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ORIGINAL RESEARCH

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Fused in sarcoma (FUS) expression may predict survival outcomes of patients with advanced squamous cell cervical carcinoma

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Abstract

Background: There is an increasing number of studies addressing the relationship between Fused in Sarcoma (FUS) expression and cancer, owing to its role in preserving DNA/RNA stability. However, the disparity in FUS/*FUS* oncogenic *vs.* tumor-suppressive roles may be attributed to the complex molecular pathways associated with FUS regulation in different cancer types, and its role in cervical carcinogenesis remains largely unexplored. **Methods**: We determined FUS protein expression in specimens of 61 patients with advanced cervical cancer. Long-term (>10 years) clinical follow-up data for these patients were available, and we determined disease-free, cancer-related and overall survival as related to FUS expression. **Results**: There were no significant associations between FUS expression and patients' age, tumor grade, and acute/late toxicity events related to treatment (either radiation alone or chemoradiation). However, multivariate Cox regression analysis for disease-free survival (recurrence), overall survival (death) and cancer-related survival showed that patients with high average FUS expression fared significantly better than their counterparts with low average FUS expression, both in terms of disease-free survival (Hazard Ratio (HR) = 0.31; 95% Confidence Interval (CI): 0.12 to 0.77; $p = 0.01$) and cancer-related survival (HR = 0.41; 95% CI: 0.17 to 0.98; $p = 0.04$). **Conclusions**: Our study shows that high FUS protein expression in advanced cervical cancer specimens is a potent harbinger of better prognosis, and can as such be used in clinical practice to help characterize patients and, possibly, plan treatment and follow-up strategies.

Keywords

Fused in sarcoma; Cervical cancer; Prognosis; Long-term follow-up data

1. Introduction

RNA metabolism and DNA repair are regulated by several genes [1, 2]. In this context, the Fused in Sarcoma (*FUS*) oncogene, located on chromosome 16 and initially identified in human liposarcoma, has been described to encode a 526 amino acid DNA/RNA binding protein which seems to be involved in gene transcription, RNA transport and translation [3–5]. Of special interest in oncology, it seems that FUS helps safeguard genomic stability; during DNA damage repair, FUS is one of the proteins firstly recruited to the DNA damage site [6, 7]. Knockdown of *FUS* during cell growth leads to defects in DNA damage recovery [8]. Loss of FUS in the nucleus affects transcription, alternative splicing and also DNA repair [9]. The Cancer Genome Atlas (TCGA) Sarcoma Collection (TCGA-SARC) includes data on Fused in Sarcoma (FUS), a DNA/RNA binding protein involved in RNA metabolism and DNA repair. FUS combines prion-like properties with a multifunctional DNA/RNA-binding domain and regulates RNA metabolism, including transcription, pre-mRNA splicing, mRNA transport, and translation. FUS has been identified as a potential biomarker for sarcoma diagnosis and prognosis and further research on FUS and its role in sarcoma development and progression is ongoing [10].

Because of its role in preserving DNA stability, there is an increasing number of studies addressing the relationship between FUS expression and cancer, such as in cervical, brain (glioma), liposarcoma and lung cancers [11–15]. Conflicting results, however, have been reported, depending on the particular cancer type studied and whether FUS-related mRNA, protein expression, or genomic stability are being evaluated. For instance, C-terminal mutations in *FUS* have been shown to explain 5–10% of cases of familial amyotrophic sclerosis [16], but *FUS* mutations or single nucleotide polymorphisms

(SNPs) were not found in 96 cases of liposarcoma [13], leading the authors of that study to assume that mutations in *FUS* may not play a role in sarcomagenesis. Later, one study described that elevated FUS expression was negatively associated with prognosis of patients with non-small cell lung cancer [14], and one experimental study revealed that the knockdown of *FUS* inhibited the viability, migration and tube formation of glioma cells [12]. Further, studies on prostate and cervical cancer have suggested potential associations between FUS/*FUS*. Haile *et al.* [16] (2011) found that FUS is a co-activator of androgen receptor in prostate cancer [17], and Chen *et al*. [10] (2019) reported that circRNA_0000285 promotes cervical cancer by up-regulating FUS in human tissues [11].

