Pathology & Oncology Research (2020) 26:1975–1981
https://doi.org/10.1007/s12253-019-00736-8

SHORT COMMUNICATION

Expression and Clinical Significance of Translation Regulatory Long Non-Coding RNA 1 (*TRERNA1*) in Ependymomas

Prit Benny Malgulwar[1] · Aruna Nambirajan[1] · Manmohan Singh[2] · Vaishali Suri[1] · Chitra Sarkar[1] · Mehar Chand Sharma[1]

Received: 15 January 2019 / Accepted: 27 August 2019 / Published online: 5 September 2019

Abstract

Long noncoding RNAs (lncRNA) have emerged as vital molecules governing epithelial-to-mesenchymal transition (EMT) in cancers. Translation regulatory RNA 1 (*TRERNA1*) is one such lncRNA known to enhance the transcriptional activity of the EMT-transcription factor, Snail. We have previously demonstrated differential upregulation of EMT-transcription factors and cadherin switching across various clinico-pathologic-molecular subclasses of ependymomas (EPN). With an aim to analyze the correlation between the expression of *TRERNA1* in EPNs, we performed gene expression analysis for *TRERNA1* on 75 Grade II/III EPNs and correlated with tumor site, *C11orf95-RELA* fusions, age, MIB-1 proliferative indices, and outcome wherever available. Upregulation of gene expression levels of *TRERNA1* was seen in intracranial EPNs, with highest expression levels in pediatric posterior fossa EPNs. High *TRERNA1* expression was found associated with higher proliferative indices ($p = 0.034$) and shorter progression free survival ($p = 0.002$). Our study, for the first time, demonstrates an association between *TRERNA1* expressions and pediatric posterior fossa EPNs. Further in-vivo and in-vitro studies are required to confirm these findings and evaluate *TRERNA1* as a novel biomarker and potential therapeutic target in childhood PF-EPNs.

Keywords Ependymoma · *TRERNA1* · lncRNA · Epithelial to mesenchymal transition

Introduction

Ependymomas (EPNs) are uncommon gliomas with poorly defined prognostic criteria [1–6]. Incomplete surgical resection, pediatric age and intracranial tumor locations have been accepted as poor prognostic factors [2, 3]. DNA methylation profiling has recently identified nine distinct molecular subgroups which have been shown to outperform routine histopathological grading in prognostication [7]. Of these groups, supratentorial (ST) Grade II/III EPNs harboring fusions of the *RELA* gene (ST-EPN-*RELA* subgroup) and posterior fossa (PF) Grade II/III EPNs with a CpG island methylator phenotype (PF-EPN-A subgroup), both of which predominate in the pediatric age group, are associated with dismal prognosis [7–9]. The poor clinical outcomes despite advances in neurosurgical techniques, ineffectiveness of chemotherapy, and the toxic effects of radiotherapy in children, have created an urgent need to identify newer predictive biomarkers with potential for therapeutic targeting in these two subgroups [10].

Epithelial to mesenchymal transition (EMT) is a transdifferentiation cellular process required for normal cellular homeostasis during development. In cancers, high expression of canonical regulators of EMT such as *SNAI1*/Snail is associated with tumor invasion and metastasis, augmented stemness and chemo-resistance, and increased proliferation [11–13]. *SNAI1* is a member of the Snail zinc-finger family and acts as a transcriptional repressor for E-cadherin and is one of the key regulators of cell adhesion, migration and EMT [13]. In a previous study, we identified and established the occurrence of an EMT-phenotype in EPNs and showed that EMT-transcription factors including Snail are most upregulated in the aggressive *RELA*-fusion positive ST EPNs and PF EPNs [14]. While a preliminary experiment yielded

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s12253-019-00736-8) contains supplementary material, which is available to authorized users.

✉ Mehar Chand Sharma
sharmamehar@yahoo.co.in

[1] Department of Pathology, All India Institute of Medical Sciences, Ansari Nagar, New Delhi 110029, India

[2] Department of Neurosurgery, All India Institute of Medical Sciences, New Delhi 110029, India

compelling evidence that Snail is a direct target of *C11orf95-RELA* fusion driven NF-KB pathway aberrant signaling in ST-EPN-*RELA* EPNs [15], the exact mechanisms by which Snail expression is upregulated in PF EPNs is not known.

Nearly 70–90% of RNA transcribed from genomic DNA do not code for proteins. Excluding the well characterized microRNAs, ribosomal-RNAs and transfer-RNAs, the majority of these non-coding RNA transcripts are longer than 200 nucleotides and are referred to as long non-coding RNAs (lncRNA) [16]. Originally recognized as key factors in physiological regulation of genomic imprinting and X-inactivation by means of epigenetic silencing [16–18], recent explorations into the mechanisms of action of lncRNAs have revealed versatile functions in regulating transcription, translation, and even, post-translational protein stability [18], They are increasingly being implicated as tumor suppressors/oncogenes in different malignancies, and significantly altered lncRNA profiles have been noted in gliomas as well [19, 20].

