Pathology - Research and Practice 215 (2019) 152640

Contents lists available at ScienceDirect

Pathology - Research and Practice

journal homepage: www.elsevier.com/locate/prp

Case report

Immunofluorescence analysis of DNA damage response protein p53-binding protein 1 in a case of uterine dedifferentiated leiomyosarcoma arising from leiomyoma

Katsuya Matsuda[a,*], Yuko Akazawa[a], Yuka Yamaguchi[b], Zhanna Mussazhanova[a], Hirokazu Kurohama[a], Nozomi Ueki[a], Michiharu Kohno[c], Ai Fukushima[c], Itsuki Kajimura[d], Hiroko Hiraki[e], Takahiro Matsuwaki[d], Sayaka Kawashita[f], Akira Kinoshita[g], Masahiro Nakashima[a]

[a] Department of Tumor and Diagnostic Pathology, Atomic Bomb Disease Institute, Nagasaki University, Nagasaki, Japan
[b] Resident Doctor, Sasebo City General Hospital, Nagasaki, Japan
[c] Department of Obstetrics and Gynecology, Saiseikai Nagasaki Hospital, Nagasaki, Japan
[d] Department of Obstetrics and Gynecology, Japan Community Health care Organization Isahaya General Hospital, Nagasaki, Japan
[e] AI Women's Clinic, Nagasaki, Japan
[f] Department of Obstetrics and Gynecology, Sasebo City General Hospital, Nagasaki, Japan
[g] Department of Human Genetics, Atomic Bomb Disease Institute, Nagasaki University, Nagasaki, Japan

ARTICLE INFO

Keywords:
p53-binding protein 1
DNA damage response
Dedifferentiated leiomyosarcoma
Genomic instability

ABSTRACT

Aims: Genomic instability has been indicated during the dedifferentiation process from leiomyoma (LM) to leiomyosarcoma (LMS). Previously, we have described that nuclear expression pattern of DNA damage response protein p53-binding protein 1 (53BP1), detected by immunofluorescence, reflects the magnitude of genomic instability during malignancy. Here, we present a case of LMS arising from LM with molecular analysis of 53BP1, which showed transitional magnitude of DNA damage response within a tumor.

Methods and results: A fifty-year-old female with abdominal mass underwent hysterectomy. Histologically, the tumor consisted of LMS with highly atypical multinucleated giant cells as well as an LM component with transitional atypical spindle cells in the border area. LMS showed diffuse nuclear staining of 53BP1 expression, which has been previously described as high DNA damage response pattern. In contrast, the LM component lacked 53BP1 immunoreactivity and focal expression was observed in transitional lesion. Furthermore, double-labelled immunofluorescence revealed co-localization of 53BP1 with p53 and Ki-67 in the LMS component, which indicated abnormal DNA damage response in proliferative state.

Conclusions: This study revealed that diffuse-type 53BP1 expression may be beneficial to estimate genomic instability during dedifferentiation from LM to DLMS.

1. Introduction

The importance of a functional DNA damage response pathway in tumor suppression is well established. Defective or abnormal DNA damage response can result in genomic instability, leading to the development of cancers [1,2]. The p53-binding protein 1 (53BP1), belonging to a family of evolutionarily conserved DNA damage response proteins, localizes at sites of DNA double-strand breaks and forms nuclear foci (NF) [3,4]. Previously, we have reported that the type of 53BP1 expression alters during carcinogenic process, including that in thyroid [5–8], skin [9], cervical [10], and bladder cancers [11]. Notably, high DNA damage response types including diffuse-type with heterogeneous and brightly diffuse nuclear staining were found to be closely associated with a higher level of malignant potency [11]. Furthermore, 53BP1 and proliferation marker Ki-67 show frequent co-localization in carcinoma cells, whereas this is rarely observed in benign lesions. As DNA damage

Abbreviations: MRI, Magnetic resonance imaging; 53BP1, 53 binding protein-1; DLMS, dedifferentiated leiomyosarcoma; LM, leiomyoma; αSMA, alpha-smooth muscle actin; IF, immunofluorescence; LMS, leiomyosarcoma; MED12, mediator complex subunit 12