In 2010, a group of 66 genes whose expression pattern differed between normal and E7-expressing cervical cancer cells was identified. Among these genes, *FUS* was up-regulated in high-grade squamous intraepithelial cells and invasive cervical cancer cells [18]. However, more than a decade later, the relationship between FUS and cervical carcinogenesis, as well as the relationship between FUS expression and cervical cancer behavior remain elusive. We took advantage of vast clinical follow-up data from a long-term randomized clinical trial-originally aimed at exploring the efficacy of chemoradiation versus radiation alone for the treatment of advanced (stage IIIB—International Federation of Gynecology and Obstetrics (FIGO) 2009) [19] cervical cancer—in order to further explore the association between FUS expression and cervical cancer prognosis [20, 21]. Based on the previous studies on the relationship between FUS and carcinogenesis, it was our hypothesis that FUS expression in cervical cancer cells could provide prognostic information. However, due to the heterogeneity of previous findings, it remains unclear whether such expression would be a harbinger or worse or better prognosis in clinical practice. The present report shows our findings on such relationships.

2. Material and methods

2.1 Selection of cases

For this study, we selected patients who had participated in a randomized clinical trial aimed at comparing radiotherapy alone (RT) *vs.* chemoradiotherapy (CRT) for the treatment of advanced cervical cancer (Concomitant cisplatin plus radiotherapy and high-dose-rate brachytherapy versus radiotherapy alone for stage IIIB squamous cell cervical cancer: a randomized controlled trial) [20]. This study was conducted in the Women's Hospital, CAISM (Centro de Atenção Integral à Saúde da Mulher), State University of Campinas (São Paulo, Brazil). The protocol was approved by the hospital institutional review board on 08 April 2003 and by the local research ethics committee on 16 September 2003 (Faculdade de Ciências Médicas da Unicamp, number 238/2003). All patients who met the inclusion criteria and provided written informed consent were invited to participate. The recruitment period was from September 2003 to July 2010, and patients were followed up until January 2018. For this present study, only patients with available paraffin-embedded cervical specimens were included. Trial and patient details were described elsewhere [20]. Of the 147 patients originally included in the trial, only 61 had cervical material available for analysis (31 in the CRT group, 30 in RT group). Ethics approval for the present analyses was obtained from regulatory authorities in Brazil (Research Ethics Committee approval CAAE# 55014816.5.0000.5404, 11 December 2017). All methods were carried out in accordance with guidelines and regulations from the hospital institutional review board and Ethics Committee. Follow-up assessments were performed every 4 months in the first 2 years following the treatment, every 6 months in the third year and therefore once a year.

2.2 Immunohistochemistry assay for FUS detection

The immunohistochemical assays were performed at the Cancer Innovation Laboratory, Centro de Investigação Translacional em Oncologia, Instituto do Câncer do Estado de São Paulo Octavio Frias de Oliveira—ICESP, Universidade de São Paulo, Brazil, by automated reaction using the UltraView Universal DAB Detection Kit® (Y09284, Ventana Medical Systems, Inc., Roche, Tucson, AZ, USA) and the Ventana BenchMark GX equipment (a powerful and versatile automated tissue preparation and staining instrument, Roche Diagnostics, Mannheim, BW, Germany), according to the manufacturer's instructions. In summary, the slides were submitted to an antigenic recovery process, carried out with a tris-based buffer, pH 8.0 (Ultra Cell Conditioning Solution—Roche Diagnostics, Y18099, Mannheim, BW, Germany) under heat for 30 min. Next, rabbit polyclonal antibodies directed against FUS protein (ab13533, Abcam, Cambridge, MA, USA) were used in a 1:1000 concentration for 32 min. Positive reactions were visualized with a cocktail (UltraView Universal HRP Multimer—Roche Diagnostics, Y15571, Mannheim, BW, Germany) containing peroxidase conjugated anti-mouse and anti-rabbit secondary antibodies in the presence of diaminobenzidine tetrahydrochloride (DAB), resulting in a brown precipitate. The slides were counterstained with hematoxylin for 20 min. This entire process was performed inside the Ventana BenchMark GX equipment in a closed system. Samples of cervical squamous cell carcinomas previously tested for FUS expression by immunohistochemistry were used as a reaction control and were incubated with or without anti-FUS antibodies (data not shown). The immunohistochemical positivity reaction was performed by a quantitative method using Image J® [22] and evaluated by an experienced pathologist blinded to clinical and pathological data.

