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The Impact of Parkin as a Possible Future Biomarker in Five Solid Pediatric Tumors

Castro EM[1,2,3], Rocha RMVM[2], Taques GR[2], Zanoni A[2], Percicote AP[2], Pereira IRPD[3], Boscoli SS[3], Poncio L[2], França Junior N[4], Benevides APK[4], Dalke DBZ[4], Da Silva-Camargo CCV[4], Elífio-Esposito S[4], Werner B[2], Sotomaior VS[4], Noronha L[4], Machado-Souza C[1]*

[1]*Instituto de Pesquisa Pelé Pequeno Príncipe, Brazil*
[2]*Federal University of Paraná, Brazil*
[3]*Faculdades Pequeno Príncipe, Brazil*
[4]*Pontifícia Universidade Católica do Paraná, Brazil*

Abstract

Pediatric neoplasms represent an important group of childhood diseases. Biomarkers with prognostic function can help to manage this complex process. In this context, the tissue expression of parkin, could be used as a prognostic biomarker for the individual in the main solid pediatric tumors. We aimed to investigate the correlation between the tissue expression of parkin and the clinical-pathological characteristics, and to determine if parkin can be used as a prognostic marker. We assessed immune histochemical analysis of parkin in five solid pediatric tumors. High tissue expression of parkin was associated with positive prognostic factors for astrocytoma's and nephronblastomas, while in medulloblastomas and neuroblastomas; the same underlying aspect was associated with poor prognostic factors. Choroid plexus tumors showed no association. Parkin showed favorable behavior in patients with in astrocytoma's and nephroblastomas. In medulloblastomas and neuroblastomas, results showed the opposite. Research may enable an analysis of the overall behavior of this molecule as a prognostic tool.

Keywords: Parkin • Choroid plexus tumors • Astrocytoma • Medulloblastoma • Neuroblastoma • Nephroblastoma

Introduction

Childhood cancer can be considered infrequent when compared to adult tumors, and they correspond to nearly 3% of all malignant tumors [1]. The International Classification of Childhood Cancer [2,3] proposed the classification in 11 groups and in this article, we present five of them: Choroid plexus tumors (IIIa), Astrocytomas (IIIb), Meduloblastoma (IIIc); Neuroblastoma (IVa), and Nephroblastomas (Va) which are the main types of solid tumors in the Brazilian pediatric population.

Recent advances in molecular strategies and analytical platforms, including genomics, epigenomics, proteomics, and metabolomics, have identified an increasing number of potential biomarkers. Over the past 50 years, there has been a significant improvement in outcomes for children with cancer, driven mainly by better tumor stratification associated with the plurality of individualized treatment approaches [4]. In this context, the search for representative biomarkers can be challenging, especially concerning pediatric cancer. The use of biomarkers for prognosis has been the goal of many researchers to identify risk populations and help to predict unexpected outcomes.

Parkin is a protein encoded by the *Parkin RBR E3 Ubiquitin Protein Ligase* (*PRKN*), a gene discovered two decades ago [5]. The primary function of parkin is involved with proteasomal degradation, inducing ubiquitination of damaged proteins [6-8]. Parkin controls degradation in many cellular processes, such as cell cycle control through the degradation of cyclins (D and E). Other functions have already been described for this molecule such as regulation of cell proliferation and migration, protection against oxidative stress, mitochondrial homeostasis (mitophagy), xenophagy and tumor suppression [9-12]. Parkin also degrades the protein *p21*. *p21* is essential in preventing the accumulation of mutations and the generation of genetic instability. High levels of parkin may result in low levels of *p21*, and its consequence would be an accumulation of genetic alterations in the tumor cells [13].

The tissue expression of parkin has been shown to have a classic protective role in some neoplastic contexts and this relationship still needs to be better established so that in the future it can be incorporated as a possible prognostic biomarker. Future research using parkin as a possible prognostic biomarker may allow the correct aspect in the association of this molecule in pediatric tumors, and the use of parkin as a prognostic biomarker may early indicate the association of individuals with different types of outcomes. The objective of this study is to investigate the association between parkin tissue expression and the clinical-pathological characteristics in five types of primary pediatric tumors to determine if there is any support for a potential use as a prognostic biomarker.