* Corresponding author at: Department of Tumor and Diagnostic Pathology, Atomic Bomb Disease Institute, Nagasaki University, 1-12-4 Sakamoto, Nagasaki 852-8523, Japan.
E-mail address: katsuya@nagasaki-u.ac.jp (K. Matsuda).

https://doi.org/10.1016/j.prp.2019.152640
Received 3 July 2019; Received in revised form 29 August 2019; Accepted 15 September 2019

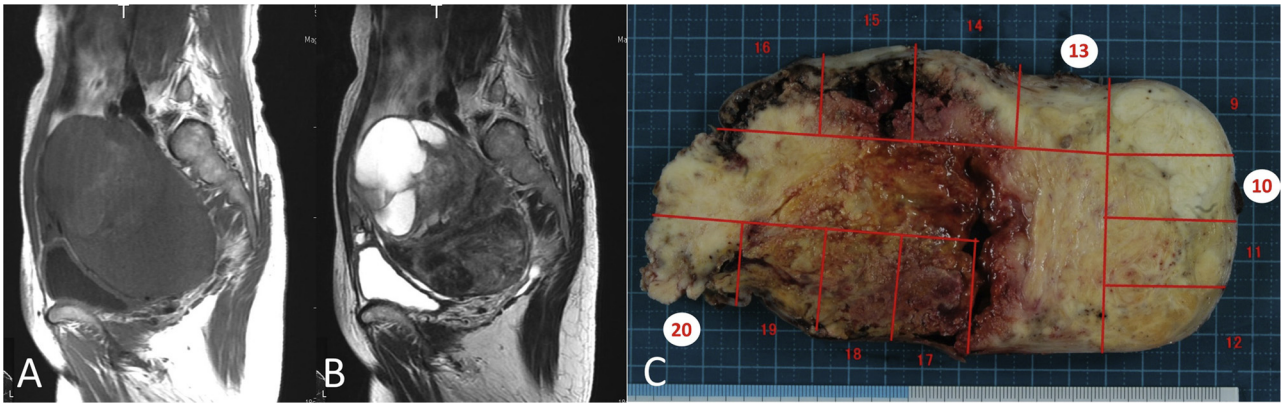

Fig. 1. Sagittal MRI of the pelvis (A) T1-weighted image and (B) T2-weighted image. MRI detected homogenous solid area adjacent to the irregular mass with high-intensity area (B), suggesting hemorrhage. (C) Gross finding of the tumor *(cut surface)* showing whitish-grey solid mass with distinct fragile reddish area containing hemorrhage and necrosis.

