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Distribution of *TERT* alternative splicing (AS) variants in pediatric brain tumors

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Summary. *Introduction:* The mechanism of telomerase regulation remains unclear, but has been suggested that *TERT* (telomerase reverse transcriptase) is regulated by alternative splicing (AS). Besides the full-length (FL) transcript, alternatively spliced variants have been described within the reverse transcriptase domain of *TERT* including, deletion alpha (α), beta deletion (β^-), and alpha beta deletions ($\alpha^-\beta^-$). Medulloblastoma (MB) and Ependymoma (EP) are two of more frequent brain tumors of childhood. We investigated and described the principal TERT transcripts; FL, α^-, β^- and $\alpha^-\beta^-$, and whether or not the presence of these patterns could be associated to clinical pathological characteristics and survival of pediatric EP e MB. *Methods:* We selected 58 MB and 43 EP samples. *TERT* AS variants were amplified by nested PCR (polymerase chain reaction) and the amplified products were electrophoresed on 2% agarose gel. *Results:* In general, around 5% of the samples of each group of tumors exhibited exclusively FL variant. *TERT* variants with deletion, exclusively or combined with others patterns, were detected in 70% of MB and 39% of EP tumors. 27% of MB and 60% EP did not show any of the patterns. We did not observed significant association between *TERT* splicing variants and clinical pathological characteristics of MB e EP tumors. *Discussion:* Since FL transcript is the only associated with reverse transcriptase activity, our results suggest that the association of *TERT* mRNA expression to clinical pathological characteristics of patients must be analyzed with caution. Further investigations will help to elucidate the complex mechanism involving AS of *TERT* gene and the function of deleted variants in tumorigenesis of pediatric brain tumors.

Key words: medulloblastoma, ependymoma, pediatric brain tumor, *TERT*, alternative splicing, therapeutic target

Introduction

Human telomerase is a ribonucleoprotein polymerase containing a protein catalytic subunit, the human telomerase reverse transcriptase (TERT), and an RNA component (TERC), that elongates telomeres by adding hexameric 5′-TTAGGG-3′ tandem repeats to the chromosomal ends (1, 2). The mechanism of regulation of telomerase remains unclear, but has been suggested that during development *TERT* is in part regulated by alternative splicing (AS) (3).

TERT gene on human chromosome 5p15.33 contains 16 exons can be spliced into multiple isoforms (3). To date, 22 isoforms of *TERT* have been identi-

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fied (4-6). Besides the full-length (FL) transcript with all 16 exons, none of the identified alternative spliced forms has reverse transcriptase activity and they cannot elongate telomeres (7, 8). The alternatively spliced variants within the reverse transcriptase domain of *TERT* include minus alpha (α^-), minus beta (β^-), or both minus alpha beta ($\alpha^-\beta^-$). These *TERT* splicing variants can lack reverse transcriptase function and their expression can modify telomerase activity levels (7-9). The inframe α^- deletion derived protein is a dominant negative inhibitor of telomerase activity, as would be expected if it forms heterodimers with the FL transcript-derived protein (8). The reading-frameshifting β^- deletion (182 bp) and $\alpha^-\beta^-$ deletion (218 bp) are believed to produce truncated proteins and may be subject to nonsense-mediated mRNA decay due to the premature stop codon (8, 10, 11).

Deletion in *TERT* variants are detected in a number of cancers and tumor cell lines and additionally during development, displaying expression patterns that reduce telomerase activity levels and may influence variations in telomere lengths (3-9). Several studies have been proposing *TERT* mRNA expression as an important prognostic factor with impact in the survival and clinical pathological characteristics of various neoplasias, including brain tumors. However, none of them identified the pattern of AS of *TERT* mRNA in pediatric brain tumors (12-21).

Between the pediatric brain tumors, Medulloblastoma (MB) is the most common embryonic neuroepithelial tumor of the cerebellum and added to other neuroectodermal tumors, accounts for 16-25% of cases. Approximately, one third of the cases remain incurable with negative impact in patients with higher long-term survival (12, 16). Of all primary tumors of the central nervous system in children, around 10% are Ependymoma (EP). This tumor arise from the ependymal lining of the ventricular system or the central canal of the spinal cord and its behavior is extremely variable, ranging from an aggressive course to prolonged survival with multiple relapses (16, 18). The clinical management of these tumors remains one of the more difficult in pediatric oncology (12, 16, 18).

