TOP2A copy number and TOP2A expression in uterine benign smooth muscle tumours and leiomyosarcoma

Glauco Baiocchi,[1] Fernando Luís Visoni Poliseli,[1] Louise De Brot,[2] Henrique Mantoan,[1] Beatriz Nunes Schiavon,[1] Carlos Chaves Faloppa,[1] Jose Vassallo,[2] Fernando Augusto Soares,[2] Isabela Werneck Cunha[2]

▶ Additional material is published online only. To view please visit the journal online (http://dx.doi.org/10.1136/jclinpath-2015-203561).

[1]Department of Gynecologic Oncology, AC Camargo Cancer Center, Sao Paulo, Brazil
[2]Department of Anatomic Pathology, AC Camargo Cancer Center, Sao Paulo, Brazil

Correspondence to
Dr Glauco Baiocchi, Departamento de Ginecologia Oncológica, AC Camargo Cancer Center, Rua Antonio Prudente, 211, São Paulo 01509-010, Brazil; glbaiocchi@yahoo.com.br, glauco.baiocchi@accamargo.org.br

Received 8 December 2015
Revised 15 February 2016
Accepted 3 March 2016
Published Online First 18 March 2016

ABSTRACT

Aims To examine *TOP2A* copy number, TOP2A expression, and its prognostic value in uterine leiomyosarcoma (LMS) and other benign smooth muscle tumours.

Methods We analysed 37 patients treated for uterine LMS with immunohistochemistry for protein expression and fluorescence in situ hybridisation (FISH) for copy number. Twelve cases of leiomyoma variants (LMVs), 4 smooth muscle tumours of uncertain malignant potential (STUMP) and 23 leiomyomas (LMs) were also included.

Results Eighteen patients with LMS (48.6%) were International Federation of Gynecology and Obstetrics (FIGO) stage I, six (16.2%) were stage II, four (10.8%) were stage III, and nine (24.3%) were stage IV. Twenty-one (56.8%) patients with LMS showed high expression of TOP2A. Greater TOP2A levels were found in patients with stage ≥II disease compared with stage I and also in high mitotic index tumours (>20/10 HPF (high power field)). Eleven (36.7%) cases had abnormal *TOP2A* copy numbers. There was no link between *TOP2A* copy number and TOP2A expression. All patients with benign smooth muscle tumours had low TOP2A immunohistochemical expression and one (7.7%) patient had *TOP2A* amplification. TOP2A expression and *TOP2A* copy number had no impact on disease outcomes. Only the presence of disease outside of the uterus negatively impacted survival compared with early disease (53.4 vs 15.8 months; p<0.001).

Conclusions TOP2A is highly expressed in advanced LMS but not in non-malignant diseases. TOP2A expression does not correlate with FISH results and does not predict outcome. TOP2A levels are higher in high-mitotic index tumours and in more advanced stages of disease.

INTRODUCTION

Uterine sarcomas are rare tumours that account for 3–7% of uterine cancers; uterine leiomyosarcoma (LMS) constitutes approximately 40% of all uterine sarcomas.[1] Most women with LMS present with a tumour that is limited to the pelvis. However, even after complete resection, the risk for recurrence in high-grade LMS is 50–80% at 2 years.[1–3]

The minimum pathological criteria for a diagnosis of LMS is usually problematic, because in certain cases, it might fail to be differentiated from various benign smooth muscle uterine tumours that have atypical histologic features and unusual growth patterns and from smooth muscle tumours of uncertain malignant potential (STUMPs). Since 2003, the WHO diagnostic criteria[4] have been used to distinguish these unusual histologic variants of leiomyoma (LM), which have been previously misdiagnosed as well differentiated or low-grade LMS.

Although the aggressiveness of uterine sarcomas is well recognised, their histopathological diversity and rarity have contributed to the lack of a consensus on their prognostic factors and optimal treatment.[1 5] Recently, several immunohistochemical and molecular markers of LMS have been reported,[6–10] which might improve classification, sarcogenesis knowledge and guide target-driven therapies for LMS.

TOP2A encodes for an essential enzyme in the regulation of DNA structure and cell proliferation; it is a direct molecular target of anthracyclines.[11–14] Previous studies have reported its function in carcinogenesis and in the chemotherapeutic response in breast cancer and other primary tumours.[15–20] However, TOP2A function has not been examined with regard to uterine LMS.

Our aim was to determine the prognostic value of TOP2A immunohistochemical expression and gene amplification status in LMS. We also examined the diagnostic value of TOP2A in other benign uterine smooth muscle tumours, such as LMs, leiomyomas variants (LMVs) and STUMPs.