Materials and Methods

Databases

The cases selected for this study comprised Formalin Fixed Paraffin Embedded (FFPE) samples of five types of pediatric tumors, mostly obtained at the tertiary referral children's hospital (Pequeno Príncipe Children's Hospital) in South Brazil. Thirty-three patients with choroid plexus tumors (1992 to 2010); one hundred and eight pediatric astrocytomas (2003 to 2015); twenty-nine meduloblastomas (1998 to 2009); ninety neuroblastic tumors (neuroblastoma/ganglioneuroblastoma/ganglioneuroma) (2001 to 2014) and seventy-seven patients diagnosed with nephroblastoma (1994 and 2012) were analyzed. This study brings together five ethics committee approvals. The Human Research Ethics Committee approved this study (Registration number: 3.573.221). Table 1 shows the main characteristics of these samples.

***Address for Correspondence:** Dr. Cleber Machado de Souza, Instituto de Pesquisa Pelé Pequeno Príncipe - Av. Silva Jardim, 1632 – Curitiba-PR-CEP-80250-060, Brazil; E-mail: cleberius@gmail.com*

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Tissue Microarray (TMA) and Immunohistochemistry (IHC)

From each case, hematoxylin-eosin (H&E) slides were prepared. Two different neoplastic areas free from tissue processing artifacts or necrosis were selected for TMAs block construction. Each TMA block included five to eight cases, with two 3 mm samples from each case. One conventional slide from each TMA was stained using H&E, and the other slides were separated for immunohistochemical study following the procedure described by da Silva-Camargo et al. [14]. The TMA slides were subjected to antigen retrieval and incubated with the monoclonal mouse anti-parkin antibody (1:100, Abcam®, Cambridge, MA, USA). As a positive control, samples of high-grade colon cancer expressing large quantities of parkin were used. As a negative control, the primary antibody was omitted.

Morphological analysis of protein expression

Parkin immune expression was evaluated by quantitative and semi-quantitative analysis (Tables 2 and 3). Some adaptations were made in the different types of neoplasms addressed. The morphometric evaluation was used for quantitative analysis and the Allred score, initially described in breast carcinomas, for semi-quantitative analysis [15].

The quantitative analysis (morphometry) was based on images obtained by the slide scanner model Axio Scan Z1 (Zeiss; Germany), in high power field (HPF - 40x objective), for digital documentation in Tagged Image File Format (TIFF). After digitization, the software generated images in the format of photomicrography, which was selected and analyzed through color morphometry, using the Image Proplus™ analyzer program (Rockville, MD, USA). Immuno-positive areas in square micrometers of each photomicrograph were compiled and transformed into a percent by HPF.

The semi-quantitative analysis was made by the Allred score, and it was obtained by summing two scores (proportion and intensity of positivity), ranging from 0 to 8. The proportion score is subdivided according to the percentage of stained cells: score 0 - 0% stained cells, score 1 - < 1%, score 2 - 1-10%, score 3 - 11-33%, score 4 - 34-66% and score 5 - > 66%. While the intensity of positivity is evaluated: negative - score 0, weak - score 1, moderate - score 2, and strong - score 3 [15].

The results of the quantitative and semi-quantitative analyzes for all the tumors were compared to clinical-pathological variables of prognostic importance, such as the presence of metastases, local staging, treatment, histological type, presence of anaplasia, risk group, nodal involvement, and outcome.

Statistical analysis

Descriptive analysis was performed with the absolute and relative frequencies of the qualitative variables. The results of quantitative variables were described by medians, minimum values, maximum values. Student's t-test was used to compare the result obtained through quantitative and semi-quantitative analysis to two groups of qualitative variables and the Kruskal-Wallis test for comparison with three groups or more with quantitative variables. Kaplan-Meier's curves were built to assess median survival with log-rank testing. Data were analyzed using the computer program The R Project for Statistical Computing. Data were analyzed by IBM® SPSS Statistics v.20.0 software.

Results

Parkin immune histochemical tissue reactions were positive in all specimens analyzed, and tissue expression presented with ample variable intensity and proportion in cytoplasm and nucleus of neoplastic cells (Figure 1).