Although several investigations of telomerase activity and/or expression in brain tumors of childhood have been made, to the best of our knowledge, this is the first study identifying the pattern of AS of *TERT* mRNA in pediatric brain tumors (12-27). Thus, we here aim to investigate and describe *TERT* transcripts, FL, α^-, β^- and $\alpha^-\beta^-$, and whether or not the presence of these isoforms could be associated to clinic pathological characteristics and survival of pediatric EP e MB.

Methodology

For the analysis of *TERT* transcripts, we selected a subgroup of 58 Medulloblastoma (MB) samples, and 43 Ependymoma (EP) samples. All samples used in this study were obtained from patients treated at the Pediatric Oncology Institute/Grupo de Apoio ao Adolescente e a Criança com Câncer - Federal University of São Paulo (IOP/GRAACC-UNIFESP). This was a retrospective study of samples collected sequentially between 2002 and 2013. Three cell lines (DAOY, SAOS, U2OS) were used as controls. Samples from each MB and EP were collected after informed consent was signed by patients/guardians according to the university's institutional review board (IRB/Federal University of São Paulo nº 333.158).

RT-PCR and nested PCR

TERT AS variants were amplified by nested PCR using primers designed according to GenBank, using Primer accession n°AF015950, based in previously published protocol (11, 28). The first round of amplification spanned a region that included all α^- and β^- deletion sites with forward primer 5'GCTGCTCAGGTCTTTCTTTTAT3' and reverse primer 5'GGAGGATCTTGTAGATGTTGGT3'. PCR was performed in 25 µL of reaction mixture using 1 µL of cDNA and 1U GoTaq polymerase (Promega, Madison, WI, USA) by incubation at 94°C for 2 minutes, followed by 25 amplification cycles of 94°C for 30 seconds, 54°C for 30 seconds, and 72°C for 90 seconds, and a final extension at 72°C for 5 minutes. This second round of PCR was carried out with 1 µL of the first-round PCR product, nested primer set and Taq. The nested primer set, forward 5'CCGCCTGAGCTGTACTTTGTC3' and reverse 5'CAGAGCAGCGTGGAGAGGAT3', produced four possible products, FL

(418 bp), α^- (382 bp), β^- (236 bp), and $\alpha^-\beta^-$ (200 bp). This round was performed by incubation at 94°C for 2 minutes, followed by 35 amplification cycles of 94°C for 20 seconds, 59°C for 20 seconds, and 72°C for 30 seconds, and a final extension at 72°C for 2 minutes. Amplified products were electrophoresed on 2% agarose gel, stained with Gel Red (Biotium, Hayward, CA, USA), for size products identification.

Statistical analyses

Data analysis was performed using GraphPad Prism software, version 5 (San Diego, CA). Overall survival was defined as the time from diagnosis until the date of either the last follow-up or death. For the event free survival analysis, the duration was defined as the time from diagnosis until the occurrence of metastasis or local relapse. Overall survival and event-free survival curves were generated by applying the Kaplan-Meier method, and were then compared by the log rank test. Categorical data (age at diagnosis, gender, histological subtype, risk, and status for MB; age at diagnosis, gender, histological subtype, morphological classification, surgery extension, treatment, tumor location, and status for EP) and *TERT* AS patterns were studied using chi-square or Fisher exact tests. For this, different associations between the categorical variables, clinical-pathological characteristics and presence of the transcripts of the *TERT* variants, were tested. Statistical significance was taken as $p<0.05$. To provide level of confidence, we calculated the effect size and statistical power of tests, using R Core Team (2016) (URL http://www.R-project.org/).

Results

We analyzed 43 EP tumor samples and 58 MB tumor samples. A summary of the clinical pathological characteristics is demonstrated in Table 1. The complete data of the patients included in this study is in Tables 2 and 3. Statistical analysis are summarized in the Tables 4 and 5.

TERT AS variant patterns in MB tumors

For the 58 MB samples, 28 (48%) were considered high risk group, and 27 (46%) were low risk group.

Table 1. Clinical pathological characteristics of MB and EP tumor samples.