MATERIALS AND METHODS

Patient characteristics

This retrospective analysis included individuals with uterine LMS who were admitted to the Department of Gynecologic Oncology, AC Camargo Cancer Center, from January 1982 to December 2010. The institutional review board approved this study. Clinical data were retrieved from the medical records.

All pathology slides were reviewed. Of 43 uterine LMS cases with paraffin blocks that were available in our files, 37 were classified as LMS after histological review and 6 were reclassified as LMV or STUMP, as per WHO criteria.[4] We included an additional three STUMP cases and six LMVs. The final analysis comprised 12 cases of LMVs, 4 STUMPs, and 37 LMSs. The LMVs were five mitotically active LMs, four cellular LMs and three LMs with bizarre nuclei.[4]

Tissue microarray construction

H&E-stained sections of malignant and non-malignant specimens were reviewed, and the areas

To cite: Baiocchi G, Poliseli FLV, De Brot L, *et al*. *J Clin Pathol* 2016;**69**:884–889.

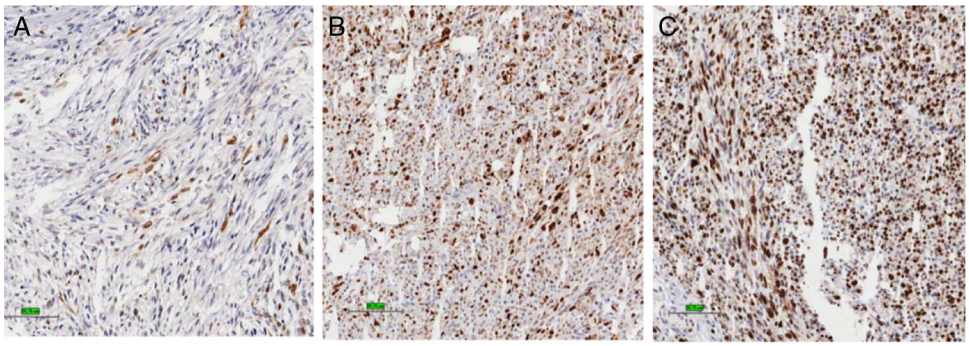

Figure 1 Microphotography of TOP2A immunohistochemical expression. (A) 1+ (1–25% positive cells); (B) 2+ (26–50% positive cells); (C) 3+ (>50% positive cells).

of the tumours were marked on the slides. For each paraffin block, two tissue cores (1 mm in diameter) were sampled from each marked area in the donor block and mounted into a recipient paraffin block using a custom-made instrument (Beecher Instruments, Silver Springs, Maryland, USA). In the resulting block, the tissue cylinders were aligned and marked for identification on a chart. Cores were spaced at intervals of 0.2 mm.

Immunohistochemical staining

Five-micrometer tissue microarray construction (TMA) sections were transferred to an adhesive-coated slide system (Instrumentics Inc, Hackensack, New Jersey, USA), and antigens were detected using a second-generation biotin-free polymer detection system (Advance; Dako), as described.[21] The reactions were accompanied by a positive control and two negative controls—one that lacked the primary antibody and another that lacked the secondary antibody.

The primary antibody for TOP2A (monoclonal, clone 1E2; Gene Tex, Irvine, California, USA, diluted to 1:800) generated nuclear immunostaining, which was evaluated semi-quantitatively, based on the percentage of positive cells. The section was scored 0 when there was no stain; 1 for nuclear staining in 1–25% of tumour cells; 2 if 26–50% of tumour cells were stained; and 3 if >50% of tumour cells were positive.[21] A score was calculated as the mean of two cores. Samples were considered to be positive if they received a score of ≥2 and negative if <2. All slides were analysed by a single pathologist (IWC) who was blinded to the clinical data by light microscopy (figure 1).

Fluorescence in situ hybridisation

TMA sections were analysed by fluorescence in situ hybridisation (FISH) using triple probes to TOP2A, HER-2/neu and the centromeric region of chromosome 17 (Kreatech Diagnostics, Amsterdam, the Netherlands). Briefly, deparaffinised and hydrated sections were incubated for 20 min at room temperature, 30 min at 82°C in 0.2 mmol/L HCl, 2 min in distilled water and 3 min in 29 citrate buffer. Next, the slides were subjected to protease digestion, immersed in 10% buffered formalin for 10 min, dehydrated and incubated with the probes—initially at 75°C for 5 min and then at 37°C overnight. The next day, the slides were rinsed in posthybridisation solution for 3 min at 74°C, and the nuclei were counterstained with DAPI.