The comparison of the tissue immune expression of parkin (quantitative and semi-quantitative methods) in *Choroid plexus tumor* (IIIa Group; n=33; Table 1) showed no significant difference between carcinomas (n=29) and papilloma's (n=4) (Tables 2 and 3). Outcome data was obtained for 23 of 29 choroid plexus carcinoma: 60.9% (14/23) died, 8.7% were alive for more than 1 year (2/23), 21.7% were alive for more than 5 years (5/23), and 2 (8.7%) were alive for more than 10 years (2/23) (Table 1). The outcome was compared with parkin immune expression (quantitative and semi-quantitative methods) and showed no significant results (Table 4).

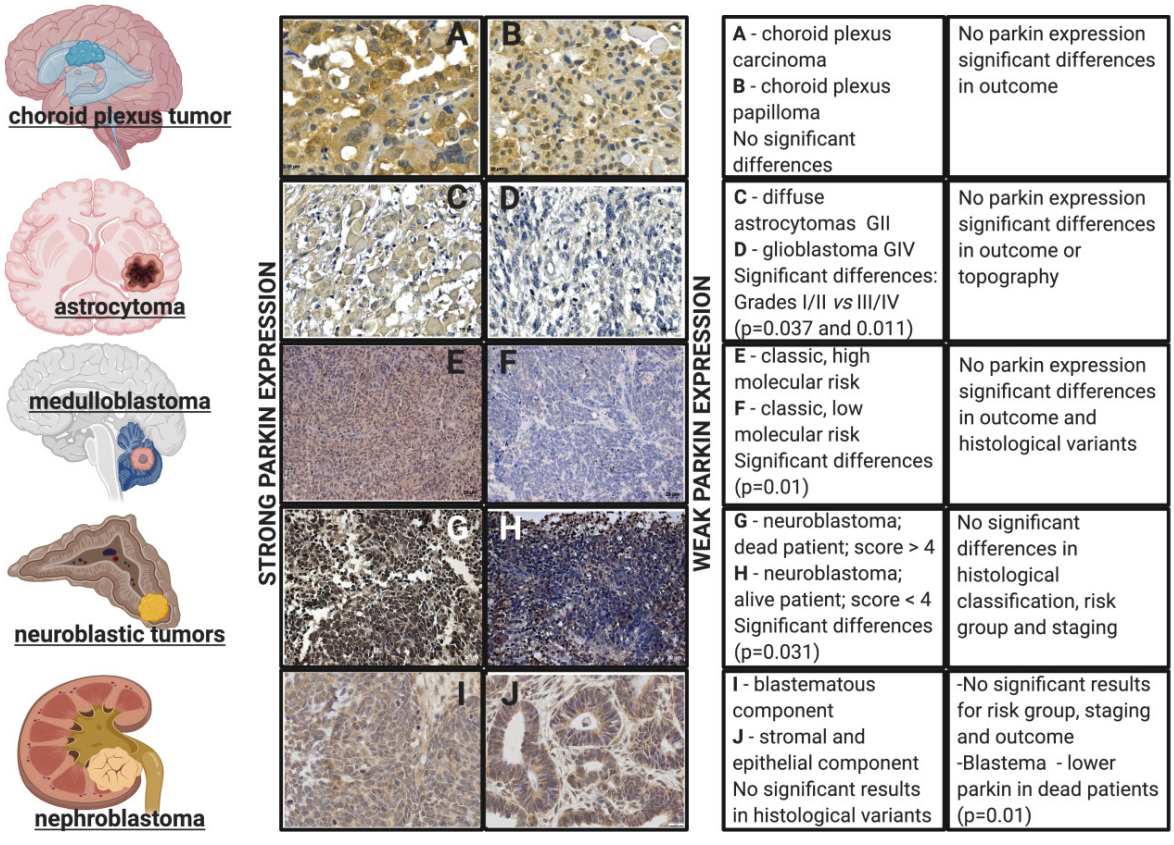

Figure 1. Schematic figure showing five pediatric cancers localization and describing the main results.

Table 1. Descriptive statistics for the clinical variables in the group of patients.