Medulloblastoma (MB)			Ependymoma (EP)		
	N	%		N	%
Total number of samples	58	100	Total number of samples	43	100
Risk			Location		
HR	28	48	*PF*	28	65
LR	27	46	*ST*	10	23
Status			*IM*	5	12
Alive	26	45	Status		
Dead	31	53	*Alive*	21	49
Histology			*Dead*	21	49
classic	45	77	Histology		
desmoplasic	3	5	*GI and GII*	33	77
anaplasic/large cells	3	5	*GIII*	10	23
nodular	7	12			
TERT transcript patterns			*TERT* transcript patterns		
NE	16	28	*NE*	26	60
FL	3	5	*FL*	2	5
FL + $\alpha^-/\beta^-/\alpha^-\beta^-$	38	65	*FL* + $\alpha^-/\beta^-/\alpha^-\beta^-$	15	35
$\alpha^-/\beta^-/\alpha^-\beta^-$	7	12	$\alpha^-/\beta^-/\alpha^-\beta^-$	8	18

IM= Intramedular, PF= Posterior fossa, ST= Supratentorial, PR= Partial resection, TR= Total resection, LR= Low risk, HR= High risk, GI= grade I, GII= grade II, GIII= grade III, NE= No mRNA expression, α^-= variant with deletion minus alpha, β^-= variant with deletion minus beta, $\alpha^-\beta^-$= variant with deletion minus alpha both minus alpha beta

Table 2. Clinical pathological characteristics and *TERT* alternative splicing variant patterns data of MB patients.

Patient	Age at diagnosis (years)	Histology	Risk	Staging	Overall Survival	Status	FL/α^-/β^-/$\alpha^-\beta^-$
1	7,1	Classic	LR	R0M0	70,17	Dead	NE
2	13	Classic	HR	R+M0	12,73	Dead	FL/α^-/β^-
3	4	Desmoplasic	LR	R0M0	148,73	Alive	FL/α^-/β^-/$\alpha^-\beta^-$
4	11	Classic	HR	R+M2	9,40	Dead	NE
5	13	Classic	LR	R0M0	28,10	Dead	FL/α^-/β^-/$\alpha^-\beta^-$
6	2,7	NI	NI	NI	1,87	NI	NE
7	7	Classic	LR	R0M0	152,33	Alive	FL/α^-/β^-
8	1,6	Classic	HR	R+M+	3,53	Dead	FL/α^-/β^-/$\alpha^-\beta^-$
9	9	Classic	HR	R0M+	0,77	Dead	NE
10	6	Classic	LR	R0M0	151,80	Alive	FL/α^-/β^-
11	18	Classic	NI	NI	7,63	Dead	FL/α^-/β^-/$\alpha^-\beta^-$
12	7	Classic	HR	R+M+	57,87	Dead	FL/α^-/β^-/$\alpha^-\beta^-$
13	11	Classic	LR	R0M0	11,60	Dead	β^-
14	5	Classic	HR	R+M+	5,40	Dead	NE
15	13	Anaplasic/Large Cells	LR	R0M0	16,77	Dead	NE
16	3, 4	Classic	HR	R+M+	21,93	Dead	NE
17	15	Classic	LR	R0M0	14,43	Dead	FL/α^-/β^-
18	2,11	Classic	HR	R+M+	3,07	Dead	FL/α^-/β^-/$\alpha^-\beta^-$
19	16	Classic	LR	R0M0	64,90	Dead	β^-
20	1,6	Extensive nodularity	HR	R+M+	1,23	Dead	β^-
21	5	Extensive nodularity	HR	R0M+	123,47	Alive	NE
22	8	Anaplasic/Large Cells	LR	R0M0	11,17	Dead	FL/α^-/β^-
23	7	Large Cells	LR	R0M0	97,00	Dead	FL/α^-/β^-
24	15	Classic	LR	R0M0	6,57	Dead	FL
25	10	Classic	LR	R0M0	114,07	Alive	NE
26	6	Classic	LR	R0M0	113,10	Alive	FL/α^-/β^-/$\alpha^-\beta^-$
27	14	Classic	HR	R+M0	40,27	Alive	FL
28	5	Classic	LR	R0M0	37,53	Dead	FL/α^-/β^-/$\alpha^-\beta^-$
29	16	Classic	LR	R0M0	108,60	Alive	FL/α^-/β^-/$\alpha^-\beta^-$
30	13	Classic	LR	R0M0	2,80	Dead	FL/β^-
31	8	Classic	LR	R0M0	104,53	Alive	FL/α^-/β^-/$\alpha^-\beta^-$
32	9	Classic	LR	R0M0	102,03	Alive	FL/α^-/β^-/$\alpha^-\beta^-$
33	1	Mixed (classic/desmoplasic)	HR	R0M0	3,97	Dead	NE
34	5	Classic	HR	R+M0	95,83	Alive	FL/β^-
35	7	Classic	HR	R+M0	98,53	Alive	FL/α^-/β^-/$\alpha^-\beta^-$
36	7	Classic	LR	R0M0	93,83	Alive	FL
37	5	Classic	HR	R0M+	90,67	Alive	FL/α^-/β^-/$\alpha^-\beta^-$
38	9	Classic	HR	R+M0	90,13	Alive	FL/α^-/β^-/$\alpha^-\beta^-$
39	16	Classic	HR	R+M+	65,53	Dead	FL/α^-/β^-/$\alpha^-\beta^-$
40	6	Classic	LR	R0M0	3,63	Dead	FL/β^-/$\alpha^-\beta^-$
41	4	Classic	HR	R+M0	42,97	Dead	FL
42	2	Classic	HR	R0M+	37,80	Dead	FL/α^-/β^-
43	1	Extensive nodularity	HR	R+M0	5,13	Dead	FL/α^-/β^-
44	13	Classic	LR	R0M0	1370,43	Alive	NE
45	5	Classic	LR	R0M0	50,37	Alive	β^-
46	11	Classic	LR	R0M0	11,07	Dead	NE
47	0,3	Extensive nodularity	HR	R+M0	61,47	Alive	NE
48	5	Classic	LR	R0M0	45,60	Alive	NE
49	3,2	Desmoplasic	HR	R0M0	27,40	Dead	FL/α^-/β^-
50	3,3	Classic	HR	R0M0	37,67	Alive	α^-/β^-/$\alpha^-\beta^-$
51	9	Classic	LR	R0M0	37,20	Alive	FL/α^-/β^-
52	4,5	Classic	LR	R0M0	35,60	Alive	NE
53	1,9	Extensive nodularity	HR	R0M0	30,60	Alive	NE
54	0,11	Extensive nodularity	HR	R0M0	32,00	Alive	FL/β^-
55	8,7	Classic	LR	R0M0	32,73	Alive	β^-
56	2,1	Extensive nodularity	HR	R+M0	11,07	Dead	FL/α^-/β^-