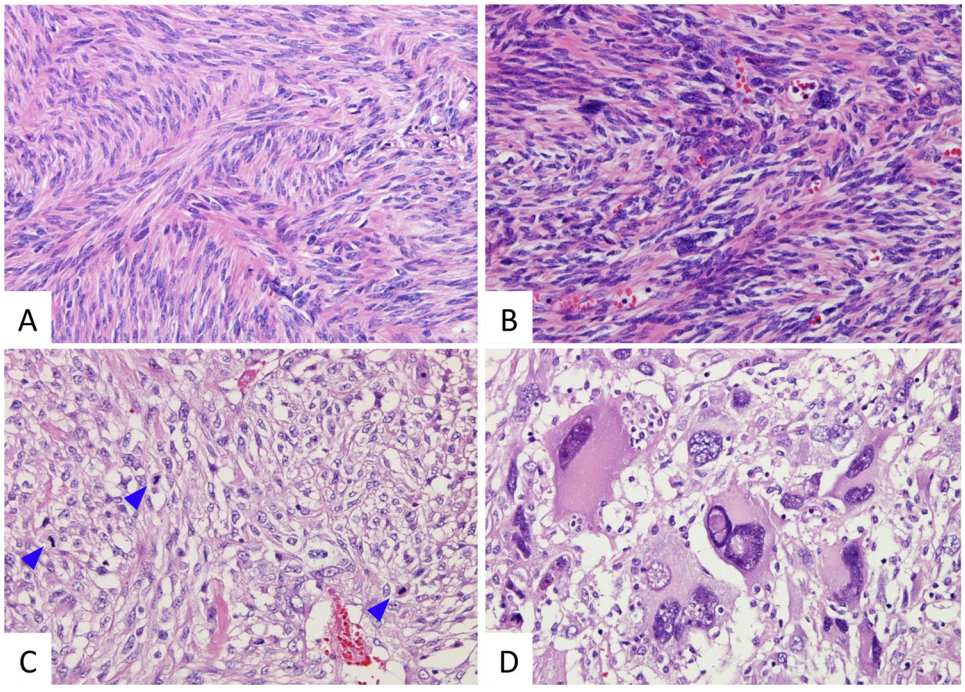

Fig. 2. Microscopic findings of the tumor. (A: Section #10 in Fig. 1C) Interlacing fascicles of spindle cells with blunt nucleus and no mitotic figure in the solid area, suggesting leiomyoma. (H&E staining; original magnification ×20). (B: section#13 in Fig. 1C) Tumor cells in transitional lesion presenting gradual increase of cellular atypia toward reddish and necrotic area (H&E staining; original magnification ×200). (C, D: section#20 in Fig. 1C) Tumor exhibiting highly atypical multinucleated giant cells with large bizarre nuclei and frequent mitoses in the reddish lesion, suggesting leiomyosarcoma (H&E staining; original magnification C × 200, D × 400).

response occurs during cell cycle arrest, the co-localization of 53BP1 expression and Ki-67 can be considered an indicator of abnormal DNA damage response pathway, which is subsequently associated with carcinogenesis via genomic instability.

Dedifferentiation is defined by the occurrence of a high-grade or undifferentiated tumor from a low-grade/borderline neoplasm [12], and is occasionally associated with the presence of highly atypical multinucleated giant cells, indicating aneuploidy as well as genomic instability. Here, we present a rare case of uterine dedifferentiated leiomyosarcoma (DLMS), showing highly atypical multinucleated giant cells with the presence of leiomyoma (LM) elements. Although such case of uterine tumor is extremely rare, previous molecular studies support the occurrence of DLMS from adjacent LM in the uterus [13]. In addition, previous reports suggest a role of p53 mutation and p16 in dedifferentiated chondrosarcoma [14]. The present case highlights the significance of the type of 53BP1 expression as an ancillary molecular pathological marker during tumorigenesis, particularly in dedifferentiation.

2. Case presentation

The patient was a 50-year-old multipara woman who presented with increasing abdominal mass. Magnetic resonance imaging revealed a bulky pelvic mass (12 × 10 × 15 cm) in the dorsal right side of the uterus. The tumor was iso-intense on T1-weighted images, which contained a few high-intensity zones. T2-weighted images showed iso-intense mass with high-intensity component (Fig. 1A, B). Preoperative diagnosis was sarcoma. Total hysterectomy and bilateral oophorectomy were performed. The tumor had developed in the uterine part adjacent to the right broad ligament and protruded to the serosal surface with peritoneal dissemination. The weight of the resected tumor was 1060 g. The patient underwent combination chemotherapy of gemcitabine and docetaxel after the surgery. One year post surgery, multiple recurrent tumors developed in the peritoneum. Although immunotherapy was additionally performed, the patient deceased 6 months after recurrence due to multiple organ failure induced by tumor progression.