Mammalian genomes are occupied with thousands of enhancers that organize cell-type-specific gene expression programs [21]. Enhancer RNAs (eRNAs) are a newly identified subgroup of lncRNAs transcribed by RNA polymerase II from the domain of these transcription enhancers and are a major type of cis-regulatory elements in the genome [22]. *TRERNA1* (Translation Regulatory Long Non-Coding RNA 1) is one such lncRNA that has been found to regulate the expression of EMT master-transcription factor *SNAI1*/Snail [23]. Transcribed from chromosome 20q13.13 (coordinates 50,040,707- 50,041,629), *TRERNA1* appears to function as an enhancer RNA augmenting the transcriptional activity of the adjacent *SNAI1* gene (chromosome 20q13.13, coordinates 49,982,976-49,988,886) in a cis-dependent manner [17, 21].

The role of *TRERNA1* has not been studied previously in EPNs and its association with EMT phenotype in EPNs is unknown. In this study, we analyzed the expression level of *TRERNA1* in different clinico-pathological-molecular subgroups of EPNs and attempted to decipher its significance in the pathogenesis of EPNs.

Methods

Sample Collection The study was of retrospective design and ethically approved by the Institute Ethics Committee (Ref No: IESC/T-211/05/05/2015) and has been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments. Informed consent was obtained from all patients prior to their inclusion in the study. Fresh tumor samples from patients operated in the Department of Neurosurgery are collected routinely from the operation theatre at the time of surgery. Portions of resected tumors are snap-frozen in liquid nitrogen and stored at −80 °C until use, and the remaining tissue is fixed in 10% buffered neutral formalin and paraffin-embedded for routine histopathology and immunohistochemistry. Cases diagnosed as EPNs between 2003 and 2016 were retrieved from the archives and corresponding Hematoxylin and Eosin (H&E) stained slides were reviewed for reconfirmation of diagnosis by two neuropathologists (AN and MCS) according to the 2016 World Health Organization (WHO) classification of CNS tumors [24]. WHO Grade I subependymomas and myxopapillary ependymomas were excluded from analysis. Scrapings of ependymal lining of lateral ventricles obtained from autopsies conducted in the Department of Forensic Medicine and normal brain tissues from epilepsy surgery performed in Department of Neurosurgery were used as normal controls in gene expression analysis.

RNA Isolation and cDNA Conversion Total RNA was isolated using mirVana™ miRNA Isolation Kit(M/S Ambion, Life Technologies, USA) as per manufacturer's protocol. One μg of total RNA was reverse transcribed using Superscript VILO cDNA Synthesis Kit (M/S Invitrogen, Life Technologies, USA).

Gene Expression Analysis for *TRERNA1* Quantitative Realtime PCR (qPCR) was performed using Syber-green with Agilent Mx3005P system (Agilent Technologies, USA). The differences in expression between patients and controls (delta Ct) were calculated using the comparative method and the level of *TRERNA1* fold change was calculated using $2-\Delta\Delta Ct$ method, using *ACTB* and *GAPDH* as housekeeping genes. The primer sequences for the transcripts analyzed are provided in Supplementary Table-1.

Immunohistochemistry for MIB1 Immunohistochemical studies were performed on 4-μ-thick formalin-fixed, paraffin-embedded tumor sections using antibodies directed against MIB-1 (Dako, Denmark; 1:200). Labeled streptavidin biotin kit (Universal) was used as a detection system (Dako, Denmark). MIB-1 labeling index (L1) was calculated as percentage after counting 1000 tumor cell nuclei in different hot spots. Labeling index of more than 10% tumor cell nuclei was considered high [2].

Detection of C11orf95-RELA Fusions in Supratentorial EPNs qRT-PCR for the two most common C11orf95-RELA fusion transcripts were performed as previously described [9].

Clinico-Pathologic-Molecular Subgrouping of EPNs Molecular subgrouping of EPNs was attempted based on the classification proposed by Pajtler KW, et al. [7]. Due to non-availability of methylation profiling, we attempted to classify EPNs into proxy molecular subgroups. Supratentorial grade II/III EPNs with *C11orf95-RELA* fusions were classified as 'ST-RELA+', while the cases without fusion were grouped as 'ST-RELA-'.

Pediatric posterior fossa EPNs (≤18 years at diagnosis) were categorized as PF-A while adult posterior fossa EPNs (>18 years at diagnosis) were categorized as 'PF-B'. Spinal Grade II/III EPNs were grouped as 'SP'.

Survival and Statistical Analysis

Graph Pad Prism version 5.0 for Windows and SPSS version 11.5 for Windows was used for statistical, box plot and Kaplan–Meier curve analysis. *P*-values less than 0.05 were considered significant. For representation of *p* value in graphs, following symbols were used: p = <0.05–0.01-*, *p* = 0.01–0.001-**, p = <0.001-***.