2.3 Analysis of immunohistochemical reactions

Stained slides were evaluated by an experienced pathologist (LBEC). The pathologist logged the intensity of staining and the percentage of stained cells in the representative areas of squamous cell carcinoma. Evaluation covered the ratio of stained cells to the total number of cells and staining intensity in the representative areas of squamous cell carcinoma. Staining intensity was classified as 0 (no staining), 1 (weak), 2 (moderate) or 3 (strong) (Fig. 1). Microphotographs of the 3 best fields at medium magnification (×20) were taken from

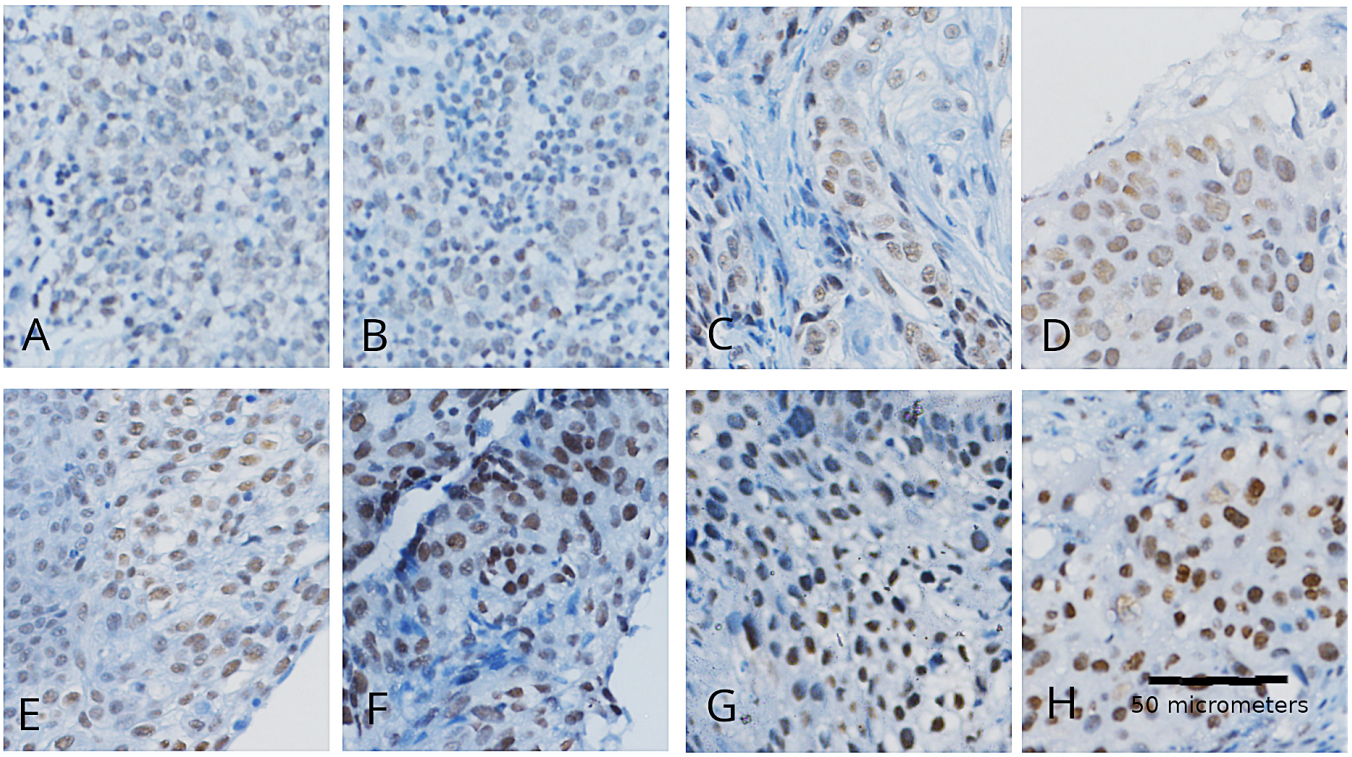

FIGURE 1. Staining intensity. (A) Squamous cell carcinoma with negative FUS staining (20×). (B) Nests of neoplastic cells exhibiting weak nuclear FUS staining and less than 50% of positive cells. (C) Nests of neoplastic cells exhibiting weak nuclear FUS staining and between 50–75% of positive cells. (D) Nests of neoplastic cells exhibiting weak nuclear FUS staining and more than 90% of positive cells. (E) Nests of neoplastic cells exhibiting moderate nuclear FUS staining and between 50–75% of positive cells. (F) Nests of neoplastic cells exhibiting moderate and difuse nuclear FUS expression. (G) Nests of neoplastic cells exhibiting strong nuclear FUS staining and between 50–75% of positive cells. (H) Nests of neoplastic cells exhibiting strong and diffuse nuclear FUS expression.

