P53 expression and survivin subcellular localization contribute to the diagnosis and prognosis of patients with astrocytomas

Roberta Soares Faccion[1,2]*, Paula Sabbo Bernardo[1]*, Giselle Pinto de Faria Lopes[3]*, Leonardo Soares Bastos[4], Cristina Lordello Teixeira[5], José Antonio de Oliveira[6], Priscila Valverde[7], Luiz Gustavo Dubois[8], Leila Chimelli[9], Raquel Ciuvalschi Maia[1]#

*** These three authors contributed equally to this work.**

1. Laboratório de Hemato-Oncologia Celular e Molecular, Programa de Hemato-Oncologia Molecular, Hospital do Câncer I, Centro de Pesquisas do Instituto Nacional de Câncer José Alencar Gomes da Silva (INCA), Rio de Janeiro, Brazil. 2. Programa de Pós-Graduação em Ciências Biológicas – Fisiologia, Instituto de Biofísica Carlos Chagas Filho, Centro de Ciências da Saúde, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil. 3. Departamento de Biorregulação, Universidade Federal da Bahia (UFBA)/ Departamento de Ciências Morfológicas, Universidade Federal do Estado do Rio de Janeiro (UNIRIO), Brazil. 4. Programa de Computação Científica (PROCC), Presidência, Fundação Oswaldo Cruz (Fiocruz), Rio de Janeiro, Brazil. 5. Serviço de Patologia Clínica, Hospital do Câncer I, INCA, Rio de Janeiro, Brazil. 6. Serviço de Neurocirurgia, INCA, Rio de Janeiro, Brazil. 7. Divisão de Patologia, INCA, Rio de Janeiro, Brazil. 8. Laboratório de Biomedicina do Cérebro, Instituto Estadual do Cérebro Paulo Niemeyer (IECPN), Rio de Janeiro, Brazil. 9. Laboratório de Neuropatologia, Instituto Estadual do Cérebro Paulo Niemeyer (IECPN), Rio de Janeiro, Brazil.

E-mail: robsfaccion@gmail.com

ABSTRACT

Diffuse astrocytic tumors are the most frequent subtype of primary central nervous system (CNS) tumors. Diffuse astrocytoma (DA), anaplastic astrocytoma (AA) and glioblastoma (GB) are diffuse astrocytic tumors derived from glial cells or precursors. Despite continuous efforts from the scientific community, these tumors still have a poor prognosis. Histological classification is still challenging, and prognostic factors are still limited to age and tumor subtype. Biomarkers that could improve histological classification, especially that could help differentiate AA from the other subtypes, and/or serve as prognostic factors are much needed. Furthermore, the relationship between survivin and p53 in diffuse astrocytic tumors' progression and survival is still controversial. The present study aims to investigate the role of these proteins in the accuracy of histologic classification of diffuse astrocytic tumors and treatment response. One hundred thirty-three formalin-fixed-paraffin-embedded diffuse astrocytic tumor samples were obtained from patients (Table 1). Tumor samples were reviewed and stained for p53, survivin, and IDH by immunohistochemistry. Expression levels of p53 and survivin subcellular localization were correlated with histological classification (Figure 1, Table 2 and Table 3) and patient outcomes (Table 4). Age and histological subtype were the only clinical features that had a prognostic impact (Figure 2, Table 4 and Table 5). High nuclear survivin and p53 correlated with AA (Table 3). High cytoplasmic survivin correlated with GB (Table 3). This was consistent with lower survival of patients whose tumors displayed high cytoplasmic survivin and with the fact that high cytoplasmic survivin was not an independent prognostic factor (Figure 3, Table 4 and Table 6). Furthermore, patients whose tumors expressed high cytoplasmic survivin, high nuclear survivin or high p53 and did not receive radiotherapy had worse short-term and long-term survival (Figure 4, Table 7 and Table 8). Our results suggest that survivin subcellular localization and p53 expression improve the accuracy of histological classification (Figure 5). Patients whose tumors overexpress these proteins benefit from radiotherapy regardless of age and histological classification. Furthermore, patients whose tumors overexpress survivin may benefit from novel targeted anti-survivin treatment.