(continued)

Table 2 *(continued)*. Clinical pathological characteristics and *TERT* alternative splicing variant patterns data of MB patients.

Patient	Age at diagnosis (years)	Histology	Risk	Staging	Overall Survival	Status	FL/α^-/β^-/$\alpha^-\beta^-$
57	3,4	Classic	HR	R+M+	13,70	Alive	α^-/β^-/$\alpha^-\beta^-$
58	0,9	Classic	HR	R0M+	4,53	Dead	FL/α^-/β^-/$\alpha^-\beta^-$

LR= Low risk, HR= High risk, R0M0= no residual disease and no metastasis, R+M0= radiological residual disease alone, R0M+= presence of metastasis, R+M+= presence of residual disease and metastasis, NE= No mRNA expression, FL= Full Length, α^-= variant with deletion minus alpha, β^-= variant with deletion minus beta, $\alpha^-\beta^-$= variant with deletion minus alpha both minus alpha beta

Table 3: Clinical pathological characteristics and *TERT* alternative splicing variant patterns data of EP patients.

Patient	Gender	Age at diagnosis (years)	Diagnosis	Classification	Surgical extension	Recidive	Status	Overall Survival (months)	FL/α^-/β^-/$\alpha^-\beta^-$
1	F	1,4	IM	GII	PR	Yes	Dead	52,97	FL/β^-
2	F	1,9	PF	GII	TR	Yes	NI	107,90	β^-
3	M	17,2	PF	GII	PR	Yes	Dead	20,93	NE
4	M	2,7	PF	GII	TR	Yes	Dead	17,87	NE
5	F	8,8	ST	GII	TR	No	Alive	153,67	FL/α^-/β^-
6	M	0,8	ST	GII	NI	NI	Dead	0,47	FL/α^-/β^-/$\alpha^-\beta^-$
7	F	0,7	ST	GII	TR	No	Dead	12,30	NE
8	F	5,8	PF	GII	PR	Yes	NI	NI	β^-
9	M	5,1	PF	GII	TR	No	Alive	132,23	β^-
10	M	15,6	PF	GII	TR	No	Dead	124,67	FL
11	M	4,1	ST	GII	PR	Yes	Dead	31,57	NE
12	M	1,2	PF	GII	PR	Yes	Dead	94,83	NE
13	M	14,1	IM	GII	PR	No	Dead	114,70	NE
14	F	12,5	ST	GIII	TR	Yes	Dead	75,13	β^-
15	M	3,4	PF	GII	PR	Yes	Dead	18,03	β^-
16	M	16,1	ST	GIII	TR	Yes	Dead	70,80	NE
17	F	5,3	PF	GIII	TR	No	Alive	51,77	NE
18	F	9,8	PF	GII	TR	Yes	Dead	57,80	NE
19	M	0,10	PF	GIII	PR	Yes	Dead	98,23	FL/β^-
20	M	15,7	IM	GI	PR	No	Alive	100,73	NE
21	F	1,4	ST	GII	PR	No	Alive	90,47	NE
22	M	6,6	PF	GIII	PR	Yes	Dead	19,90	NE
23	M	NI	PF	GIII	NI	Yes	Alive	81,13	β^-
24	M	1,8	PF	GII	PR	Yes	Alive	100,37	NE
25	M	7,1	PF	GII	TR	No	Alive	28,97	FL
26	M	22	IM	GII	TR	No	Alive	62,30	β^-
27	F	0,4	ST	GII	PR	No	Alive	70,50	NE
28	M	13,6	PF	GII	TR	No	Alive	12,77	NE
29	M	1,3	PF	GII	PR	No	Alive	67,50	NE
30	F	6,8	ST	GIII	PR	No	Alive	66,13	NE
31	M	1,1	PF	GII	PR	Yes	Dead	28,43	NE