the lesions in each case, obtained through a 995 Nikon digital camera (Kingston Technology Corporation, Fountain Valley, CA, USA). Using the open source image processing software ImageJ [22] the counting of positive cells and the total number of cells per field were carried out in each picture, to obtain the positive cell ratio in each case. For statistical reasons, the percentage of positive cells was further categorized into <50%, 50–75% and >90%. Finally, we multiplied the percentage of stained cells by staining intensity, in three distinct fields, and calculated the average of that product (hereinafter referred to as average of three fields).

2.4 Statistical analysis

In order to determine the best threshold for the average of three fields to diagnose recurrence, we produced a Receiver Operating Characteristic (ROC), with the resulting optimal cutoff point being 0.233. This cutoff point was used in subsequent analysis to separate patients into two groups, according to the average of three fields (Low if ≤0.233 and High if >0.233). In Tables 1 and 2, we compared the frequencies of key patients' and tumor characteristics as related to either staining intensity, percentage of stained cells and average expression of 3 fields using Chi-squares (or Fisher's exact test where appropriate). In Table 3, we used Cox Proportional Hazard models to evaluate the disease-free, overall survival and cancer-related survival of the patients, as related to FUS expression (average of 3 fields), age, tumor grade and trial allocation group. All calculations were performed using the R Environment for Statistical Computing [23], assuming $p < 0.05$ (95% confidence intervals) as significant.

3. Results

Table 1 shows FUS protein expression characteristics (staining intensity, percentage of stained cells and the average expression of 3 fields) as related to patient age, tumor grade, treatment allocation (either CRT or RT), acute and late toxicity events. There were no significant associations between any of FUS expression characteristics and the variables analyzed.

In Table 2, we show the relation between FUS expression characteristics and disease outcomes. A low average FUS expression in 3 fields was marginally associated with disease relapse (57.7% of the women with low average expression relapsed, contrasted to only 31.4% of those with high average expression) ($p = 0.07$) and local recurrence (34.6% *vs.* 11.4%, respectively) ($p = 0.05$). In addition, 93.8% of the patients with low average FUS expression died due to cancer, contrasted to only 66.7% of the patients with high average FUS expression, but this association was not formally significant ($p = 0.09$).

TABLE 1. Expression of FUS protein according to patient's age, tumor grade, treatment performed and toxicity.

	n	Staining intensity			Percentage of stained cells				Average expression of 3 fields		
		Weak n (%)	Moderate/Strong n (%)	*p*	<50% n (%)	50–75% n (%)	>90% n (%)	*p*	Low n (%)	High n (%)	*p*
Age (yr)											
<50	19	13 (27.1)	6 (53.8)	0.23$^{\alpha}$	10 (27.0)	5 (55.6)	4 (26.7)	0.27$^{\beta}$	5 (19.2)	14 (40)	0.12$^{\alpha}$
≥50	42	35 (72.9)	7 (46.2)		27 (73.0)	4 (44.4)	11 (73.3)		21 (80.8)	21 (60)	
Tumor grade											
1	4	3 (6.4)	1 (7.7)		1 (2.8)	2 (22.2)	1 (6.7)		0 (0.0)	4 (11.4)	
2	45	35 (74.5)	10 (76.9)	1.00$^{\beta}$	28 (77.8)	5 (55.6)	12 (80.0)	0.28$^{\beta}$	19 (76.0)	26 (74.3)	0.15$^{\beta}$
3	11	9 (19.1)	2 (15.4)		7 (19.4)	2 (22.2)	2 (13.3)		6 (24.0)	5 (14.3)	
Missing*		1			1				1		
Treatment											
CRT	31	23 (47.9)	8 (61.5)	0.57$^{\alpha}$	16 (43.2)	8 (88.9)	7 (46.7)	0.05$^{\beta}$	12 (46.2)	19 (54.3)	0.71$^{\alpha}$
RT	30	25 (52.1)	5 (38.5)		21 (56.8)	1 (11.1)	8 (53.3)		14 (53.8)	16 (45.7)	
Acute toxicity											
Yes	16	13 (28.3)	3 (27.3)	1.00$^{\beta}$	12 (33.3)	1 (12.5)	3 (23.1)	0.57$^{\beta}$	10 (40.0)	6 (18.8)	0.14$^{\alpha}$
No	41	33 (71.7)	8 (72.7)		24 (66.7)	7 (87.5)	10 (76.9)		15 (60.0)	26 (81.2)	
Missing*		2	2		1	1	2		1	3	
Late toxicity*											
Yes	25	21 (55.3)	4 (66.7)	0.53$^{\beta}$	17 (45.9)	2 (22.2)	6 (46.2)	0.44$^{\beta}$	10 (38.5)	15 (45.5)	0.78$^{\alpha}$
No	34	26 (44.7)	8 (33.3)		20 (54.1)	7 (77.8)	7 (53.8)		16 (61.5)	18 (54.5)	
Missing*		1	1				2			2	