KEYWORDS: Astrocytomas, survivin, p53.

Table 1: Patients' clinical and demographic data.

Characteristics	Absolute and relative frequencies (%)
Age	
Median	52
Mean	51.8
< 50 years	56 (42%)
≥ 50 years	77 (58%)
Gender	
Male	79 (59%)
Female	54 (41%)
Histological Grade	
Astrocytoma Grade II	25 (19%)
Astrocytoma Grade III	27 (20%)
Glioblastoma	81 (61%)
Resection surgery extension	
None	47 (35%)
Subtotal	41 (31%)
Total	45 (34%)
Radiotherapy	
No	41 (31%)
Yes	92 (69%)
Chemotherapy	
No	125 (94%)
Yes	8 (6%)
Recurrence	
No	80 (60%)
Yes	45 (34%)
N.A.	8 (6%)

N.A.: Not Available.

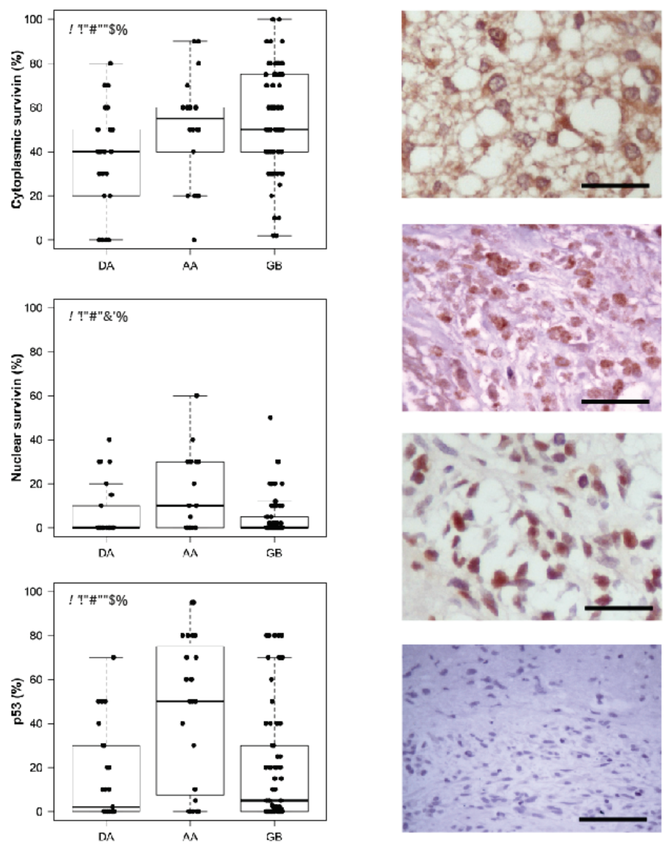


Figure 1. Expression of survivin and p53 in diffuse astrocytic tumors. Left-side graphs represent a merge of column scatters plots with box-and-whiskers graph of the same data. The upper graph displays the percentage of cytoplasmic survivin expression in diffuse astrocytic tumors cases sorted by histological classification. The middle graph displays the percentage of nuclear survivin expression in diffuse astrocytic tumors cases sorted by histological classification. Lower graph displays the percentage of p53 expression in diffuse astrocytic tumors cases sorted by histological classification. Right-side pictures are examples of a cytoplasmic survivin-expressing tumor, nuclear survivin-expressing tumor, p53 expression tumor, and a negative case, respectively from top to bottom. Size bars represent four µm for the positives cases and ten µm for the negative case. Differences were considered significant when p.<0.05 (Kruskall-Wallis test).