Results

A total of 75 cases of Grade II and III EPNs were included in the study. The clinicopathological features are summarized in Table 1. Overall median age at diagnosis was 11.5 years (range 1–62 years) with male:female ratio of 2.2:1. The tumors were located in the supratentorium (48%), posterior fossa (41.3%) and spinal cord (10.6%). Type 1 and 2 *C11orf95-RELA* fusion transcripts were identified in 72.2% of supratentorial EPNs (ST-*RELA*+). This subgroup showed a preponderance of pediatric (80%) age group, male gender (68%) and grade III (76%) histology. Survival data was available for 62.2% (47/75) of the patients with median follow-up duration of 17 months (range 1–72 months).

Gene Expression Analysis for *TRERNA1*

The gene expression of *TRERNA1* was significantly upregulated in intracranial subgroups of EPN, i.e. ST-*RELA*+, ST-*RELA*-, PF-A and PF-B as compared to normal brain ($p < 0.05$) (Fig. 1a), while there was no significant change in *TRERNA1* expression in SP subgroup. The highest median fold change of *TRERNA1* expression was observed in PF-A subgroup and this was significantly higher than all other subgroups i.e. ST-*RELA*+ ($p = 0.011$), ST-*RELA*- ($p = 0.005$), PF-B (p = <0.001) and SP (p = <0.001).

Correlation of *TRERNA1* with Proliferation Marker (MIB1)

High expression levels of *TRERNA1*was found to be associated with high MIB-1 labelling index >10 ($p = 0.034$) (Fig. 2a).

Survival Analysis

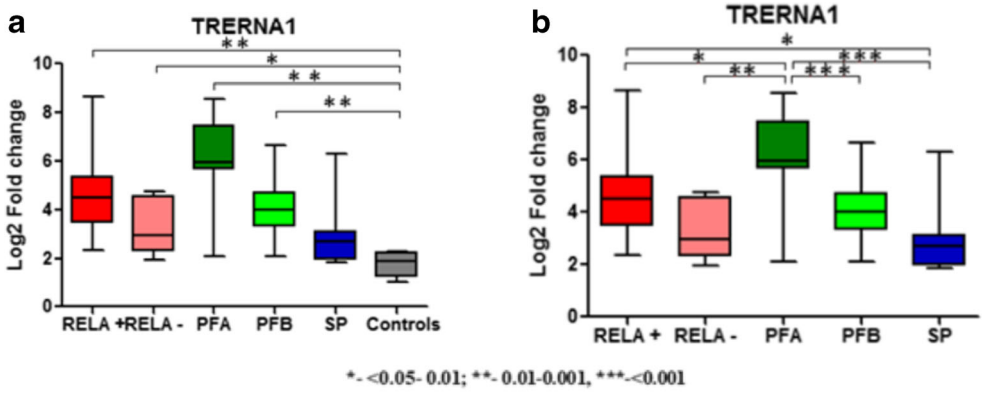
Intracranial subgroups showed significantly shorter progression free survival as compared to spinal EPNs ($p = 0.031$) (Fig. 2b). For survival analysis of *TRERNA1* in EPNs, the median was used as a cut-off value to divide the samples in two groups: high expression (with expression more than the median cut-off) and low expression (with expression less than the median cut-off). The group with high expression of *TRERNA1* in EPNs was found to be linked with shorter progression free survival on univariate analysis ($p = 0.002$) (Fig. 2c). A multivariate analysis could not be performed due to small number of events.

Discussion

Many previous studies including ours [14], have reported enrichment of EMT related signaling pathways such as Notch [25], Interleukin-6/STAT3 [26], PI3K-AKT [27], NF-kB [26, 28] and pathways related to extracellular matrix, hypoxia and chemotaxis [8] in pediatric posterior fossa ependymomas;

Table 1 Clinicopathological details of selected cases

Variable	Supratentorial (*n* = 36)		Infratentorial (*n* = 31)		Spinal (*n* = 8)
	ST-RELA+	ST-RELA -	PF-A	PF-B	SP
Total number of cases	*n* = 26	*n* = 10	*n* = 19	*n* = 12	n = 8
Age					
Median (Range)	10 (1–45)	14.5(2–59)	9(2–18)	34 (21–62)	22.5 (9–55)
Pediatrics (<18 years)	22 (80%)	7 (70%)	19 (100%)	0	3 (37.5%)
Adults (>18 years)	4 (20%)	3 (30%)	0	12 (100%)	5 (62.5%)
Gender					
Male	18 (68%)	5 (50%)	15 (78.9%)	6 (50%)	7 (87.5%)
Female	8 (32%)	5 (50%)	4 (21%)	6 (50%)	1 (12.5%)
Grade					
Grade II	5 (24%)	3 (30%)	5 (26.3%)	7 (58.3%)	6 (75%)
Grade III	21 (76%)	7 (70%)	14 (73.6%)	5 (41.6%)	2 (25%)



c

TRERNA1	Controls	ST-RELA+	ST-RELA-	PF-A	PF-B	SP
Controls		0.001	0.024	0.003	0.009	0.072
ST-RELA+		-	0.269	0.011	0.192	0.012
ST-RELA-		-	-	0.005	0.980	0.280
PF-A		-	-	-	<0.001	<0.001
PF-B		-	-	-	-	0.106
SP		-	-	-	-	-