32	M	19	IM	GII	NI	NI	Alive	68,57	NE
33	M	17,1	PF	GII	TR	No	Alive	59,90	FL/α^-/β^-
34	F	10,2	PF	GIII	PR	Yes	Alive	72,57	NE
35	F	3,8	IM	GII	PR	Yes	Alive	55,47	NE
36	M	1,8	PF	GII	PR	No	Alive	58,90	NE
37	M	0,1	ST	GIII	PR	No	Alive	52,77	α^-/β^-/$\alpha^-\beta^-$
38	M	8	PF	GII	PR	No	Alive	48,80	NE
39	F	8,11	PF	GII	PR	NI	Dead	0,90	FL/$\alpha^-\beta^-$
40	M	1,1	PF	GII	PR	Yes	Dead	31,20	NE
41	M	4,6	PF	GII	TR	NI	Dead	20,23	NE
42	M	7,9	PF	GIII	TR	Yes	Dead	19,87	FL/α^-/β^-
43	M	11	PF	GII	TR	Yes	Dead	37,83	NE

IM= Intramedular, PF= Posterior fossa, ST= Supratentorial, PR= Partial resection, TR= Total resection, GI= grade I, GII= grade II, GIII= grade III, NI= No information, FL= Full Length, NE= No mRNA expression, α^-= variant with deletion minus alpha, β^-= variant with deletion minus beta, $\alpha^-\beta^-$= variant with deletion minus alpha both minus alpha beta.

Table 4. Expression of *TERT* transcripts according to clinical parameters of MB patients.

	NE		FL		FL+Variants		Variants		Total		p	DF	Effect Size	Power (%)
Histology														
Anaplasic/ Large Cells	1	6,7%	-	-	1	3,2%	-	-	2	3,5%				
Classic	10	66,7%	4	100,0%	24	77,4%	6	85,7%	44	77,2%				
Desmoplasic	-	-	-	-	2	6,5%	-	-	2	3,5%	0,5540	15	0,376955	0,3670 (36)
Extensive nodularity	3	20,0%	-	-	3	9,7%	1	14,3%	7	12,3%				
Large Cells	-	-	-	-	1	3,2%	-	-	1	1,8%				
Mixed (classic/ desmoplasic)	1	6,7%	-	-	-	-	-	-	1	1,8%				
Total	15	100,0%	4	100,0%	31	100,0%	7	100,0%	57	100,0%				
Risk														
HR	8	53,3%	2	50,0%	15	50,0%	3	42,9%	28	50,0%				
LR	7	46,7%	2	50,0%	15	50,0%	4	57,1%	28	50,0%	0,9134	3	0,061168	0,0625 (6)
Total	15	100,0%	4	100,0%	30	100,0%	7	100,0%	56	100,0%				
Status														
Alive	7	46,7%	2	50,0%	13	41,9%	4	57,1%	26	45,6%				
Dead	8	53,3%	2	50,0%	18	58,1%	3	42,9%	31	54,4%	0,7846	3	0,101035	0,0865 (8)
Total	15	100,0%	4	100,0%	31	100,0%	7	100,0%	57	100,0%				

NE= No expression, FL= Full Length, DF=degree of freedom, HR= High risk, LR= Low risk

Also, 26 (44%) patients are alive, 31 (53%) are dead, and 1 (2%) had no information. Of these 58 tumor samples, 45 (77%) were considered classic histology, 3 (5%) were desmoplasic histology, 3 (5%) were anaplasic/large cells and 7 (12%) were nodular histology.