CRT: Chemoradiotherapy; RT: Radiotherapy.

**For some patients there was no available information on tumor grade, acute or late toxicities. Missing information is listed for each category. Statistical calculations are based on available data.*

$^{\alpha}$*Chi-squared test.* $^{\beta}$*Fisher's exact test.*

TABLE 2. Expression of FUS protein according to the occurrence of relapse, local recurrence, distant recurrence, death and death due to cancer.

	n	Staining intensity			Percentage of stained cells				Average expression of 3 fields		
		Weak n (%)	Moderate/Strong n (%)	*p*	<50% n (%)	50–75% n (%)	>90% n (%)	*p*	Low n (%)	High n (%)	*p*
Relapse											
Yes	26	20 (41.7)	6 (46.2)	1.00[α]	17 (45.9)	3 (33.3)	6 (40.0)	0.81[β]	15 (57.7)	11 (31.4)	0.07[α]
No	35	28 (58.3)	7 (53.8)		20 (54.1)	6 (66.7)	9 (60.0)		11 (42.3)	24 (68.6)	
Local recurrence											
Yes	13	10 (20.8)	3 (23.1)	1.00[β]	10 (27.0)	2 (22.2)	1 (6.7)	0.32[β]	9 (34.6)	4 (11.4)	0.05[β]
No	48	38 (79.2)	10 (76.9)		27 (73.0)	7 (77.8)	14 (93.3)		17 (65.4)	31 (88.6)	
Distant recurrence											
Yes	18	12 (25.0)	6 (46.2)	0.25[α]	9 (24.3)	3 (33.3)	6 (40.0)	0.52[β]	7 (26.9)	11 (31.4)	0.92[α]
No	43	36 (75.0)	7 (53.8)		28 (75.7)	6 (66.7)	9 (60.0)		19 (73.1)	24 (68.6)	
Death											
Yes	34	26 (54.2)	8 (61.5)	0.87[α]	18 (48.6)	7 (77.8)	9 (60.0)	0.26[α]	16 (61.5)	18 (51.4)	0.59[α]
No	27	22 (45.8)	5 (38.5)		19 (51.4)	2 (22.2)	6 (40.0)		10 (38.5)	17 (48.6)	
Death due to cancer											
Yes	27	20 (76.9)	7 (87.5)	1.00[β]	16 (88.9)	5 (71.4)	6 (66.7)	0.38[β]	15 (93.8)	12 (66.7)	0.09[β]
No	7	6 (23.1)	1 (12.5)		2 (11.1)	2 (28.6)	3 (33.3)		1 (6.2)	6 (33.3)	

[α] *Chi-squared test.* [β] *Fisher's exact test.*

TABLE 3. Multivariate Cox regression analysis for disease-free survival, overall survival and overall survival due to cancer according to patient's age, tumor grade, treatment performed and average expression of 3 fields.

Categories	Disease-free survival			Overall Survival			Cancer-related survival		
	HR[#]	95% CI	*p*-value	HR[##]	95% CI	*p*-value	HR[###]	95% CI	*p*-value
Age (yr)									
≥50	Ref.	(1.26 to 9.45)	0.01	Ref.	(0.80 to 4.58)	0.14	Ref.	(1.41 to 10.42)	0.008
<50	3.45			1.92			3.83		
Tumor grade*									
1 and 2	Ref.	(0.56 to 3.98)	0.42	Ref.	(0.62 to 3.11)	0.42	Ref.	(0.55 to 3.61)	0.46
3	1.49			1.39			1.41		
Treatment									
CRT	Ref.	(1.01 to 5.39)	0.05	Ref.	(0.59 to 2.58)	0.56	Ref.	(0.89 to 4.64)	0.09
RT	2.30			1.24			2.03		
Average expression of 3 fields									
Low	Ref.	0.12 to 0.77	0.01	Ref.	(0.29 to 1.46)	0.30	Ref.	(0.17 to 0.98)	0.04
High	0.31			0.65			0.41		