Fig. 1 Box plot for gene expression analysis of *TRERNA1* using qRT-PCR data in relation to molecular subgroups: Realtime Quantitative PCR analysis for *TRERNA1* expression as compared to (**a**) controls and (**b**) molecular subgroups with individual *p* values depicted in Table (**c**) . For representation of p value in graphs, following symbols were used: p = <0.05–0.01-*, *p* = 0.01–0.001-**, p = <0.001-***

however the mechanisms of upregulation have remained largely unexplored. In the present study, we assessed the expression of *TRERNA1,* across different clinico-pathologic-molecular subgroups of EPN. By gene expression analysis, we found upregulated expression of *TRERNA1* in all EPNs with maximal overexpression in pediatric PF EPNs. *TRERNA1* expression significantly correlated with high proliferative indices and a shorter progression free survival on univariate analysis. Our data suggests a significant role for *TRERNA1* in pediatric posterior fossa EPNs and possibly playing a role in dysregulation of Snail expression and resultant augmentation of the EMT phenotype.

The role of many lncRNAs in augmenting [20, 29–35] or reversing EMT-phenotype [35, 36] has been well studied in various solid epithelial malignancies [18, 29–35], gliomas [20] and melanomas [30]. In these studies, expression levels of lncRNAs correlated with higher clinical stage, distant metastases or tumor aggressiveness [30–32], and in many of them, functional lncRNA knockdown assays showed significant alteration in the proliferation, invasiveness and migratory capabilities of tumor cells in-vitro [20, 29–33] and in-vivo [30–33]. Altered protein expression levels of EMT-related factors such as E-cadherin, Vimentin, and N-cadherin [29–31, 35], and/or dysregulation of EMT-related pathways

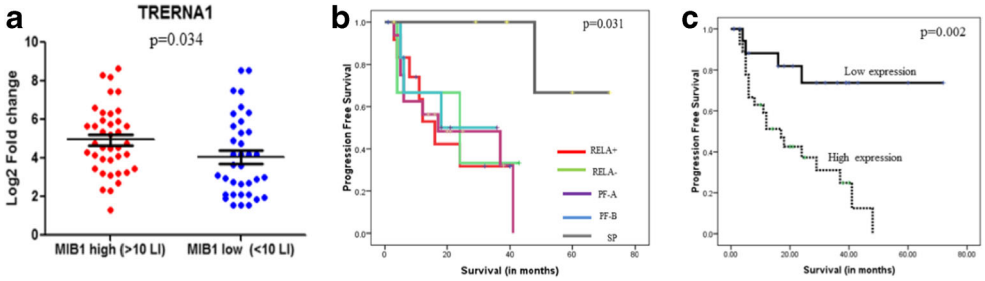


Fig. 2 Association of *TRERNA1* expression with proliferation marker (MIB1) and survival: Scatter plots showing upregulation of *TRERNA1* expression in high MIB1 LI (>10) as compared to low MIB1 LI (<10) (**a**), Univariate progression free survival analysis for molecular subgroups of ependymomas (**b**) and *TRERNA1* expression (**c**)

including JAK-STAT3 [29], EZH2-Notch1 [33], TGF-Beta [36], WNT/Beta-catenin [36] and NF-KB [36] were also demonstrated in some studies, strengthening the evidence for the role of lncRNAs in EMT. However, knowledge of the exact mechanisms by which these lncRNAs mediate changes in EMT phenotype is limited [18]. Some lncRNAs appear to directly interact with and silence specific miRNAs with antagonistic functions [35]. HOTAIR and MEG8 appear to bind with EZH2 and recruit PRC2 to anti-EMT genes, including those encoding miRNAs, leading to H3K27 tri-methylation and gene silencing [31].