Table 2: Expression of p53 and survivin proteins in tumor cells according to histopathological grade

Grade	II	III	IV	Pearson's Chi-square	df	*p*-value
IDH						
Negative	1	2	71	76.52	2	<0.0001
Positive	8	21	4			
Survivin cytoplasmic expression[a]						
< 25%	8	5	5	10.50	2	0.0052*
≥ 25%	17	16	68			
Survivin nuclear expression						
< 10%	18	10	57	8.73	2	0.0127*
≥ 10%	7	12	16			
Survivin expression exclusively in the cytoplasm[b]						
Cytoplasm <25% or Cytoplasm ≥ 25% and Nucleus ≥ 10%	15	16	21	16.87	2	0.0002*
Cytoplasm ≥ 25% and Nucleus < 10%	10	6	52			
p53 expression						
< 25%	18	7	45	11.77	2	0.0028*
≥ 25%	7	16	21			

Protein expression was evaluated as the percentage of positive tumor cells.
[a] The case was considered positive when survivin was present in the cytoplasm of tumor cells, regardless of nucleus positivity.
[b] The case was considered positive when survivin was present exclusively in the cytoplasm of tumor cells, hence negative in the nucleus.
*Differences were considered significant when *p*-value<0.05

Table 3: Multinomial logistic regression model of histological grading according to p53 and survivin expression

	Odds Ratio[a] II *versus* III (95% CI)	Odds Ratio[a] II *versus* IV (95% CI)	Odds Ratio[a] III *versus* IV (95% CI)
Cytoplasmic ≥ 25%	1.56 (0.42 – 5.8)	4.73 (1.21 – 18.55)	3.04 (0.73 – 12.6)
Nuclear survivin ≥ 10%	3.87 (1.08 – 13.88)	1.07 (0.31 – 3.64)	IV versus III 3.63 (1.24 – 10.6)
Cytoplasmic survivin ≥ 25% and Nuclear survivin < 10%	0.45 (0.13 – 1.64)	2.22 (0.76 – 6.5)	III *versus* IV 4.91 (1.61 – 14.96)
p53 ≥ 20% and nuclear survivin ≥ 10%	6.74 (1.4 – 32.49)	0.32 (0.04 – 2.58)	IV *versus* III 21.01 (3.97 – 111.23)

CI: Confidence interval. [a]Odds ratio was adjusted for patients with respect to age. "A *versus* B": Risk of B having A as base.

Table 4: Twelve-months and 15-years probability of survival of glioma patients' according to clinical, demographic, histopathological and treatment response data

Characteristics	12-month OS rate (%)	*p*-value	15-years OS rate (%)	*p*-value
Overall survival	51% (43-60)	-	3.1% (1-10)	-
Age				
< 50 years	74% (63-87)	<0.001*	6.7% (2-22)	<0.001*
≥ 50 years	34% (25-47)		0%	
Gender				
Male	54% (43-66)	0.337	3.3% (0.8-13)	0.793
Female	47% (35-62)		3.5% (0.5-22)	
Histological Classification				
Diffuse Astrocytoma (DA)	92% (82-100)	<0.001*	15% (5-46)	<0.001*
Anaplastic Astrocytoma (AA)	62% (46-84)		0%	
Glioblastoma (GB)	34% (25-46)		0%	
Resection surgery extension				
None	52% (39-69)	0.575	6.7% (1.8-2.5)	0.383
Parcial	57% (43-75)		3.0% (0.4-20)	
Total	43% (31-62)		2.6% (0.4-18)	
Radiotherapy				
No	49% (36-68)	0.073[a]	0%	0.9272
Yes	52% (43-64)		12%	
Cytoplasmic survivin ≥ 25%				
Negative	60% (41-88)	0.208	18% (6-56)	0.011*
Positive	48% (39-59)		1.3% (0.2-9)	
Nuclear survivin ≥ 10%				
Negative	51% (41-63)	0.394	3% (0.5-16)	0.464
Positive	47% (33-67)		3.7% (0.5-25)	
p53 expression ≥ 25%				
Negative	51% (40-65)	0.711	7.0% (2.5-19)	0.335
Positive	48% (34-66)		0%	

Protein expression was evaluated as the percentage of positive tumor cells. *Differences were considered significant when *p*-value<0.05. [a]Radiotherapy had a borderline impact on 12 -months survival.