In the group of MB samples, we observed the expression of at least one of *TERT* transcripts investigated in 41 (70%) of the 58 analyzed. In total, only 3/58 (5%) of samples exhibited exclusively FL variant. FL pattern combined with the presence of variants with deletion; inhibitory α^- deletion, nonfunctional β^- and $\alpha^-\beta^-$deletions were detected in 31/58 (53%) of the samples. 7/58 (12%) of the samples showed exclusively variants with deletion and 16/58 (27%) did not show any of the patterns (Figures 1 and 2). We did not observed significant association between *TERT* splicing variants and clinical pathological characteristics of MB patients (Table 4).

TERT AS variant patterns in EP tumors

Of 43 EP tumor samples, 28 (65%) were located at posterior fossa, 10 (23%) were supratentorial location, and 5 (11%) were intramedular. Among these patients, 21 (48%) are alive, 21 (48%) are dead, and 1 (2%) had no information. Of 43 tumor samples, 33 (76%) were considered grade I and II, and 10 (23%) were considered grade III. The treatment was based on chemotherapy for 24 (55%) patients and radiotherapy for 28 (65%) patients.

In the group of EP samples, we observed the expression of at least one of TERT transcripts investigated in 17 (39%) of the 43 analyzed. In total, only 2/43 (4%) of samples exhibited exclusively FL variant. FL pattern combined with the presence of the variants with deletion; inhibitory α^- deletion, nonfunctional β^- and $\alpha^-\beta^-$deletions were detected in 7/43 (16%) of the samples. 8/43 (19%) of the samples showed exclusively variants with deletion and 26/43 (60%) did not show any of the patterns (Figures 1 and 2). We did not observed significant association between *TERT* splicing variants and clinical pathological characteristics of EP patients (Table 5).

Table 5. Expression of *TERT* transcripts according to clinical parameters of EP patients.

	NE		FL		FL+Variants		Variants		Total		p	DF	Effect Size	Power (%)
Diagnosis														
IM	4	15,4%	-	-	1	14,3%	1	12,5%	6	14,0%				
PF	16	61,5%	2	100,0%	4	57,1%	5	62,5%	27	62,8%	>0,999	6	0,178759	0,1123 (11)
ST	6	23,1%	-	-	2	28,6%	2	25,0%	10	23,3%				
Total	26	100,0%	2	100,0%	7	100,0%	8	100,0%	43	100,0%				
Classification														
GI	1	3,8%	-	-	-	-	-	-	1	2,3%				
GII	20	76,9%	2	100,0%	5	71,4%	5	62,5%	32	74,4%	0,828	6	0,238308	0,1718 (17)
GIII	5	19,2%	-	-	2	28,6%	3	37,5%	10	23,3%				
Total	26	100,0%	2	100,0%	7	100,0%	8	100,0%	43	100,0%				
Surgical extension														
NI	1	3,8%	-	-	1	14,3%	1	12,5%	3	7,0%				
PR	17	65,4%	-	-	3	42,9%	3	37,5%	23	53,5%	0,267	6	0,381992	0,4219 (42)
TR	8	30,8%	2	100,0%	3	42,9%	4	50,0%	17	39,5%				
Total	26	100,0%	2	100,0%	7	100,0%	8	100,0%	43	100,0%				
Recidive														
No	11	45,8%	2	100,0%	2	40,0%	3	37,5%	18	46,2%				
Yes	13	54,2%	-	-	3	60,0%	5	62,5%	21	53,8%	0,611	3	0,260748	0,2455 (24)
Total	24	100,0%	2	100,0%	5	100,0%	8	100,0%	39	100,0%				
Status														
Alive	13	50,0%	1	50,0%	2	28,6%	4	66,7%	20	48,8%				
Dead	13	50,0%	1	50,0%	5	71,4%	2	33,3%	21	51,2%	0,642	3	0,216915	0,1865 (18)
Total	26	100,0%	2	100,0%	7	100,0%	6	100,0%	41	100,0%				

NE= No expression, FL= Full Length, DF=degree of freedom, IM= Intramedular, PF= Posterior fossa, ST= Supra tentorial, GI= Grade I, GII= Grade II, GIII= Grade III, NI=No information, PR= Partial resection, TR= Total resection

Discussion

In particular, numerous findings have been published on the prognostic value of *TERT* expression in pediatric MB and EP (14, 16-18, 22, 27). In many of these studies, *TERT* expression is present in 42% and 76% of the MB and EP samples, respectively, and has been proposed as a strong prognostic biomarker of poor survival. However, to the best of our knowledge, neither of these studies has taken into consideration the identification of *TERT* AS variant patterns (12, 15, 19-23, 25-27).