A role for *TRERNA1* in cancer has been previously documented in breast cancer [37], colorectal cancer [37], gastric cancer [23] and chronic lymphocytic leukemia [38], with ours being the first study documenting a possible pathogenic role in ependymomas. In an analysis of 12 paired primary and metastatic tumors of breast carcinomas, Gumireddy et al. found markedly higher *TRERNA1* levels in the metastatic tumor samples. They found that enforced expression of *TRERNA1* in non-invasive and non-metastatic breast cancer cell lines increased cell migration and invasion through matrigel, while knockdown suppressed the same. They also demonstrated that silencing of *TRERNA1* using siRNA reduced the incidence of lung metastases in mice transplanted with breast cancer cells [37]. Similar results were obtained by Wu et al. in their study of 48 gastric carcinoma samples and cell lines [23]. Originally discovered by Orom et al. as an enhancer RNA for the adjacent *SNAI1* gene wherein silencing of *TRERNA1* (ncRNA-a7) resulted in specific reduction in Snail levels, reduction in cell migration in vitro, and upregulation of snail target genes in A549 lung cancer cell lines, *TRERNA1* was one of the first lncRNAs discovered to augment transcription of adjacent protein coding genes in a cis-dependant manner, likely by virtue of sequence or structural homology [17]. Further, Wu et al. also demonstrated in gastric cancer cell lines that *TRERNA1* can recruit EZH2 and directly cause epigenetic silencing of *CDH1* (E-cadherin) gene promoter by PRC2 mediated histone H3K27 trimethylation [23]. On the other hand, Gumireddy et al. found that alteration of *TRERNA1* expression levels did not have any effect on Snail mRNA levels in breast cancer cell lines, but rather enhanced EMT–phenotype by effecting polysomal redistribution of E-cadherin mRNA, reducing the translation efficiency and decreased E-cadherin protein levels [37]. Our study reports overexpression of *TRERNA1* levels in ependymomas, and, further opens window for in-vitro and in-vivo studies.

In the present study, we also observed an association of *TRERNA1* with higher proliferative indices and poor prognostic outcome on univariate analysis. While activation of Snail-mediated EMT can lead to increased proliferation and poor outcome by itself, *TRERNA1* may be capable of effects independent of Snail. In breast and gastric cancer cell lines, *TRERNA1* has been found to be present in the nuclear and cytoplasmic compartments suggesting that it may utilize different mechanisms of gene regulation targeting transcription and translation within the same tumor cells [37]. Interestingly, overexpression of *TRERNA1* has also been implicated in chemo-resistance in a recent study where Miller et al. in their analysis of over 30,000 lncRNAs in 144 patients with chronic lymphocytic leukemia, identified that *TRERNA1* expressing CLL cell lines showed less evidence of DNA damage when exposed to fludarabine as compared to those lacking *TRERNA1*, thus, leading to increased chemo-résistance and shorter progression free survival in patients receiving chemotherapy [38]. However, this study does not delve into the exact mechanisms by which *TRERNA1* executes this effect.

Conclusion

Despite the rapidly expanding knowledge on lncRNA biology, the underlying mechanisms by which lncRNA contributes to carcinogenesis and progression of cancer are still not clear. While some light has been shed on the roles of *TRERNA1* in transcription and translation processes [15, 21, 35], the target genes/mRNAs may be different in EPNs. In this context, the present study requires further extensive functional in-vivo experiments to confirm the interaction between *TRERNA1* and Snail. This would help to confirm the prognostic relevance and assess the implications of therapeutic targeting of either of these molecules.

Acknowledgements The authors acknowledge all consultants from the Departments of Pathology and Neurosurgery, AIIMS; all technical staff from the Neuropathology laboratory, AIIMS and Department of Biostatistics, AIIMS.

Funding Information The authors are thankful to Neuro Sciences Centre, AIIMS, AIIMS Intramural Research grants, and Science and Engineering Research Board (SERB) (EMR/2016/003365) for financial support of the work. Prit Benny Malgulwar is a Senior Research Fellowship awardee (No.3/2/3/284/2014/NCD-III) from Indian Council of Medical Research (ICMR), India. Aruna Nambirajan is a Senior Research Associate (8948A/17) under the Scientists' Pool Scheme of Council of Scientific and Industrial Research (CSIR), India.

Compliance with Ethical Standards

Conflict of Interest None

References

1. Kilday JP, Rahman R, Dyer S, Ridley L, Lowe J, Coyle B, Grundy R (2009) Pediatric ependymoma: biological perspectives. Mol Cancer Res 7(6):765–786
2. Ramaswamy V, Hielscher T, Mack SC, Lassaletta A, Lin T, Pajtler KW, Jones DTW, Luu B, Cavalli FMG, Aldape K, Remke M, Mynarek M, Rutkowski S, Gururangan S, McLendon RE, Lipp