Slides were analysed on a fluorescence microscope (BX61; Olympus, Center Valley, Pennsylvania, USA), and the images were captured on a Q-Color 5 Olympus digital camera. For each case, 50 cells were counted, and the final results were scored with regard to normal gene count (N), gene amplification (A), gene deletion (D) and chromosomal polysomy (P). Amplification was recorded when the ratio of signals between the target gene and centromeric marker exceeded 2, trisomy was defined as a frequency of centromeric and gene signals of 3, and polysomy was a frequency of centromeric and gene marker signals of more than 4 per cell, but with a ratio equal to 1 (figure 2).

Statistical analysis

The database was constructed using SPSS, V.20.0 for Mac (SPSS, Inc., Chicago, Illinois, USA). Follow-up time was the interval from the date of surgery to the last date for which information was available. The associations between immunostaining and other variables were analysed by χ^2 or Fisher's exact test. Survival curves were generated using the Kaplan–Meier method and compared by log-rank test. The associations between mean scores for benign and malignant smooth cell tumours were analysed by non-parametric Kruskal–Wallis test. For patients with

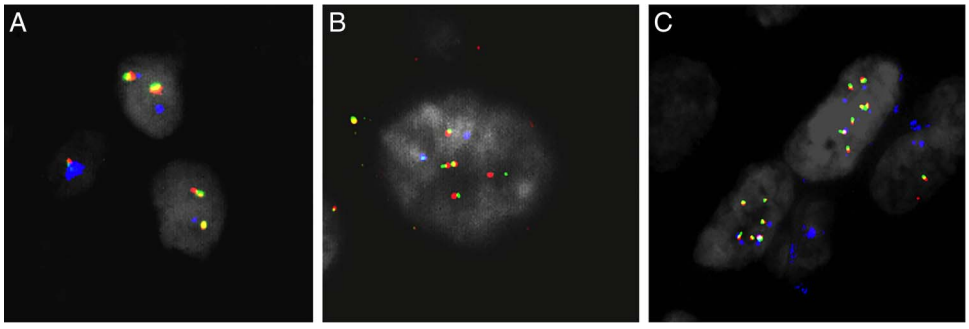

Figure 2 Microphotography showing fluorescence in situ hybridisation (FISH) where *TOP2A* is marked in green, *HER2* gene in red and chromosome 17 centromeres in blue. (A) Case of leiomyosarcoma with no *TOP2A* gene amplification; (B) case of leiomyosarcoma with *TOP2A* gene amplification; (C) case of leiomyosarcoma with chromosome 17 polysomia.

LMS, overall survival was defined as the time from surgery to the date of death or last follow-up. For all tests, a significance level of 5% was assumed.

RESULTS

Clinical characteristics

The clinical and pathological variables of our study sample are summarised in table 1.

The median age of patients with LMS was 50.2 years (range 27–84), and 20 (54.1%) patients had postmenopausal status. The most common primary symptoms were vaginal bleeding in 10 (27%) and pelvic mass in 4 (10.8%) patients. Sixteen (43.2%) patients had their first surgery at our institution. Four (10.8%) patients had enlarged lymph nodes that were detected during surgery, and lymph node dissection was performed in five (13.9%) cases.

Eighteen (48.6%) cases had International Federation of Gynecology and Obstetrics (FIGO) stage I disease, six (16.2%) had stage II disease, four (10.8%) had stage III disease, and nine (24.3%) had stage IV disease. Sixteen (43.2%) patients received adjuvant chemotherapy—five with anthracyclines—and six (17.1%) patients received adjuvant radiotherapy.

Four (22.2%) patients with stage I disease received adjuvant chemotherapy, none of whom received anthracyclines. Of the patients with stage II disease, three (50%) received chemotherapy (two with anthracyclines). All patients with stage III disease received chemotherapy without anthracyclines. Five (77.8%) patients with stage IV disease received palliative chemotherapy —three with anthracyclines and four without. Two patients with stage IV disease received only palliative treatment after surgery due to poor performance status.

After a median follow-up of 21.4 months (range 1–184), 26 (70.3%) patients died from disease, 2 (5.4%) deaths were related to treatment and 1 (2.7%) died due to other causes. Six (16.2%) patients were alive with no evidence of disease and two were lost to follow-up (5.4%).