**The evaluation of FUS expression was based on the percentage of stained cells and the staining intensity.*
[#]HR = 26 patients relapsed during observation.
[##]34 patients died during observation.
[###]27 patients died due to cancer during observation.
CI: confidence interval of 95%; HR: Hazard Ratio; Ref.: referential; CRT: Chemoradiotherapy; RT: Radiotherapy.

Table 3 shows the multivariate Cox regression analysis for disease-free survival (recurrence), overall survival (death) and cancer-related survival according to patient's age, tumor grade, treatment performed and average expression of 3 fields. Patients with high average FUS expression fared significantly better than their counterparts with low average FUS expression, both in terms of disease-free survival (HR = 0.31; 95% CI: 0.12 to 0.77; $p = 0.01$) and cancer-related survival (HR = 0.41; 95% CI: 0.17 to 0.98; $p = 0.04$). In addition, women allocated to the RT-only group had a worse disease-free survival compared to those allocated to the CRT group (HR = 2.30; 95% CI: = 1.01 to 5.39; $p = 0.05$). Younger (<50 years) women also had a poorer prognosis contrasted to their older counterparts: disease free-survival (HR = 3.45, 95% CI: 1.26 to 9.45; $p = 0.01$) and cancer-related survival (HR = 3.83; 95% CI: 1.41 to 10.42; $p = 0.008$).

4. Discussion

After examining our long-term prospective data, we were able to infer that patients with advanced cervical cancer with high FUS expression in their pathological samples had significantly better disease-free and cancer-related survival probabilities compared to those without a high FUS expression level. Other factors associated with survival were age <50 years and being treated with radiotherapy alone, but the association between FUS expression and survival persisted even after adjustments for age and the treatment allocation. We also found that FUS expression must be determined at several hotspot expression points, since only when examining the average expression of 3 fields we obtained a significant association with survival. Some previous studies focused on the laboratorial aspects of FUS interaction with cancer [24–26], and less than a handful examined the clinical implications of FUS expression. In this regard, our study is a major stride towards understanding whether FUS protein expression may be used as a tool to prognosticate cervical cancer, since we used a large clinical database on prospectively followed cervical cancer patients to evaluate whether the molecule bore a relationship with survival.

It is worth noting that, in relation to cervical cancer, knowledge about biomarkers is still incipient when compared to that related to other neoplasms. One of the potential markers to be used in the characterization and clinical management of cervical neoplasms is the oncogene FUS, also known as TLS (Translocated in Liposarcoma), which was initially identified in liposarcomas as a fusion protein (FUS-CHOP) caused by a chromosomal translocation. It is a nucleoprotein involved in DNA and RNA metabolism, including DNA repair, and the regulation of transcription, RNA splicing, and export to the cytoplasm. Translocation in the transcriptional activation domain results in protein fusion and has been implicated in carcinogenesis in numerous neoplasms [27]. The FUS protein has 526 amino acids and is encoded by 15 exons located on chromosome 16. Studies have already shown that it binds to DNA, RNA and proteins, acting in several stages, from gene expression to protein translation [28]. In 1994, a group of researchers discovered that the FUS protein acts as a transcriptional activator of oncogenic fusion proteins, raising the hypothesis that FUS could be linked to the regulation of cellular transcription [29]. Subsequently, several studies demonstrated the interaction between FUS, RNA-polymerase II and TFIID (a transcription factor that is involved in the transcription initiation complex), suggesting that the FUS protein exerts an influence on cellular transcription in general [7, 10, 30–36].