ES, Dunham C, Hukin J, Eisenstat DD, Fulton D, van Landeghem FKH, Santi M, van Veelen MLC, van Meir EG, Osuka S, Fan X, Muraszko KM, Tirapelli DPC, Oba-Shinjo SM, Marie SKN, Carlotti CG, Lee JY, Rao AAN, Giannini C, Faria CC, Nunes S, Mora J, Hamilton RL, Hauser P, Jabado N, Petrecca K, Jung S, Massimi L, Zollo M, Cinalli G, Bognár L, Klekner A, Hortobágyi T, Leary S, Ermoian RP, Olson JM, Leonard JR, Gardner C, Grajkowska WA, Chambless LB, Cain J, Eberhart CG, Ahsan S, Massimino M, Giangaspero F, Buttarelli FR, Packer RJ, Emery L, Yong WH, Soto H, Liau LM, Everson R, Grossbach A, Shalaby T, Grotzer M, Karajannis MA, Zagzag D, Wheeler H, von Hoff K, Alonso MM, Tuñon T, Schüller U, Zitterbart K, Sterba J, Chan JA, Guzman M, Elbabaa SK, Colman H, Dhall G, Fisher PG, Fouladi M, Gajjar A, Goldman S, Hwang E, Kool M, Ladha H, Vera-Bolanos E, Wani K, Lieberman F, Mikkelsen T, Omuro AM, Pollack IF, Prados M, Robins HI, Soffietti R, Wu J, Metellus P, Tabori U, Bartels U, Bouffet E, Hawkins CE, Rutka JT, Dirks P, Pfister SM, Merchant TE, Gilbert MR, Armstrong TS, Korshunov A, Ellison DW, Taylor MD (2016) Therapeutic impact of Cytoreductive surgery and irradiation of posterior Fossa Ependymoma in the molecular era: a retrospective multicohort analysis. J Clin Oncol 34:2468–2477

