiansen S, Varela M, Campodonico P, Pallotta MG, Lastiri J, et al. Alteration of serum and tumoral neural cell adhesion molecule (NCAM) isoforms in patients with brain tumors. J Neuro-Oncol. 2007;83:135–44.
29. Scarpino S, Di Napoli A, Melotti F, Talerico C, Cancrini A, Ruco L. Papillary carcinoma of the thyroid: low expression of NCAM (CD56) is associated with downregulation of VEGF-D production by tumour cells. J Pathol. 2007;212:411–9.
30. Huerta S, Srivatsan ES, Venkatesan N, Peters J, Moatamed F, Renner S, et al. Alternative mRNA splicing in colon cancer causes loss of expression of neural cell adhesion molecule. Surgery. 2001;130:834–43.