Regarding benign smooth muscle tumours, the median age of patients with LM was 46.8 years (range 26–74) and the median tumour size was 4.5 cm (range 1.2–11). The median age of patients with LMV (n=12) and STUMP (n=4) was 44.6 years (range 27–59) and the median tumour size was 9.4 cm (range 2.5–35). Three patients with LMV experienced a local recurrence and all were treated with surgery. Notably, because two of these cases were formerly considered LMSs, one also received radiotherapy and the other received chemotherapy after surgery. At the last follow-up, they were alive and free of disease after 184 and 118 months, respectively, corroborating the diagnosis of LMV.

There was no difference in age (p=0.24) or tumour size (p=0.08) between patients with LM and LMV/STUMP. Patients with LMSs were older (mean 52.9 vs 45.5 years; p=0.01) and their tumours were larger (mean 12.4 vs 6.9 cm; p=0.041) compared with those with benign smooth muscle disease (LM and LMV/STUMP) (table 2).

Pathological characteristics

Of the patients with LMSs, the median tumour size was 8 cm (range 1–60); high cellularity was found in 25 (67.6%) cases and intense atypia was seen in 19 (51.4%) cases. Thirteen (35.1%) cases had a mitotic index >20/10 HPF (high power field), and 34 (91.8%) cases developed coagulative necrosis (table 1).

We observed high immunohistochemical expression of TOP2A in 21 (56.8%) cases with LMSs and low expression in 16 (43.2%). TOP2A expression correlated with a mitotic index >20/10 HPF (p=0.036) and the presence of extrauterine disease (≥ stage II) (p=0.033) but was unrelated to other clinical-pathological parameters, such as age, tumour size, cellularity, atypia grade and the presence of necrosis (table 3).

Table 1 Clinical and pathological characteristics of the 37 patients with uterine leiomyosarcoma

Variable	No. of patients (%)
Post menopause	
No	17 (45.9)
Yes	20 (54.1)
Symptoms	
Asymptomatic	4 (10.8)
Pelvic pain	10 (27.0)
Vaginal bleeding	15 (40.5)
Vaginal discharge	1 (2.7)
Pelvic mass	4 (10.8)
NA	3 (8.1)
Presence of lymph node enlargement	
No	19 (51.4)
Yes	4 (10.8)
NA	14 (37.8)
Lymph node dissection	
No	32 (86.4)
Yes	5 (13.6)
FIGO stage	
I	18 (48.6)
II	6 (16.2)
III	4 (10.8)
IV	9 (24.3)
Adjuvant chemo	
No	19 (54.3)
Anthracyclines	6 (17.1)
No anthracyclines	10 (28.6)
Adjuvant EBRT	
No	29 (82.9)
Yes	6 (17.1)
Adjuvant HDR	
No	31 (88.6)
Yes	4 (11.4)
Atypia grade	
Mild/moderate	18 (48.6)
Intense	19 (51.4)
Coagulative necrosis	
Absence	3 (8.2)
Presence	34 (91.8)
Mitotic index	
≤20/10 HPF	13 (35.1)
>20/10 HPF	24 (64.9)
High cellularity	
No	12 (32.4)
Yes	25 (67.6)
TOP2A expression	
Low	16 (43.2%)
High	21 (56.8%)
TOP2A gene	
Normal	19 (63.3%)
Amplification	8 (26.6%)
Polysomia	2 (6.6%)
Trisomy	1 (3.3%)

EBRT, external beam radiotherapy; HPF, high power field; NA, data not evaluable.

Table 2 Clinical and pathological characteristics of the patients with uterine LMs, LMVs/STUMP and leiomyosarcomas

	LM	LMV/STUMP	LMS	p Value
Age, years (range)	46.8 (26–74)	44.6 (27–59)	50.2 (27–84)	0.01
Tumour size, cm (range)	4.5 (1.2–11)	9.4 (2.5–35)	8 (1–60)	0.041
TOP2A high expression	0	0	21 (56.8%)	
Altered *TOP2A* gene	0	1 (7.7%)*	11 (36.6%)†	

*Case of leiomyoma variant (leiomyoma with bizarre nuclei); 13 cases were suitable for FISH analysis.
†30 cases of leiomyosarcomas were suitable for FISH analysis.
FISH, fluorescence in situ hybridisation; LM, leiomyoma; LMV, leiomyoma variant; LMS, leiomyosarcoma; STUMP, smooth muscle tumour of uncertain malignant potential.