3. Zamecnik J, Snuderl M, Eckschlager T, Chanova M, Hladikova M, Tichy M, Kodet R (2003) Paediatric intracranial ependymomas: prognostic relevance of histological, immunohistochemical, and flow cytometric factors. Mod Pathol 16:980–991
4. Gupta RK, Sharma MC, Suri V, Kakkar A, Singh M, Sarkar C (2014) Study of chromosome 9q gain, notch pathway regulators and Tenascin-C in ependymomas. J Neuro-Oncol 116:267–274
5. Nambirajan A, Sharma MC, Gupta RK, Suri V, Singh M, Sarkar C (2014) Study of stem cell marker nestin and its correlation with vascular endothelial growth factor and microvascular density in ependymomas. Neuropathol Appl Neurobiol 40:714–725
6. Rajeshwari M, Sharma MC, Kakkar A, Nambirajan A, Suri V, Sarkar C, Singh M, Saran RK, Gupta RK (2016) Evaluation of chromosome 1q gain in intracranial ependymomas. J Neuro-Oncol 127:271–278
7. Pajtler KW, Witt H, Sill M, Jones DT, Hovestadt V, Kratochwil F, Wani K, Tatevossian R, Punchihewa C, Johann P, Reimand J, Warnatz HJ, Ryzhova M, Mack S, Ramaswamy V, Capper D, Schweizer L, Sieber L, Wittmann A, Huang Z, van Sluis P, Volckmann R, Koster J, Versteeg R, Fults D, Toledano H, Avigad S, Hoffman LM, Donson AM, Foreman N, Hewer E, Zitterbart K, Gilbert M, Armstrong TS, Gupta N, Allen JC, Karajannis MA, Zagzag D, Hasselblatt M, Kulozik AE, Witt O, Collins VP, von Hoff K, Rutkowski S, Pietsch T, Bader G, Yaspo ML, von Deimling A, Lichter P, Taylor MD, Gilbertson R, Ellison DW, Aldape K, Korshunov A, Kool M, Pfister SM (2015) Molecular classification of ependymal tumors across all CNS compartments, histopathological grades, and age groups. Cancer Cell 27:728–743
8. Mack SC, Witt H, Piro RM, Gu L, Zuyderduyn S, Stütz AM, Wang X, Gallo M, Garzia L, Zayne K, Zhang X, Ramaswamy V, Jäger N, Jones DTW, Sill M, Pugh TJ, Ryzhova M, Wani KM, Shih DJH, Head R, Remke M, Bailey SD, Zichner T, Faria CC, Barszczyk M, Stark S, Seker-Cin H, Hutter S, Johann P, Bender S, Hovestadt V, Tzaridis T, Dubuc AM, Northcott PA, Peacock J, Bertrand KC, Agnihotri S, Cavalli FMG, Clarke I, Nethery-Brokx K, Creasy CL, Verma SK, Koster J, Wu X, Yao Y, Milde T, Sin-Chan P, Zuccaro J, Lau L, Pereira S, Castelo-Branco P, Hirst M, Marra MA, Roberts SS, Fults D, Massimi L, Cho YJ, van Meter T, Grajkowska W, Lach B, Kulozik AE, von Deimling A, Witt O, Scherer SW, Fan X, Muraszko KM, Kool M, Pomeroy SL, Gupta N, Phillips J, Huang A, Tabori U, Hawkins C, Malkin D, Kongkham PN, Weiss WA, Jabado N, Rutka JT, Bouffet E, Korbel JO, Lupien M, Aldape KD, Bader GD, Eils R, Lichter P, Dirks PB, Pfister SM, Korshunov A, Taylor MD (2014) Epigenomic alterations define lethal CIMP-positive ependymomas of infancy. Nature 506:445–450
9. Malgulwar PB, Nambirajan A, Pathak P, Faruq M, Rajeshwari M, Singh M, Suri V, Sarkar C, Sharma MC (2018) C11orf95-RELA fusions and upregulated NF-KB signaling characterise a subset of aggressive supratentorial ependymomas that express L1CAMand nestin. J Neuro-Oncol 138:29–39
10. Pajtler KW, Mack SC, Ramaswamy V, Smith CA, Witt H, Smith A, Hansford JR, von Hoff K, Wright KD, Hwang E, Frappaz D, Kanemura Y, Massimino M, Faure-Conter C, Modena P, Tabori U, Warren KE, Holland EC, Ichimura K, Giangaspero F, Castel D, von Deimling A, Kool M, Dirks PB, Grundy RG, Foreman NK, Gajjar A, Korshunov A, Finlay J, Gilbertson RJ, Ellison DW, Aldape KD, Merchant TE, Bouffet E, Pfister SM, Taylor MD (2017) The current consensus on the clinical management of intracranial ependymoma and its distinct molecular variants. Acta Neuropathol 133:5–12
11. Malgulwar PB, Sharma V, Tomar AS, Verma C, Nambirajan A, Singh M, Suri V, Sarkar C, Sharma MC (2018) Transcriptional co-expression regulatory network analysis for snail and slug identifies IL1R1, an inflammatory cytokine receptor, to be preferentially expressed in ST-EPN-RELA and PF-EPN-A molecular subgroups of intracranial ependymomas. Oncotarget 9:35480–35492
12. Thiery JP, Acloque H, Huang RY et al (2009) Epithelial-mesenchymal transitions in development and disease. Cell 139: 871–890
13. Kahlert UD, Nikkhah G, Maciaczyk J (2013) Epithelial-to-mesenchymal(−like) transition as a relevant molecular event in malignant gliomas. Cancer Lett 331:131–138
14. Malgulwar PB, Nambirajan A, Pathak P, Rajeshwari M, Suri V, Sarkar C, Singh M, Sharma MC (2018) Epithelial-to-mesenchymal transition related transcription factors areupregulated in ependymomas and correlate with a poor prognosis. Hum Pathol 82:149–157
15. Malgulwar PB, Pathak P, Desiraju BK, Nambirajan A, Sarkar C, Suri V, Singh M, Sharma MC (2016) EPN-13 Epithelial-to-mesenchymal transitions in childhood ependymomas mechanistically links oncogenic C11orf95-RELA fusion driven activation of SNAI1/Snail. Neuro-Oncology 18(Suppl 3):iii33. https://doi.org/10.1093/neuonc/now070.12
16. Mattick JS (2009) The genetic signatures of noncoding RNAs. PLoS Genet 5:e1000459
17. Ørom UA, Derrien T, Beringer M, Gumireddy K, Gardini A, Bussotti G, Lai F, Zytnicki M, Notredame C, Huang Q, Guigo R, Shiekhattar R (2010) Long noncoding RNAs with enhancer-like function in human cells. Cell 143:46–58
18. Lin X, Qiu J, Hua K (2018) Long non-coding RNAs as emerging regulators of epithelial to mesenchymal transition in gynecologic cancers. Biosci Trends 12:342–353
19. Zhang XQ, Leung GK (2014) Long non-coding RNAs in glioma: functional roles and clinical perspectives. Neurochem Int 77:78–85
20. Zeng J, Du T, Song Y, Gao Y, Li F, Wu R, Chen Y, Li W, Zhou H, Yang Y, Pei Z (2017) Knockdown of long noncoding RNA CCAT2 inhibits cellular proliferation, invasion, and epithelial-mesenchymal transition in glioma cells. Oncol Res 25:913–921
21. Rothschild G, Basu U (2017) Lingering questions about enhancer RNA and enhancer transcription-coupled genomic instability. Trends Genet 33:143–154
22. Liu F (2017) Enhancer-derived RNA: a primer. Genomics Proteomics Bioinformatics 15:196–200
23. Wu H, Hu Y, Liu X, Song W, Gong P, Zhang K, Chen Z, Zhou M, Shen X, Qian Y, Fan H (2017) LncRNA TRERNA1 function as an enhancer of SNAI1 promotes gastric Cancer metastasis by regulating epithelial-mesenchymal transition. Mol Ther Nucleic Acids 8: 291–299