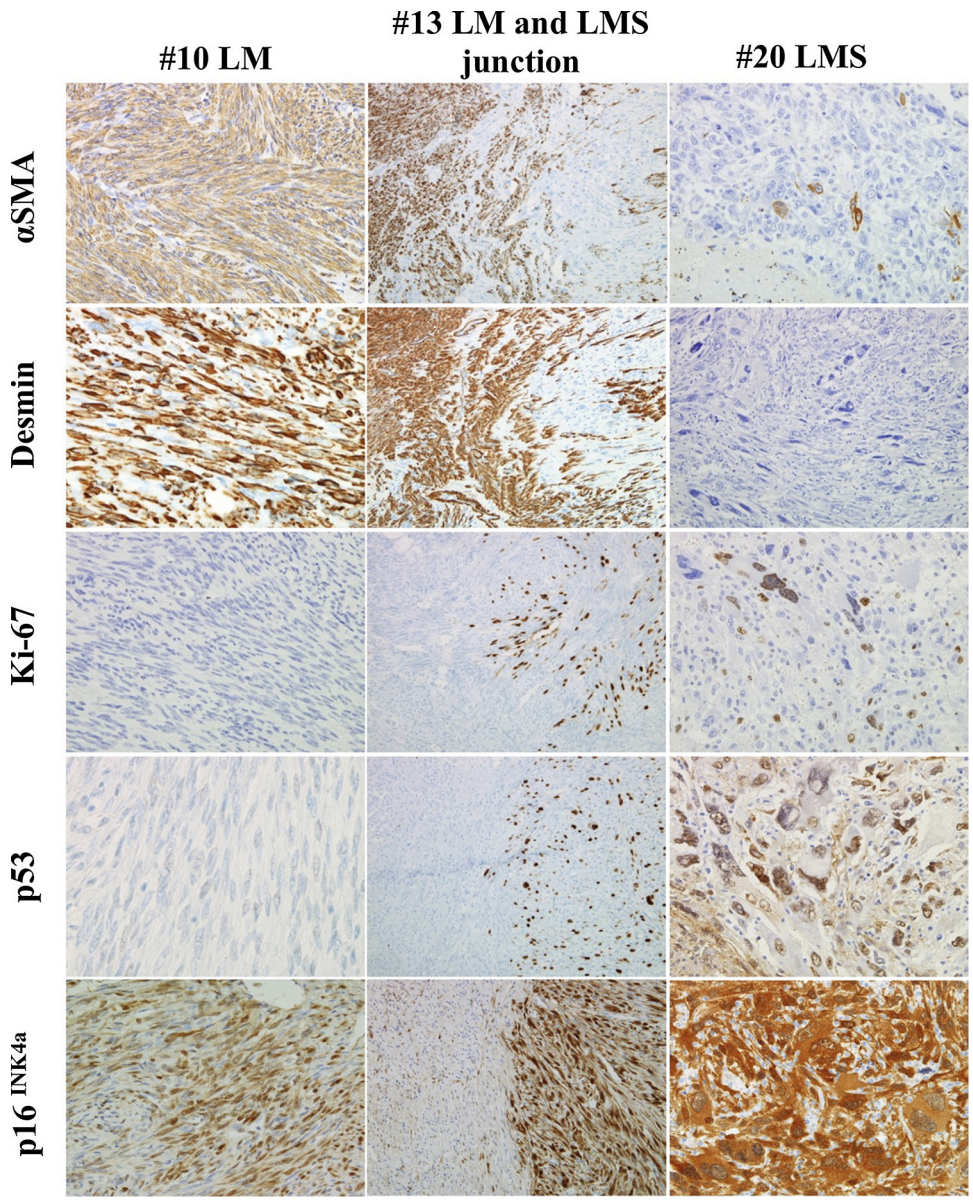

Fig. 3. Immunohistochemical analysis of the tumor. αSMA and desmin were detected in leiomyoma (LM) (section #10), whereas p53 and Ki-67 were positive in leiomyosarcoma (LMS) (section #20). $p16^{INK4a}$ was mostly positive in the LM component (section #10) and was strongly positive in leiomyosarcoma (LMS) component (section#20).

Table 1
Immunohistochemical profiles of leiomyoma and dedifferentiated leiomyosarcoma component.

	AE1/AE3	Vimentin	αSMA	Desmin	Myoglobin	S-100	hCG	KP-1	ER	PgR	Ki-67	p53	$p16^{INK4a}$
#10 LM component	–	+	+	+	–	–	–	–	–	+	0.5%	–	+
#20 DDLMS component	–	+	–	–	–	–	–	–	–	–	64.3%	+	+

LM, leiomyoma; DDLMS, dedifferentiated leiomyosarcoma; AE1/AE3, pan cytokeratin; αSMA, alpha-smooth muscle actin; hCG, human chorionic gonadotropin; KP-1, Anti-CD68 monoclonal antibody; ER, estrogen receptor; PgR, progesterone receptor.