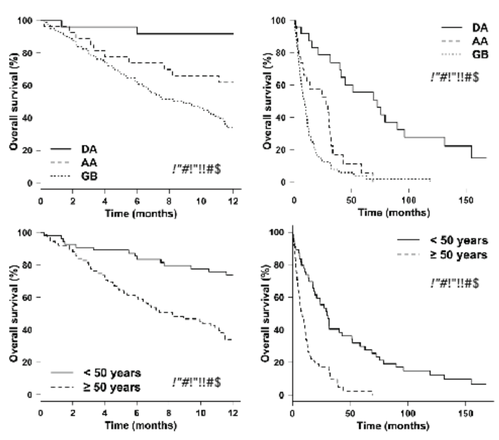


Figure 2. Kaplan-Meyer survival curves displaying the impact of subtype (upper curves) and age (lower curves) in diffuse astrocytic tumors patients' survival in 12 months (left curves) and 15 years (right curves). Age was categorically discriminated by two groups: patients younger than 50 years and patients with 50 years or older. Differences were considered significant when *p*.<0.05 (log-rank test).

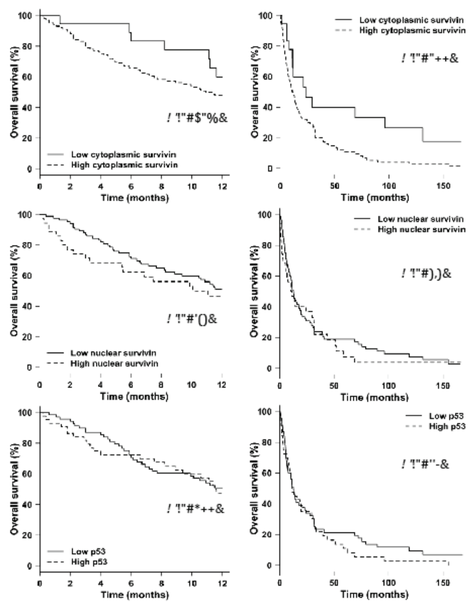


Figure 3. Kaplan-Meyer survival curves displaying the impact of survivin and p53 expression in diffuse astrocytic tumors patients' survival in 12 months (left curves) and 15 years (right curves). Differences were considered significant when *p*.<0.05 (log-rank test).

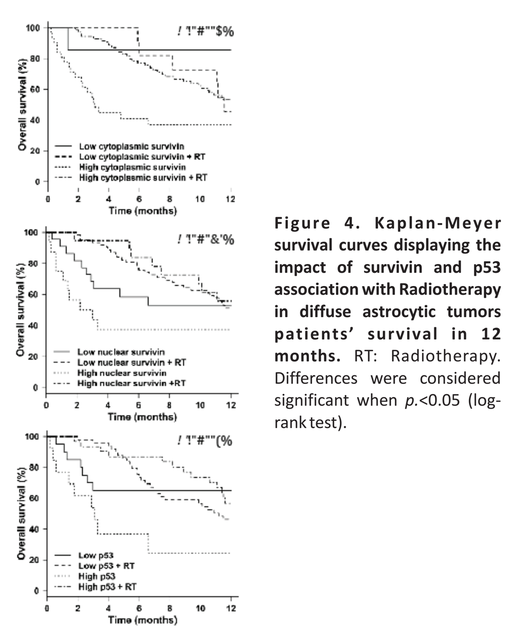
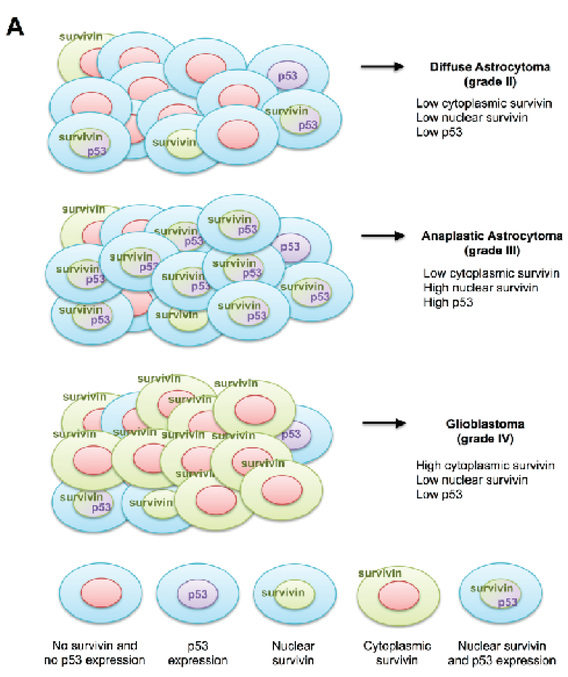