In our study, we observed *TERT* gene expression in 70% of MB and 39% of EP samples. The exclusive presence of FL form was detected in only 5% and 4% of MB and EP samples, respectively. FL transcript is the only one with reverse transcriptase activity and able to elongate telomeres. In a wide variety of telomerase-positive embryonic stem cells, adult proliferating stem cells, and cancer cells examined, only a small fraction of *TERT* transcripts are spliced into the FL form that generates the catalytically active protein (3, 29, 30). The need to fine-tune the regulation to produce "just the right amount" of telomerase may be because too little telomerase would not be enough to maintain telomere length leading to increased genomic instability in cancer cells, but too much telomerase may lead to runaway elongation of telomeres and result in adverse effects including growth inhibition of the cancer cells (31, 32).

In addition, we observed that *TERT* AS variants with deletions, α^-, β^- and $\alpha^-\beta$, exclusively or combined to FL form, were present in 53% and 16% of MB and

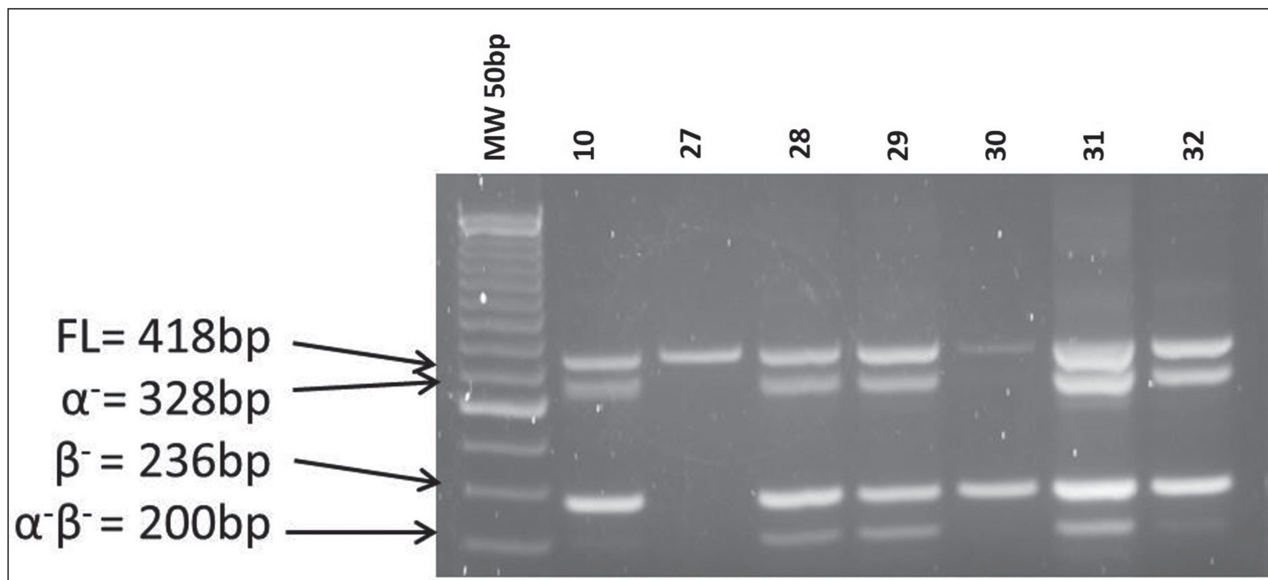

Figure 1. Identification of *TERT* transcripts in 2% agarose gel.