Thirty cases were suitable for FISH analysis. We noted gene amplification in eight cases (26.6%), polysomia in two cases (6.6%) and trisomy in one patient (3.3%). In 19 subjects (63.3%), the results were normal. The presence of copy number alterations was not associated with TOP2A immunohistochemical expression (p=0.44). Further, *TOP2A* copy number did not correlate with disease stage, tumour size, mitotic index, cellularity, atypia grade or the presence of necrosis.

All patients with LM had low TOP2A immunohistochemical expression and normal *TOP2A* copy numbers. Of the patients with atypical LM and STUMP, three did not have samples that were suitable for FISH analysis. One patient (7.7%) (LM with bizarre nuclei) showed amplification of *TOP2A*.

Survival analysis—LMSs

The median overall survival was 21.6 months (95% CI 17.0 to 26.1), and the 2-year and 5-year overall survival rates were 44% and 22.6%, respectively. There was no difference in median overall survival related to the presence of pelvic or extrapelvic disease (stages II and III) or the presence of visceral disease (stage IV) (15.9 vs 11.8 months; p=0.45). Only the presence of any extrauterine disease (≥ stage II) negatively impacted survival (p<0.001) (figure 3). Moreover, the median overall survival for patients with disease that was restricted to the uterus (stage I) and extrauterine disease (≥ stage II) was 53.4 and 15.8 months, respectively. No other clinical or pathological variable, including TOP2A expression (figure 3) and gene alterations, was linked to survival (table 4). TOP2A expression did not correlate with survival when early and advanced stage disease samples were analysed separately.

In the disease-free survival analysis, we excluded patients with visceral disease (stage IV). The overall disease-free survival was 16.9 months (95% CI 6.8 to 26.9), and the 2-year disease-free survival rate was 32%. The median disease-free survival for patients with stage I disease was 24 months (2-year rate of 46.4%) versus 11.0 months for those with stage II/III disease (p=0.002). No other variable, including TOP2A expression and gene alterations, impacted disease-free survival.

Table 3 Association between clinical-pathological variables and TOP2A immunohistochemical expression for the 37 patients with uterine leiomyosarcomas

		TOP2A expression (No. of patients)		
Variable	**Category**	**Low**	**High**	**p Value**
FISH *TOP2A*	Normal	9	10	0.44
	Abnormal	3	8	
FIGO stage	I	11	7	0.033
	II, III, IV	5	14	
Tumour size	≤8 cm	7	6	0.84
	>8 cm	6	6	
Cellularity	Low	7	5	0.19
	High	9	16	
Nuclear atypia	Grade 1 and 2	9	9	0.41
	Grade 3	7	12	
Mitotic index	≤20/10 HPF	9	4	0.036
	>20/10 HPF	7	17	

FISH, fluorescence in situ hybridisation; HPF, high power field.

DISCUSSION

LMSs are very aggressive tumours that have a poor prognosis, even for early-stage disease.[1 3 22] The principal treatment