It is important to highlight that our results somewhat contradict previous laboratory findings on how FUS operates in cervical cancer. Recently, a Chinese study showed that the expression level of FUS was positively correlated with the expression of circRNA_0000285 and, subsequently, that the knockdown of circRNA_0000285 significantly inhibited the formation and metastasis of cervical cancer in nude mice. We must emphasize, however, that those authors did not test for a direct relationship between FUS expression at the protein level and cervical cancer proliferation and metastasis [11]. On the other hand, in an attempt at summarizing data about FUS gene expression in association with prostate cancer, Ghanbarpanah and cols. [37] reported that FUS may prevent the growth of prostate cancer cells by down-regulating proliferator factors such as Cdk6 and cyclin D1, and up-regulating Cdk and p27. Further, an immuno-histochemical analysis showed that FUS expression had an inverse relationship with the degree of prostate cancer, which in turn suggests that patients whose tumors have a high FUS expression may experience less bone pain and theoretically enjoy a longer survival. These findings are in alignment with those from a study on cell cultures published in 2010 [38], in which the authors posited that FUS has some features of a putative tumor suppressor: FUS overexpression promoted growth inhibition and apoptosis of prostate cancer cells, whereas its knockdown led to prostate cancer cell proliferation.

Two studies on hepatocarcinoma have also suggested a positive association between FUS expression and better prognosis. Ma and colleagues [39] examined the association between miR-378 expression and liver cancer cell migration using real-time quantitative polymerase chain reaction (PCR), and found that miR-378 overexpression enhanced cell proliferation, migration and liver cell invasion by down-regulating FUS expression. Further, Bao and colleagues [40] examined the effects *FUS* has on hepatocellular carcinoma progression in HuH7 and MHCC97 cells, and found that overexpression of *FUS* decreased cell viability, migration, invasion and stemness, in addition to activating the Hippo pathway, which in turn is an important signaling pathway that regulates organ growth and tissue size [41]. All those phenomena associated with *FUS* overexpression resulted in significant inhibition of hepatocellular carcinoma progression.

The small sample size is a weakness of this study because it prevented the analysis of more variables, such as other prognostic factors. However, it should be noted that our study also provides long-term (in excess of ten years) fully annotated follow-up data, derived from a strictly controlled trial aimed at observing survival differences in patients who underwent either CRT or RT for the treatment of advanced cervical cancer. It is important to emphasize that the conditions that allowed the execution of the present study are no longer extant: the incidence of cervical cancer has been declining sharply in recent years, due to improvements in patients' liv-

ing conditions, screening and human papillomavirus (HPV) vaccine availability. Thus, this is an opportunistic analysis that takes full advantage of available data derived from prior studies. Since the availability of histological samples for immunohistochemistry is limited, and the inclusion of new cases is only feasible in a timespan that will mount to decades, the limitations in sample size are untreatable. These considerations notwithstanding, this study showed a clear statistical association between FUS expression and the prognosis of cervical cancer, which strongly suggests the existence of a relevant biological event.

5. Conclusions

In synthesis, our study suggests an association between FUS protein expression and cervical cancer prognosis. As mentioned above, the disparity in FUS/*FUS* oncogenic *vs.* tumor-suppressive roles may be attributed to the complex molecular pathways associated with FUS regulation in different cancer types. In the context of cervical cancer, owing to our prospective dataset, this study suggests that FUS acts as a tumor suppressor. Low cost approaches such as immunohistochemistry can be used in clinical practice to determine FUS protein expression in cervical cancer specimens, which as demonstrated here may be a predictor of longer survival in advanced cases. Confirmatory data from larger studies is pending.

AVAILABILITY OF DATA AND MATERIALS

The data presented in this study are available on request from the corresponding author. The data are not publicly available due to ethical restrictions.

AUTHOR CONTRIBUTIONS

AMDF, ACZ, MCRT, EB, LT, SHRS, LCZ—Conception and design. AMDF, MCRT, RALN, GÁFS—Collection and assembly of data. AMDF, LOS, LBEC, LCZ—Data analysis and interpretation. All authors contributed to manuscript writing. All authors gave final approval of the manuscript.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Ethics approval for the present analyses was obtained from regulatory authorities in Brazil (Research Ethics Committee approval CAAE# 55014816.5.0000.5404, 11 December 2017). All tissue samples were originally collected for diagnostic purposes and were thoroughly anonymized before the use in this study. The institutional ethics committee waived the need for signed consent because this was a risk-free retrospective study and it was no longer possible to contact many of the enrolled women. Therefore, the analysis carried out in this study does not imply any change in the clinical follow-up and medical conduct.

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CONFLICT OF INTEREST

The authors declare no conflict of interest. Luís Otávio Sarian is serving as one of the Editorial Board members of this journal. We declare that Luís Otávio Sarian had no involvement in the peer review of this article and has no access to information regarding its peer review. Full responsibility for the editorial process for this article was delegated to AEM.

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