	Choroid plexus tumors (n=33)		Astrocytoma (n=108)		Medulloblastoma (n=29)		Neuroblastoma (n= 90)		Nephroblastoma (n=77)	
Age at diagnosis[1]	11.5 (3.0-120)		101.2 (0.4-173.6)		4.0 (0.1-15.0)		32.0 (0.0-159)		33.6 (1-108)	
Gender[2]										
Female	11 (33.3)		57 (53.0)		14 (48.3)		41 (45.6)		31 (40.3)	
Male	22 (66.7)		51 (47.0)		15 (51.7)		49 (54.4)		46 (59.7)	
Histological classification[2,3]	Papilloma	4 (12.1)	Pilocytic (GI)	61 (56,5)	Classic variant	23 (79.3)	NB POOR	54 (60.0)	Diffuse anaplasia type	5 (6.5)
	Carcinoma	29 (87.9)	Diffuse (GII)	23 (21.3)	Nodular variant	3 (10.3)	NB DIFF	18 (20.0)	Regressive type	4 (5.2)
	---	---	Anaplastic (GIII)	4 (3.7)	Desmoplasic variant	2 (7.0)	GNB INTERMIX	2 (2.2)	Mixed type	37 (48.1)
	---	---	Glioblastoma (GIV)	20 (18.5)	Anaplasic variant	1 (3.4)	GNB NOD DIFF	1 (1.1)	Epithelial type	10 (13.0)
	---	---	---	---	---	---	GNB NOD POOR	1 (1.1)	Stromal type	9 (11.7)
	---	---	---	---	---	---	GN	14 (15.6)	Blastemal type	12 (15.5)
Risk group (Age and total resection)[2]										
Low	----		----		9 (31.1)		----		----	
High	----		----		20 (68.9)		----		----	
Molecular Risk group[2]										
Low risk	----		----		5 (17.3)		----		----	
Standard risk	----		----		11 (37.9)		----		----	
High risk	----		----		3 (10.3)		----		----	
Uncertain risk	----		----		10 (34.5)		----		----	
Shimada Risk group[2]										
Unfavorable	----		----		----		45 (50.0)		----	
Favorable	----		----		----		38 (42.2)		----	
Not applicable	----		----		----		7 (7.8)		----	
Risk group (Age)[2]										
< 1.5 years	----		----		----		39 (43.3)		----	
1.5 - 5 years	----		----		----		35 (38.9)		----	
> 5 years	----		----		----		16 (17,8)		----	
Risk group (Histological)[2]										
Intermediate	----		----		----		----		68 (88.3)	
High	----		----		----		----		9 (11.7)	
Staging[2]										
I	----		----				15 (16.7)		47 (61.2)	
II	----		----		----		7 (7.8)		13 (16.9)	
III	----		----		----		11 (12.2)		13 (16.9)	
IV	----		----		----		37 (41.1)		----	
IVS	----		----		----		6 (6.6)		----	
GN/ Data Not Available	----		----		----		14 (15.6)		4 (5.2)	
Outcome[2]										
Disease-free	12 (36.4)		62 (57.4)		22 (75.9)		29 (32.2)		57 (74.0)	
Death	15 (45.5)		5 (4.6)		7 (24.1)		47 (52.2)		11 (14.3)	
GN/ Data not available	6 (18.1)		41 (38.0)		----		14 (15.6)		9 (11.7)	

Legend: [1] Age in month; MEDIAN (MIN_MAX). [2] n (%). [3] Low grade=GI + GII 84 (77.8); High grade=GIII + GIV 24 (22.2). NB POOR (Neuroblastoma poor Differentiated); NB DIFF (Neuroblastoma Differentiating); GNB INTERMIX (Ganglio Neuroblastoma Intermixed Differentiating); GNB NOD DIFF (Ganglioneuroblastoma Nodular Differentiating); GNB NOD POOR (Ganglio Neuroblastoma Nodular Poor Differentiated); GN (Ganglio Neuroma).

Table 2. Immunohistochemistry morphometric results (quantitative method) of parkin according to histological classification.