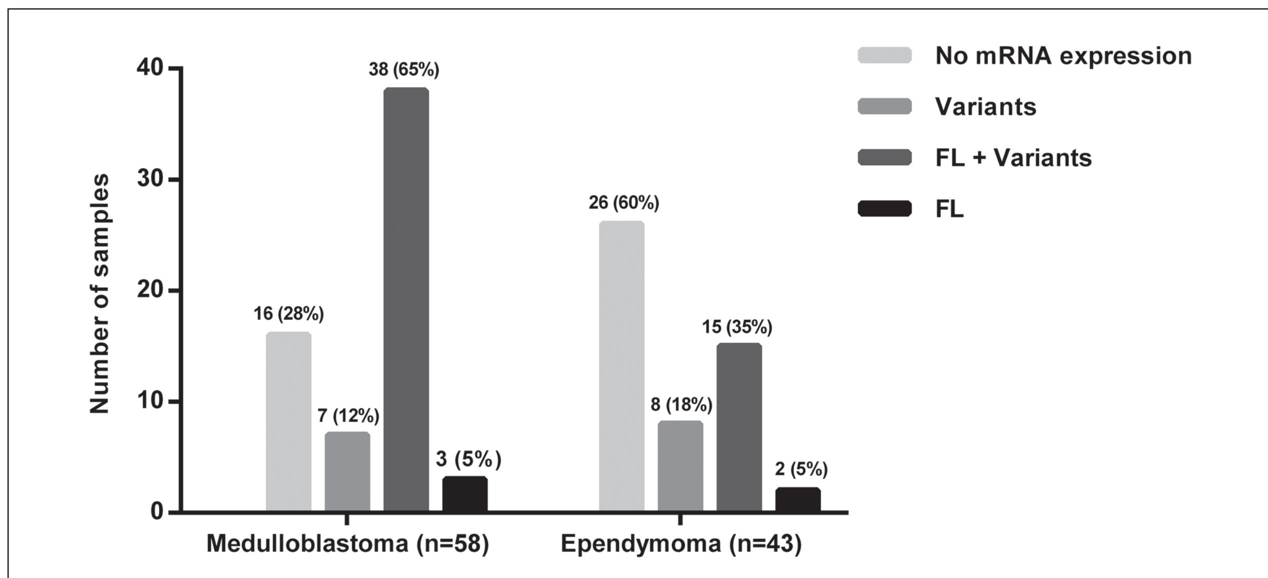

Figure 2. Distribution of *TERT* alternative splicing variant patterns in pediatric MB and. EP samples

EP tumors, respectively. We did not observed significant association between presence of *TERT* transcripts and the clinical pathological characteristics of these two groups of patients. In fact, the negative results observed are supported by the poor expressive power values found in each one of the statistical tests (Tables 4 and 5). The role of *TERT* variants in regulation of telomerase activity during tumorigenesis remains unclear and few studies have correlated *TERT* AS patterns in tumors with histopathological and clinical parameters (3, 29, 30, 32, 33). The use of different qualitative and quantitative methodologies to measure *TERT* mRNA in studies makes it difficult to directly compare interpretation of the results (7, 29, 34). Splicing variants of several proteins in tumor cells have been proposed as diagnostic or prognostic biomarkers and may provide

potential drug targets. The prospective use of more sensitive and refined methodologies, such as digital PCR, can collaborate to identify and quantify more precisely the splicing of low-abundance *TERT* transcripts (3, 29).

The establishment of associations between *TERT* AS variants and FL form and tumor clinical-biological behavior becomes even more difficult because of evidence that TERT protein has non-canonical functions that are unrelated to telomere lengthening. These in turn can be divided into the functions that still require the integrity of the catalytic site of *TERT* and the ones that do not (33). Among other functions, both, enzymatically active and inactive *TERT* modulate the Wnt pathway by acting as a transcription factor in beta-catenin complexes in positive and negative telomerase cells, indicating that this extratelomeric function is partially preserved in variant with deletion (24, 35, 36). Also, *TERT* protects normal and cancer cells from apoptosis independently of catalytic activity (37-39). Nevertheless, it is still unknown precisely the parts of *TERT* responsible for these effects and which specific variants retain these characteristics (3, 4, 7, 33, 38).

The presence of the FL form and the post-transcriptional processing of *TERT*, resulting in the variants with deletions as, α^-, β^- and $\alpha^-\beta^-$, could be a useful tool in predicting the progression of cancer. Future therapies, aimed at influencing the production of non-functional and/or dominant-negative variants, can be promising. Since FL pattern is the only associated with active telomerase enzyme, our results suggest that the association of *TERT* mRNA expression to clinic-pathological characteristics of patients, excluding the splicing alternative analysis, must be analyzed with caution. Further investigations will help to elucidate the complex mechanism involving AS of *TERT* gene and the function of variants with deletions in cancer maintenance, viability and progression, including the pediatric brain tumors.

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