Figure 4. Kaplan-Meyer survival curves displaying the impact of survivin and p53 association with Radiotherapy in diffuse astrocytic tumors patients' survival in 12 months. RT: Radiotherapy. Differences were considered significant when *p*.<0.05 (log-rank test).



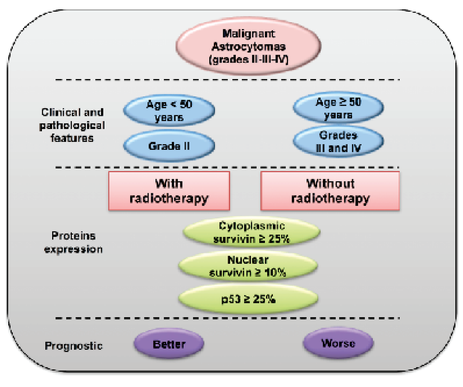


Figure 5. Schematic representation of the impact of cytoplasmic survivin, nuclear survivin and p53 expression in diffuse astrocytic tumors histological grading (A) and prognosis (B). Our data suggest that patients whose tumors express high levels of either cytoplasmic or nuclear survivin or p53 are the patients that benefit the most from radiotherapy regarding both short and long term survival (A). Our data suggest that low levels of survivin and p53 are associated with DA diffuse astrocytic tumors, high nuclear survivin, and p53 expression is associated with AA diffuse astrocytic tumors and high cytoplasmic survivin is associated with GB (B).

Table 5: Cox propotional hazard regression of 15-years survival of glioma patients' according to clinical, demographic and histopathological characteristics

Characteristics	Crude Hazard Ratio (95% CI)	Adjusted Hazard Ratio (95% CI)[a]	*p*-value
Age (continuous)	1.04 (1.03-1.06)	1.03 (1.02-1.05)	<0.001*
Histological Grade			
Astrocytoma Grade II	1.0	1.0	
Astrocytoma Grade III	3.59 (1.8-7.17)	4.28 (2.08-8.78)	<0.001*
Glioblastoma	6.17 (3.4-11.19)	5.76 (2.94-11.27)	<0.001*
Resection surgery extension			
None	1.0	1.0	
Parcial	1.03 (0.65-1.63)	0.83 (0.5-1.39)	0.487
Total	1.33 (0.85-2.1)	0.75 (0.46-1.22)	0.246
Radiotherapy			
No	1.0	1.0	0.223
Yes	1.01 (0.65-1.55)	1.32 (0.85-2.05)	

Protein expression was evaluated as the percentage of positive tumor cells. *Differences were considered significant when *p*-value<0.05. [a]Hazard Ratios were adjusted by age and histological grading.

Table 6: Cox proportional hazard regression of 15-years survival of glioma patients' according to cytoplasmic survivin subcellular localization

Characteristics	Crude Hazard Ratio (95% CI)	Adjusted Hazard Ratio (95% CI)[a]	*p*-value
Negative	1.0	1.0	0.213
Positive	1.04 (1.03-3.88)	1.48 (0.8-2.76)	
Age (continuous)	1.04 (1.03-1.06)	1.03 (1.02-1.05)	<0.001*
Histological Grade			
Astrocytoma Grade II	1.0	1.0	
Astrocytoma Grade III	3.59 (1.8-7.17)	4.39 (2.11-9.14)	<0.001*
Glioblastoma	6.17 (3.4-11.19)	4.3 (2.24-8.26)	<0.001*

Protein expression was evaluated as the percentage of positive tumor cells. *Differences were considered significant when *p*-value<0.05. [a]Hazard Ratios were adjusted by age and histological grading.