Histological Classification		Morphometry (%)	*P* value [a]
Choroid Plexus Tumors (n=33)*	Papiloma	41.6 (23.4-64.6)	0.270
	Carcinoma	33.3 (0-50.8)	
Astrocytoma (n=108)*	Low grade=GI + GII	0.7 (0.03-13.5)	**0.011**
	High grade=GIII + GIV	0.2 (0.01-7.0)	
Medulloblastoma (n=29)*	Classic	13.7 (3.1-27.4)	0.178
	Nodular variant	9.4 (1.1-17.7)	
	Desmoplasic variant	7.0 (4.0-8.5)	
	Anaplasic variant	15.8 (15.8-15.8)	
Neuroblastoma (n=90)*	NB POOR	6.4 (1.0-20.4)	0.344
	NB DIFF	7.9 (3.9-22.3)	
	GNB INTERMIX	8.0 (6.0-10.0)	
	GNB NOD DIFF	2.5 (2.5-2.5)	
	GNB NOD POOR	15.7 (15.7-15.7)	
	GN	10.4 (0.4-17.9)	
Nephroblastoma (n=77)*	Anaplastic type	11.2 (9.9-19.5)	0.802
	Blastematous type	12.6 (8.5-20.7)	
	Others**	13.1 (0.0-26.9)	

Legend: * Median (Minimum-Maximum) In Percentage By HPF; [A] Non-Parametrics Test. ** Other: Mixed; Epithelial; Stromal. NB POOR (Neuroblastoma Poor Differentiated); NB DIFF (Neuroblastoma Differentiating); GNB INTERMIX (Ganglioneuroblastoma Intermixed Differentiating); GNB NOD DIFF (Ganglioneuroblastoma Nodular Differentiating); GNB NOD POOR (Ganglioneuroblastoma Nodular Poor Differentiated); GN (Ganglioneuroma).

Table 3. Immunohistochemistry by proportion, intensity, and Allred score (semi-quantitative method) results of parkin according to histological classification.

Histological lassification		Morphology proportion [b]	*P* value[a]	Morphology intensity[b]	*P* value[a]	Allred score[b]	*P* value[a]
Choroid plexus	Papilloma	5.0 (5-5)	1	3.0 (3-3)	0.348	8.0 (8-8)	0.348
tumors (n=33)	Carcinoma	5.0 (5-5)		3.0 (1-3)		8.0 (6-8)	
Astrocytoma	Low grade	2.0 (0-3)	**0.006**	1.0 (1-2)	0.179	3.0 (1-5)	**0.037**
(n=108)	GI + GII						
	High grade	1.0 (0-3)		1.0 (1-1)		2.0 (1-4)	
	GIII + GIV						
Medulloblastoma	Classic	5.0 (3-5)	0.303	2.0 (1-3)	0.207	7.0 (4-8)	0.362
(n=29)	Nodular	4.0 (3-5)		1.5 (1-2)		5.5 (4-7)	
	Desmoplasic	4.0 (4-4)		2.0 (2-2)		6.0 (6-6)	
	Anaplasic	4.0 (4-4)		3.0 (3-3)		7.0 (7-7)	
Neuroblastoma	NB	2.0 (1-4)	0.329	1.0 (1-3)	0.826	3.5 (0-6)	0.539
(n=90)	POOR						
	NB	2.0 (1-4)		1.0 (1-3)		4.0 (0-6)	
	DIFF						
	GNB	2.0 (1-3)		1.5 (1-2)		3.5 (2-5)	
	INTERMIX						
	GNB NOD	3.0 (3-3)		2.0 (2-2)		5.0 (5-5)	
	DIFF						
	GNB NOD	3.0 (3-3)		2.0 (2-2)		5.0 (5-5)	
	POOR						
	GN	3.0 (1-4)		1.0 (1-3)		4.0 (0-6)	