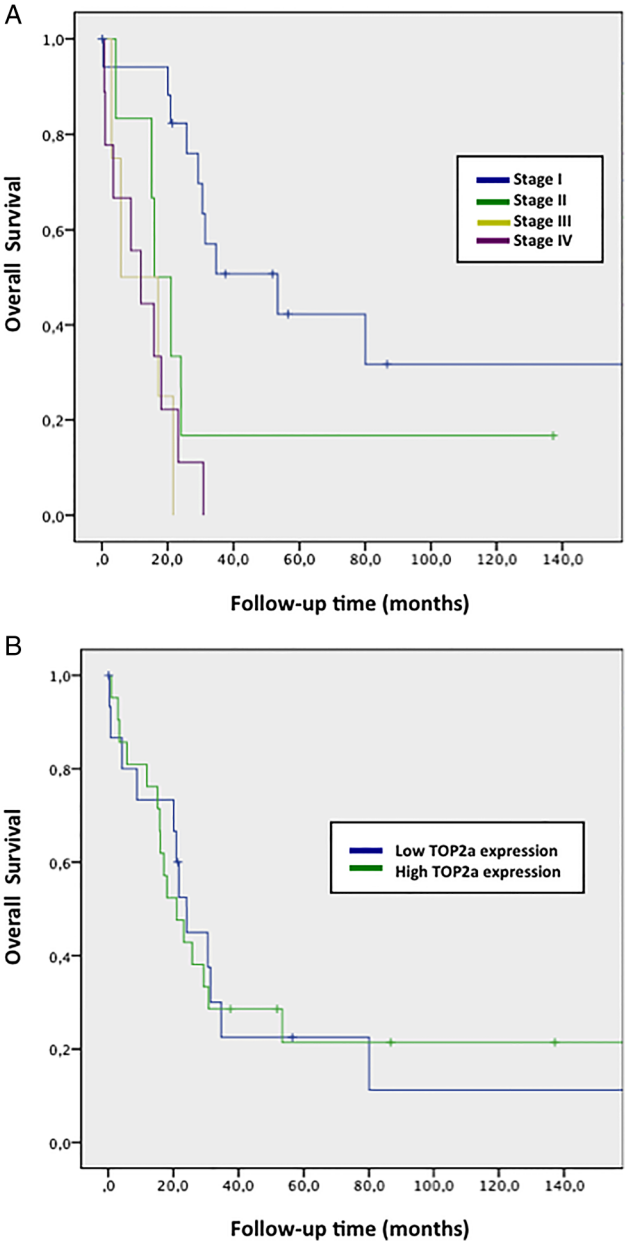

Figure 3 (A) Overall survival curves for patients with stages I, II, III and IV (p=0.033); (B) overall survival curves for patients with low and high TOP2A expression (p=0.83).

Table 4 Association between survival and clinical-pathological variables

Variable	No of patients	Median survival (months); 95% CI	p Value
Tumour size			
≤8 cm	13	29.2 (16.6 to 41.8)	0.24
>8 cm	12	17.9 (8.4 to 27.5)	
FIGO stage			
I	18	53.4 (15.6 to 91.2)	<0.001
II	6	15.9 (8.9 to 23.0)	
III	4	5.7 (1 to 19.4)	
IV	9	11.8 (2.9 to 20.8)	
FIGO stage			
I	18	53.4 (15.6 to 91.2)	<0.001
II+III	10	15.9 (13.0 to 18.9)	
IV	9	11.8 (2.9 to 20.8)	
Mitotic index			
≤20/10 HPF	13	23.2 (9.9 to 36.5)	0.50
>20/10 HPF	24	21.0 (11.8 to 30.2)	
Atypia grade			
Mild/moderate	18	23.2 (19.4 to 26.9)	0.71
Intense	19	20.0 (6.3 to 33.8)	
High cellularity			
No	12	24.0 (8.3 to 39.7)	0.58
Yes	25	20.8 (14.6 to 26.9)	
TOP2a expression			
Low	16	24.0 (18.4 to 29.6)	0.83
High	21	21.0 (11.8 to 30.2)	
TOP2A gene			
Normal	19	21.6 (15.3 to 27.9)	0.48
Abnormal	11	20.8 (10.1 to 31.4)	

Univariate analysis for the 37 patients with uterine leiomyosarcomas.
HPF, high power field.

includes total hysterectomy and debulking of the tumour if it is present outside of the uterus,[1 5] and the impact of adjuvant therapy on survival remains unknown. Radiotherapy might be useful in controlling local recurrences; chemotherapy with doxorubicin or docetaxel/gemcitabine is used for advanced or recurrent disease, with response rates of approximately 30%.[3]

Due to its rarity, the clinical and pathological prognostic factors of LMS (such as age, stage, tumour size, presence of necrosis, mitotic rate, degree of nuclear pleomorphism and vascular invasion) remain debated.[1 5 23–27] In our study, no clinical or pathological variable was a prognostic factor. Only the presence of extrauterine disease (stage ≥ II) negatively impacted survival, and notably, there was no difference in outcome between stage II, III and IV disease. We observed a very poor prognosis overall, with a median survival of 53.4 months, even for stage I disease, consistent with the previously described aggressiveness and underscoring the need to better understand LMS oncogenesis and develop biomarkers.

In our study, TOP2A had no prognostic impact in LMS but was more likely to be expressed in advanced disease—not in LMs or LMVs. This finding implicates TOP2A in sarcogenesis and tumour progression, an aspect that has not been studied extensively. TOP2A expression may also constitute an important diagnostic tool for difficult cases in which the criteria for a diagnosis of LMS are unclear.

In a large study[22] of 356 tumours that were initially considered to be LMSs, 27% were excluded on review and reclassified as benign smooth muscle tumours (LMs and variants). Of note, 8.3% of the reclassified tumours developed metastases, reflecting the difficulty in establishing objective diagnostic and prognostic criteria. Previous studies have suggested a difference in Ki67, p53 and p16 expression in uterine LMSs compared with benign smooth muscle tumours.[6–10] However, the utility of TOP2A has not been described until now.