24. Ellison DW, McLendon R, Wiestler OD et al (2016) Ependymoma. In: Louis DN, Ohgaki H, Wiestler OD, Cavenee WK (eds) WHO classification of tumours of the central nervous system, revised, 4th edn. IARC, Lyon, pp 106–112
25. Puget S, Grill J, Valent A, Bieche I, Dantas-Barbosa C, Kauffmann A, Dessen P, Lacroix L, Geoerger B, Job B, Dirven C, Varlet P, Peyre M, Dirks PB, Sainte-Rose C, Vassal G (2009) Candidate genes on chromosome 9q33-34 involved in the progression of childhood EPNEPNs. J Clin Oncol 27:1884–1892
26. Griesinger AM, Witt DA, Grob ST, Georgio Westover SR, Donson AM, Sanford B, Mulcahy Levy JM, Wong R, Moreira DC, DeSisto JA, Balakrishnan I, Hoffman LM, Handler MH, Jones KL, Vibhakar R, Venkataraman S, Foreman NK (2017) NF-κB upregulation through epigenetic silencing of LDOC1 drives tumor biology and specific immunophenotype in Group A EPN. Neuro-Oncology 19:1350–1360
27. Rogers HA, Mayne C, Chapman RJ, Kilday JP, Coyle B, Grundy RG (2013) PI3K pathway activation provides a novel therapeutic target for pediatric EPN and is an independent marker of progression-free survival. Clin Cancer Res 19:6450–6460
28. Parker M, Mohankumar KM, Punchihewa C, Weinlich R, Dalton JD, Li Y, Lee R, Tatevossian RG, Phoenix TN, Thiruvenkatam R, White E, Tang B, Orisme W, Gupta K, Rusch M, Chen X, Li Y, Nagahawhatte P, Hedlund E, Finkelstein D, Wu G, Shurtleff S, Easton J, Boggs K, Yergeau D, Vadodaria B, Mulder HL, Becksfort J, Gupta P, Huether R, Ma J, Song G, Gajjar A, Merchant T, Boop F, Smith AA, Ding L, Lu C, Ochoa K, Zhao D, Fulton RS, Fulton LL, Mardis ER, Wilson RK, Downing JR, Green DR, Zhang J, Ellison DW, Gilbertson RJ (2014) C11orf95-RELA fusions drive oncogenic NF-κB signalling in EPN. Nature 506:451–455
29. Xue J, Liao L, Yin F, Kuang H, Zhou X, Wang Y (2018) LncRNA AB073614 induces epithelial- mesenchymal transition of colorectal cancer cells via regulating the JAK/STAT3 pathway. Cancer Biomark 21:849–858
30. Shi G, Li H, Gao F, Tan Q (2018) lncRNA H19 predicts poor prognosis in patients with melanoma and regulates cell growth, invasion, migration and epithelial-mesenchymal transition in melanoma cells. Onco Targets Ther 11:3583–3595
31. Gupta RA, Shah N, Wang KC, Kim J, Horlings HM, Wong DJ, Tsai MC, Hung T, Argani P, Rinn JL, Wang Y, Brzoska P, Kong B, Li R, West RB, van de Vijver MJ, Sukumar S, Chang HY (2010) Long non-coding RNA HOTAIR reprograms chromatin state to promote cancer metastasis. Nature 464:1071–1076
32. Zheng P, Li H, Xu P et al (2017) High lncRNA HULC expression is associated with poor prognosis and promotes tumor progression by regulating epithelial-mesenchymal transition in prostate cancer. Arch Med Sci 14:679–686
33. Chen M, Xia Z, Chen C, Hu W, Yuan Y (2018) LncRNA MALAT1 promotes epithelial-to-mesenchymal transition of esophageal cancer through Ezh2-Notch1 signaling pathway. Anti-Cancer Drugs 29:767–773
34. Terashima M, Ishimura A, Wanna-Udom S, Suzuki T. MEG8 long noncoding RNA contributes to epigenetic progression of the epithelial-mesenchymal transition of lung and pancreatic cancer cells. J Biol Chem. 2018;293:18016-18030.
35. Liang WC, Ren JL, Wong CW, Chan SO, Waye MM, Fu WM, Zhang JF (2018) LncRNA-NEF antagonized epithelial to mesenchymal transition and cancer metastasis via cis-regulating FOXA2 and inactivating Wnt/β-catenin signaling. Oncogene 37:1445–1456
36. Wu W, Chen F, Cui X, Yang L, Chen J, Zhao J, Huang D, Liu J, Yang L, Zeng J, Zeng Z, Pan Y, Su F, Cai J, Ying Z, Zhao Q, Song E, Su S (2018) LncRNA NKILA suppresses TGF-β-induced epithelial-mesenchymal transition by blocking NF-κB signaling inbreast cancer. Int J Cancer 143:2213–2224
37. Gumireddy K, Li A, Yan J, Setoyama T, Johannes GJ, Orom UA, Tchou J, Liu Q, Zhang L, Speicher DW, Calin GA, Huang Q (2013) Identification of a long non-coding RNA-associated RNP complex regulating metastasis at the translational step. EMBO J 32:2672–2684
38. Miller CR, Ruppert AS, Fobare S et al (2017) The long noncoding RNA, treRNA, decreases DNA damage and is associated with poor response to chemotherapy in chronic lymphocytic leukemia. Oncotarget 8:25942–25954