		Nephroblastoma*									
(n=77)	**Component**	**Blast**	**Stromal**	**Epithelial**		**Blast**	**Stromal**	**Epithelial**		**All Tumor**	
	Anaplastic	5.0	3.3	3.3	> 0.05	2.0	1.3	1.3	> 0.05	5.0	0.705
		(5.0-5.0)	(3.3-3.3)	(3.3-3.3)		(1.7-2.0)	(1.3-1.3)	(1.3-1.3)		(5.0-7.0)	
	Blastematous	5.0	3.3	3.3		1.7	1.5	1.3		5.5	
		(1.7-5.0)	(1.3-5.0)	(1.7-5.0)		(0.3-2.3)	(0.7-2.7)	(0.3-2.7)		(5.0-7.0)	
	Others**	3.3	3.3	3.3		1.3	1.7	1.3		5.0	
		(1.7-5.0)	(1.7-5.0)	(1.3-5.0)		(0.3-2.7)	(0.3-2.3)	(0.7-2.7)		(2.0-7.0)	

Legend: b Median (Minimum-Maximum); Anon-Parametric Test. * Proportion and intensity were analyzed by component Blastematous (Blast), stromal and epithelial, and Allred score was analyzed in three components all together. ** Other: Mixed; Epithelial; Stromal. NB POOR (Neuroblastoma Poor Differentiated); NB DIFF (Neuroblastoma Differentiating); GNB INTERMIX (Ganglio Neuro Blastoma Intermixed Differentiating); GNB NOD DIFF (Ganglio Neuro Blastoma Nodular Differentiating); GNB NOD POOR (Ganglio Neuro Blastoma Nodular Poor Differentiated); GN (Ganglio Neuroma).

Table 4. Immunohistochemistry by proportion and intensity score (semi-quantitative method) results of parkin according to prognostic factors.

	Choroid Plexus Tumors (n=33)		Astrocytoma (n=108)		Medulloblastom (n=29)		Neuroblastoma (n=90)		Nephroblastoma (n=77)	
Risk group (Age and total resection)										
					Proportion	Intensity				
Low	----		----		4 (4-5)	2 (1-3)	----		----	
High	----		----		5 (3-5)	2.5 (1-3)	----		----	
Molecular					Proportion*	Intensity				
Risk group										
Low risk	----		----		4 (3-5)	2 (1-3)	----		----	
Standard risk	----		----		5 (4-5)	3 (1-3)	----		----	
High risk	----		----		5 (5-5)	2 (2-3)	----		----	
Uncertain risk	----		----		4 (3-5)	2 (1-3)	----		----	
Shimada Risk group										
							Proportion	Intensity		
Unfavorable	----		----		----		2 (1-4)	1 (1-3)	----	
Favorable	----		----		----		3 (1-4)	1 (1-3)	----	
Not applicable	----		----		----		7 (7.8)		----	
					Risk group					
(Age)							Proportion	Intensity		
< 1.5 years	----		----		----		2 (1-4)	1 (1-3)	----	
1.5 - 5 years	----		----		----		2 (1-4)	1 (1-3)	----	
> 5 years	----		----		----		3 (1.5-4)	2 (1-3)	----	
					Risk group					
(Histological)									Proportion	Intensity
Intermediate	----		----		----		----		3 (1-5)	2 (1-2)
High	----		----		----		----		3 (3-5)	2 (2-2)
Staging							Proportion	Intensity	Proportion	Intensity
I	----		----				2 (1-4)	2 (1-2)	3 (1-5)	2 (1-2)
II	----		----		----		2 (1-4)	2 (1-2)	3 (2-4)	2 (2-3)
III	----		----		----		2 (1-4)	1 (1-3)	3 (3-4)	3 (2-3)
IV	----		----		----		2 (1-4)	1 (1-3)		
IVS	----		----		----		3 (2-3)	1 (1-2)		
GN/ Data	----		----		----					
Not Available							14 (15.6)		4 (5.2)	
Outcome	Proportion	Intensity	Proportion	Intensity	Proportion	Intensity	Proportion**	Intensity	Proportion	Intensity
Disease-Free	5 (5-5)	3 (1-3)	1.5 (0-3)	1 (1-1)	4.5 (3-5)	2 (1-3)	2 (1-4)	1 (1-3)	3 (1-4)	2 (1-3)
Death	5 (5-5)	3 (1-3)	2 (0-3)	1 (1-2)	5 (4-5)	3 (2-3)	3 (1-4)	2 (1-3)	3 (2-4)	3 (2-3)
GN/ Data not available	6 (18.1)		41 (38.0)		----		14 (15.6)		9 (11.7)	

Legend: * *P*=0.010 to low risk *vs.* standard risk and low risk *vs.* high risk; ** *P*=0.033.