There are few studies on soft tissue sarcomas. TOP2A is differentially expressed in soft part tumours and higher in sarcomas compared with desmoid-type fibromatosis.[21] Positivity for TOP2A is also an independent prognostic factor of an unfavourable prognosis and is a prognostic index that can be used to evaluate overall survival.[21] However, we did not find any impact on survival in uterine LMS.

Upregulation of TOP2A does not appear to result solely from gene amplification because it did not correlate with the FISH results. This finding is consistent with studies on other solid tumours, particularly breast carcinoma[15 19] and soft tissue sarcomas,[21] but remains unexplained. We speculate that this pattern is attributed to post-transcriptional regulation.

Data on TOP2A gene expression in uterine LMSs is scarce. Shan *et al*[28] compared global patterns of gene expression in 10 myometrium samples and 10 early stage uterine LMSs. They found TOP2A overexpression in LMSs (26.6 fold) compared with normal myometrium. In another study, Skubitz *et al*[29] examined gene expression in 4 LMSs and 46 normal myometrium. TOP2A was 5–10-fold more expressed in LMS compared with normal myometrium.

Our series is the first study to analyse TOP2A immunohistochemical expression and copy number in uterine LMs and LMS. Moreover, our data can help stratify patients with benign smooth cell tumours and cancer. However, there are certain limitations, such as its retrospective setting and small sample size. We were also unable to correlate TOP2A expression to the response to chemotherapy because only 13.5% of patients received anthracycline-based regimens.

In conclusion, TOP2A is highly expressed in advanced LMS but absent from non-malignant smooth muscle disease. TOP2A expression does not correlate with *TOP2A* patterns by FISH and does not predict outcome. Future studies should recapitulate our findings and determine its expression regarding the response to anthracycline-based chemotherapy.

A greater understanding of the predictive factors of LMS will allow clinicians to identify patients who are at higher risk of recurrence and more likely to benefit from tailored treatment regimens.

Take home messages

- TOP2A is highly expressed in high-mitotic index tumours and in advanced uterine leiomyosarcoma but not in benign smooth muscle tumours.
- TOP2A expression does not predict outcome in uterine leiomyosarcoma.

Handling editor Cheok Soon Lee

Acknowledgements This study was supported by FAPESP (Fundacao de Amparo a Pesquisa do Estado de São Paulo) No. 2013/01489-3, São Paulo, Brazil.

Contributors All authors have contributed significantly to the paper, and all authors are in agreement with the content of the manuscript.

Funding FAPESP (No. 2013/01489-3).

Competing interests None declared.

Ethics approval Institutional Review Board.

Provenance and peer review Not commissioned; externally peer reviewed.