Table 7: Cox proportional hazard regression of 12-months and 15-years survival of glioma patients' according to survivin and p53 association with Radiotherapy

Characteristics	12 months			15 years		
	Crude Hazard Ratio (95% CI)	Adjusted Hazard Ratio (95% CI)[a]	*p*-value	Crude Hazard Ratio (95% CI)	Adjusted Hazard Ratio (95% CI)[a]	*p*-value
Cytoplasmic survivin < 10%	0.29 (0.04-2.13)	0.46 (0.06-3.36)	0.442	0.38 (0.14-1.06)	0.71 (0.25-1.98)	0.51
Cytoplasmic survivin < 10% + Radiotherapy	1.06 (0.44-2.54)	1.67 (0.66-4.2)	0.275	0.58 (0.29-1.17)	0.78 (0.37-1.65)	0.518
Cytoplasmic survivin = 10%	2.42 (1.37-4.3)	3.03 (1.68-5.43)	<0.001*	1.3 (0.8-2.1)	1.77 (1.09-2.88)	0.021*
Cytoplasmic survivin = 10% + Radiotherapy	1.0	1.0		1.0	1.0	
Nuclear survivin < 10%	1.49 (0.59-3.79)	1.3 (0.51-3.34)	0.582	0.87 (0.44-1.74)	0.93 (0.46-1.88)	0.842
Nuclear survivin < 10% + Radiotherapy	1.17 (0.54-2.55)	1.03 (0.47-2.26)	0.946	0.97 (0.57-1.66)	0.89 (0.52-1.53)	0.68
Nuclear survivin = 10%	2.92 (1.15-7.41)	9.85 (3.5-27.74)	<0.001*	1.34 (0.64-2.81)	3.69 (1.66-8.17)	<0.001*
Nuclear survivin = 10% + Radiotherapy	1.0	1.0	-	1.0	1.0	-
p53 < 10%	1.07 (0.43-2.67)	1.71 (0.67-4.36)	0.259	0.63 (0.32-1.24)	1.03 (0.51-2.07)	0.944
p53 < 10% + Radiotherapy	1.41 (0.73-2.75)	1.09 (0.54-2.18)	0.81	1.17 (0.73-1.87)	0.93 (0.57-1.53)	0.785
p53 = 10%	4.4 (1.86-10.42)	3.66 (1.47-9.15)	0.005*	2.85 (1.4-5.77)	2.29 (1.07-4.9)	0.032*
p53 = 10% + Radiotherapy	1.0	1.0	-	1.0	1.0	-

Protein expression was evaluated as the percentage of positive tumor cells. *Differences were considered significant when *p*-value<0.05. [a]Hazard Ratios were adjusted by age and histological grading.

Table 8: Twelve-months probability of survival of glioma patients' according to p53 and survivin expression and Radiotherapy

Characteristics	Absolute and relative frequencies (%)	12-month OS rate (%)	*p*-value
Cytoplasmic survivin < 10%	7 (6%)	86% (63-100)	0.004*
Cytoplasmic survivin < 10% + Radiotherapy	11 (9%)	46% (24-87)	
Cytoplasmic survivin = 10%	32 (27%)	37% (23-60)	
Cytoplasmic survivin = 10% + Radiotherapy	70 (58%)	53% (43-67)	
Nuclear survivin < 10%	23 (19%)	53% (35-80)	0.053[a]
Nuclear survivin < 10% + Radiotherapy	62 (52%)	51% (40-65)	
Nuclear survivin = 10%	16 (13%)	38% (20-70)	
Nuclear survivin = 10% + Radiotherapy	19 (16%)	56% (37-84)	
p53 < 10%	21 (18.5%)	65% (47-90)	0.002*
p53 < 10% + Radiotherapy	49 (43%)	46% (34-63)	
p53 = 10%	13 (11.5%)	25% (8-73)	
p53 = 10% + Radiotherapy	31 (27%)	57% (42-78)	

Protein expression was evaluated as the percentage of positive tumor cells. *Differences were considered significant when *p*-value<0.05. [a]Radiotherapy had a borderline impact on 12-months survival when the tumor presented nuclear survivin.

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