In the *Astrocytomas* (IIIb Group; n=108), parkin tissue immunoexpression, when analyzed by both methods, resulted in a significant (*P*=0.011 and *P*=0.037) increased frequency in the low-grade group (Tables 2 and 3).

From the 108 patients included in this study, 67 had available follow-up and outcome data (Table 1). The follow-up ranged from 7.2 months to 12.34 years.

Twenty-three of the *Meduloblastomas* (IIIc Group; n=29) were classic; three were nodular, two desmoplastic, and one anaplastic. Eleven meduloblastomas presented a standard molecular risk, five low risks, and three high risks (Table 1). In meduloblastomas, there were no significant differences between the tissue expression of parkin (quantitative and semi-quantitative method) and histological variants (Tables 2 and 3). Parkin tissue expression semi-quantitative analysis by Allred score (proportion of positivity) showed lower values in low molecular risk patients' tumors when compared to high (*P*=0.01) and standard molecular risk tumors (*P*=0.01) (Table 4). Parkin's Allred score (semi-quantitative method) showed a significant association (*P*=0.033) with cell size, considering large cells with intensity 8 (7 to 8), and small cells with intensity 6 (4 to 8) of tissue expression of parkin. No significant results were found when parkin tissue immuno expression was compared to outcome in patients with medulloblastoma (Table 4).

The samples for *Neuroblastic tumors* (Group IVa; n=90) were previously published by Araujo et al., but clinical-pathological aspects can be seen in Table 1 [16]. Considering parkin tissue expression (quantitative and semi-quantitative method), there were no significant differences between histological variants of neuroblastic tumors (Tables 2 and 3). No significant results were found when parkin tissue immunoexpression was compared to the risk group and staging of patients with neuroblastic tumors (Table 4).

The outcome of neuroblastoma (analysis performed without ganglio neuro blastoma/ganglio neuroma tumors) patients was compared to parkin tissue expression (by Allred score), and surviving disease-free patients showed less tissue immune expression of parkin with *P*=0.033 (Table 4). A clinical analysis in neuroblastomas (analysis performed without ganglio neuro blastoma/ ganglio neuroma tumors) involving parkin tissue expression, outcome (less/ more than five years survival), and Shimada group (unfavorable and favorable)

was performed. Outcome/survival curves that were performed for the Allred score (cutoff less than 4 or higher/equal 4) regarding parkin tissue expression in patients with neuroblastomas (analysis performed without ganglio neuro blastoma/ganglio neuroma tumors) showed that those who had parkin score higher than 4, had worse prognosis (*P*=0,031).

Table 1 shows some variables involved with *Nephroblastoma* (Group VIa; n=77). Further details can be accessed in Percicote et al. [17]. There were no differences in quantitative and semi-quantitative analysis for nephroblastoma variants concerning parkin immuno histochemical tissue expression, even when analysis of the three components was considered separately (Tables 2 and 3). No significant results were found when parkin immune expression was compared to risk group, staging, and outcome/survival of patients with nephroblastoma (Table 4).

Discussion

Parkin is a protein that was discovered around two decades ago in the context of juvenile Parkinson's disease and proteosomal degradation studies [18]. In the normal brain tissue, parkin is expressed in the cytoplasm of neurons and glia cells [19]. More recently, parkin has been found in the nuclear compartment, where it appears to act as a transcription factor [20]. Today it is known to be a multifunctional protein involved in different types of neoplasia [21-24]. Evidence of parkin involvement in the indirect regulation of different tumorigenic phenotypes could be expected.

The best-known canonical function of parkin is its control over the cell cycle through the ubiquitination of cyclins and its consequent degradation by proteasomes. Following the above, Gong and colleagues described an inverse correlation between parkin and cyclins in ovarian and breast tumors. The authors observed a decrease in parkin expression associated with increased expression of D1 and E1 cyclins with consequent tumor progression [12]. High parkin levels are expected to play a protective role when evaluating tumor growth and evolution. Parkin exhibits a protective function by accumulating in neoplasic cells and degrading excess cyclin E1, which inhibits tumor cell replication [25-28]. However, the opposite mechanism was also attributed to the presence of high levels of parkin. High levels of parkin may result in low levels of *p21*, and its consequence would be an accumulation of genetic alterations in the tumor cells [29]. In addition to the classic mechanisms of proteasomal degradation of proteins involved in the cell parkin is associated with the mitochondrial mitophagy process, adding more plurality to the functional issue surrounding parkin [30-33].