REFERENCES

1 D'Angelo E, Prat J. Uterine sarcomas: a review. *Gynecol Oncol* 2010;116:131–9.
2 Major FJ, Blessing JA, Silverberg SG, *et al*. Prognostic factors in early-stage uterine sarcoma. A Gynecologic Oncology Group study. *Cancer* 1993;71:1702–9.
3 Hensley ML, Ishill N, Soslow R, *et al*. Adjuvant gemcitabine plus docetaxel for completely resected stages I–IV high grade uterine leiomyosarcoma: results of a prospective study. *Gynecol Oncol* 2009;112:563–7.
4 Kurman RJ, Carcangiu ML, Herrington CS, *et al*. *World Health Organization classification of tumors of female reproductive organs*. 4th edn. Lyon: IARC Press, 2014.
5 Giuntoli II RL, Metzinger DS, DiMarco CS, *et al*. Retrospective review of 208 patients with leiomyosarcoma of the uterus: prognostic indicators, surgical management, and adjuvant therapy. *Gynecol Oncol* 2003;89:460–9.
6 Atkins KA, Arronte N, Darus CJ, *et al*. The use of p16 in enhancing the histologic classification of uterine smooth muscle tumors. *Am J Surg Pathol* 2008;32:98–102.
7 Bodner-Adler B, Bodner K, Czerwenka K, *et al*. Expression of p16 protein in patients with uterine smooth muscle tumors: an immunohistochemical analysis. *Gynecol Oncol* 2005;96:62–6.
8 Chen L, Yang B. Immunohistochemical analysis of p16, p53, and Ki-67 expression in uterine smooth muscle tumors. *Int J Gynecol Pathol* 2008;27:326–32.
9 Mittal K, Demopoulos RI. MIB-1 (Ki-67), p53, estrogen receptor, and progesterone receptor expression in uterine smooth muscle tumors. *Hum Pathol* 2001;32:984–7.
10 O'Neill CJ, McBride HA, Connolly LE, *et al*. Uterine leiomyosarcomas are characterized by high p16, p53 and MIB1 expression in comparison with usual leiomyomas, leiomyoma variants and smooth muscle tumours of uncertain malignant potential. *Histopathology* 2007;50:851–8.
11 Cummings J, Smyth JF. DNA topoisomerase I and II as targets for rational design of new anticancer drugs. *Ann Oncol* 1993;4:533–43.
12 Burgess DJ, Doles J, Zender L, *et al*. Topoisomerase levels determine chemotherapy response in vitro and in vivo. *Proc Natl Acad Sci USA* 2008;105:9053–8.
13 Pritchard KI, Messersmith H, Elavathil L, *et al*. HER-2 and topoisomerase II as predictors of response to chemotherapy. *J Clin Oncol* 2008;26:736–44.
14 Esteva FJ, Hortobagyi GN. Topoisomerase IIa amplification and anthacycline-based chemotherapy: the jury is still out. *J Clin Oncol* 2009;27:3416–17.
15 Järvinen TAH, Tanner M, Rantanen V, *et al*. Amplification and deletion of topoisomerase IIa associate with ErbB-2 amplification and affect sensitivity to topoisomerase II inhibitor doxorubicin in breast cancer. *Am J Pathol* 2000;156:839–47.
16 Kanta SY, Yamane T, Dobashi Y, *et al*. Topoisomerase IIa gene amplification in gastric carcinomas: correlation with the HER2 gene. An immunohistochemical, immunoblotting, and multicolor fluorescence in situ hybridization study. *Hum Pathol* 2006;37:1333–43.
17 Al-Kuraya K, Novotny H, Bavi P, *et al*. HER2, TOP2A, CCND1, EGFR and C-MYC oncogene amplification in colorectal cancer. *J Clin Pathol* 2007; 60:768–72.
18 Dimov ND, Zynger DL, Luan C, *et al*. Topoisomerase II alpha expression in testicular germ cell tumors. *Urology* 2007;69:955–61.21.
19 Mano MS, Rosa DD, De Azambuja E, *et al*. The 17q12–q21 amplicon: Her2 and topoisomerase-IIa and their importance to the biology of solid tumours. *Cancer Treat Rev* 2007;33:64–77.
20 Murphy AJ, Hughes CA, Barrett C, *et al*. Low-level TOP2A amplification in prostate cancer is associated with HER2 duplication, androgen resistance, and decreased survival. *Cancer Res* 2007;67:2893–8.
21 da Cunha IW, De Brot L, Carvalho KC, *et al*. Prognostication of soft tissue sarcomas based on chromosome 17q gene and protein status: evaluation of TOP2A, HER-2/ neu, and survivin. *Ann Surg Oncol* 2012;19:1790–9.
22 Abeler VM, Røyne O, Thoresen S, *et al*. Uterine sarcomas in Norway. A histopathological and prognostic survey of a total population from 1970 to 2000 including 419 patients. *Histopathology* 2009;54:355–64.
23 D'Angelo E, Spagnoli LG, Prat J. Comparative clinicopathologic and immunohistochemical analysis of uterine sarcomas diagnosed using the World Health Organization classification system. *Hum Pathol* 2009;40:1571–85.
24 Koivisto-Korander R, Butzow R, Koivisto AM, *et al*. Clinical outcome and prognostic factors in 100 cases of uterine sarcoma: experience in Helsinki University Central Hospital 1990–2001. *Gynecol Oncol* 2008;111:74–81.
25 Denschlag D, Masoud I, Stanimir G, *et al*. Prognostic factors and outcome in women with uterine sarcoma. *Eur J Surg Oncol* 2007;33:91–5.
26 Nordal R, Kristensen GB, Kaern J, *et al*. The prognostic significance of stage, tumor size, cellular atypia and DNA ploidy in uterine leiomyosarcoma. *Acta Oncol* 1995;34:797–802.
27 Kapp DS, Shin JY, Chan JK. Prognostic factors and survival in 1396 patients with uterine leiomyosarcomas: emphasis on impact of lymphadenectomy and oophorectomy. *Cancer* 2008;112:820–30.
28 Shan W, Akinfenwa PY, Savannah KB, *et al*. A small-molecule inhibitor targeting the mitotic spindle checkpoint impairs the growth of uterine leiomyosarcoma. *Clin Cancer Res* 2012;18:3352–65.
29 Skubitz KM, Skubitz AP. Differential gene expression in leiomyosarcoma. *Cancer* 2003;98:1029–38.