In view of these actions that involve parkin we have two possible hypotheses. One of them is that the high expression of parkin, in neoplastic tissue, could be associated with a favorable prognosis in patients with cancer, and the antagonistic idea also has strong biological plausibility [12,14,29]. In our analysis, parkin presents a protective and non-protective function in different pediatrics cancers, and this kind of variation reinforces the need for more studies concerning parkin and its role as a prognostic tool in different cancers.

In pediatric astrocytoma's, our results may indicate a potential role of parkin as a protective biomarker, since low-grade pediatric astrocytoma's showed higher parkin tissue expression, and less parkin tissue expression was associated with worse prognosis. Lin et al. established that less parkin expression indicated reduced survival rates [34]. Additionally, lower levels of parkin were associated with increased cell proliferation and poorer prognosis [35,36]. In astrocytoma's, parkin could be considered as a possible tumor grade marker, being a tool to distinguish these high- and low-grade tumors, especially in the lack of classical morphological characteristics in small specimens. It is known that glioma genesis has different molecular mechanisms in adults and children [37,38] and for this reason we could infer that the association between parkin and the prognostic factors could indicate its classic protective function.

Nephroblastomas followed the same trend seen in astrocytomas. The blastematous component of nephroblastoma, which is the component responsible for a poorer prognosis, showed less expression of parkin in tumors of patients who died when compared to tumors of disease-free surviving patients. In meduloblastomas and neuroblastomas, higher values of parkin expression were associated with worse prognosis. The reduced sample size can be the explanation for the lack of association between the expression of parkin in choroid plexus carcinoma and papilloma's and prognostic factors and outcome (patient death). Tissue biomarkers have been studied in these neoplasms, but no study has been carried out associating parkin with these tumors.

Genetic alteration in *PRKN* is common in many human neoplasms and in hereditary Parkinson's disease. In cancer, the *PRKN* gene is mutated or deleted, with copy number loss being the primary mode of alteration. Gong et al. examined the *PRKN* mutation pattern across 4934 tumors spanning 11 cancers, and *PRKN* deletions are the most common in tumors [26]. These data indicate that *PRKN* is one of the most deleted genes in human cancer. Due to these genetic aspects associated with parkin's participation in carcinogenesis, despite few references reporting expression of this protein in tumor tissue samples, our work aimed to evaluate the immune expression of this protein in five different types of solid pediatric tumors [39,40].

One of the limitations that could be attributed to our study would be the low sample size of two neoplasms (choroid plexus tumor and medulloblastoma). However, this condition reflects the low incidence that these neoplasms present in the Brazilian population of southern Brazil. Another potential limitation is that only the tissue expression of parkin in paraffinized samples (FFPE) was analyzed. The authors are aware that to consider a molecule as a biomarker, other analyzes should be performed. Our future goal will be to implement analyzes that include genetics (polymorphisms) and epigenetics (interference RNA) to complement and extend the deductive and predictive power in relation to parkin.

Conclusion

The tissue expression of parkin in the studied tumors showed an ambiguous action when considering its protective role to the patients. Parkin, acting as a protective prognostic biomarker, has been found in astrocytoma's and nephroblastomas. In medulloblastomas and neuroblastomas it was observed as a biomarker unassociated with the matter of protection. Parkin is not always protective when it comes to its association with prognostic factors and consequently, outcome, but these results reinforce the importance of knowing parkin tissue behavior to identify new possible prognostic biomarkers. The results presented here could, in the future, turn parkin into a useful prognostic biomarker in the context of solid tumors in the pediatric population.

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Conflict of Interest

The authors declare that they have no conflict of interest.

Data Availability

The data that support the findings of this study are available from the corresponding author, [CMS], upon reasonable request.

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