3. Pathological findings

Grossly, the cut surface of tumor consisted of two components: a grey-white solid mass and a fragile reddish area containing hemorrhage and necrosis (Fig. 1C). Microscopic examination revealed interlacing proliferation of spindle cells with a blunt-ended nucleus without mitotic figure in the grey-whitish area, which suggested LM (Fig. 2A). At the LM margin, adjacent to the fragile reddish area, the severity of cellular atypia of spindle cells gradually increased, indicating pleomorphism and hyperchromatia (Fig. 2B). In the fragile reddish area, tumor cells presented with frequent mitoses (12 mitoses per 15 high-power fields) (Fig. 2C) and highly atypical multinucleated giant cells with huge bizarre nuclei, suggesting LMS (Fig. 2D). The results for immunohistochemistry of this tumor are summarized in Fig. 3 and

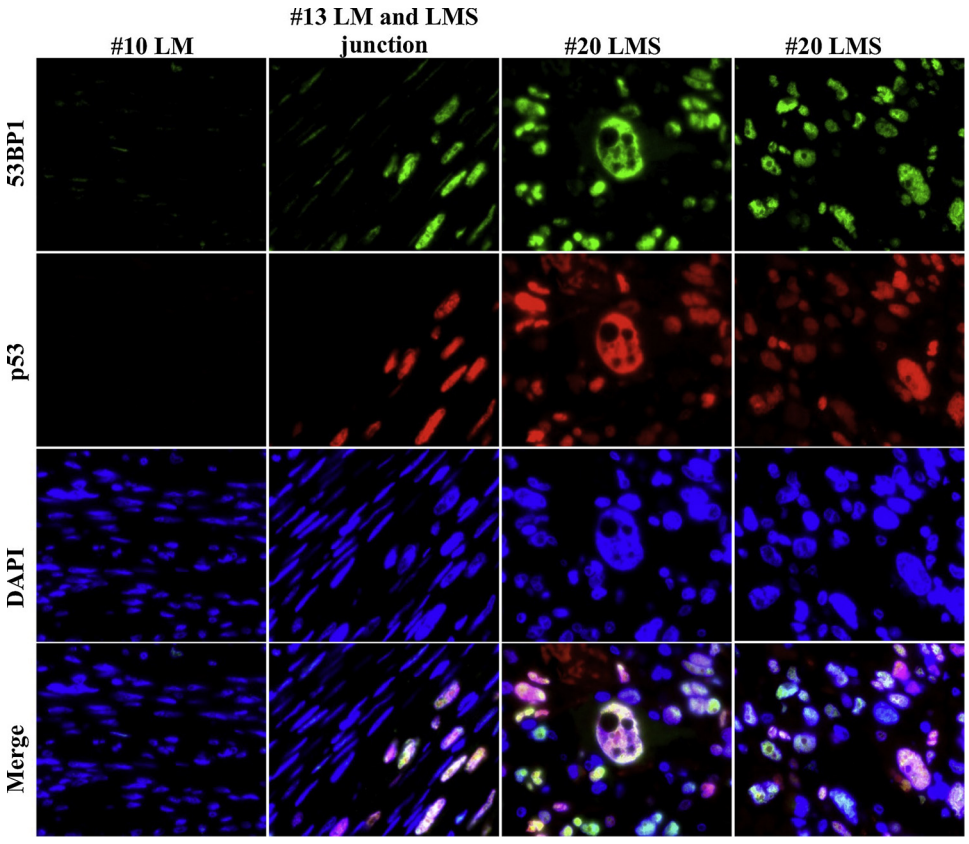

Fig. 4. Double immunofluorescence (IF) analysis of p53-binding protein 1(53BP1) and p53 in the tumor. IF showing co-localization of 53BP1 with p53 in the LMS component. LM showed minimum expression of 53BP1 with p53, whereas LMS section presented with nuclear expression of 53BP1, which was frequently co-localized with p53; the expression was also detected in the junction area.

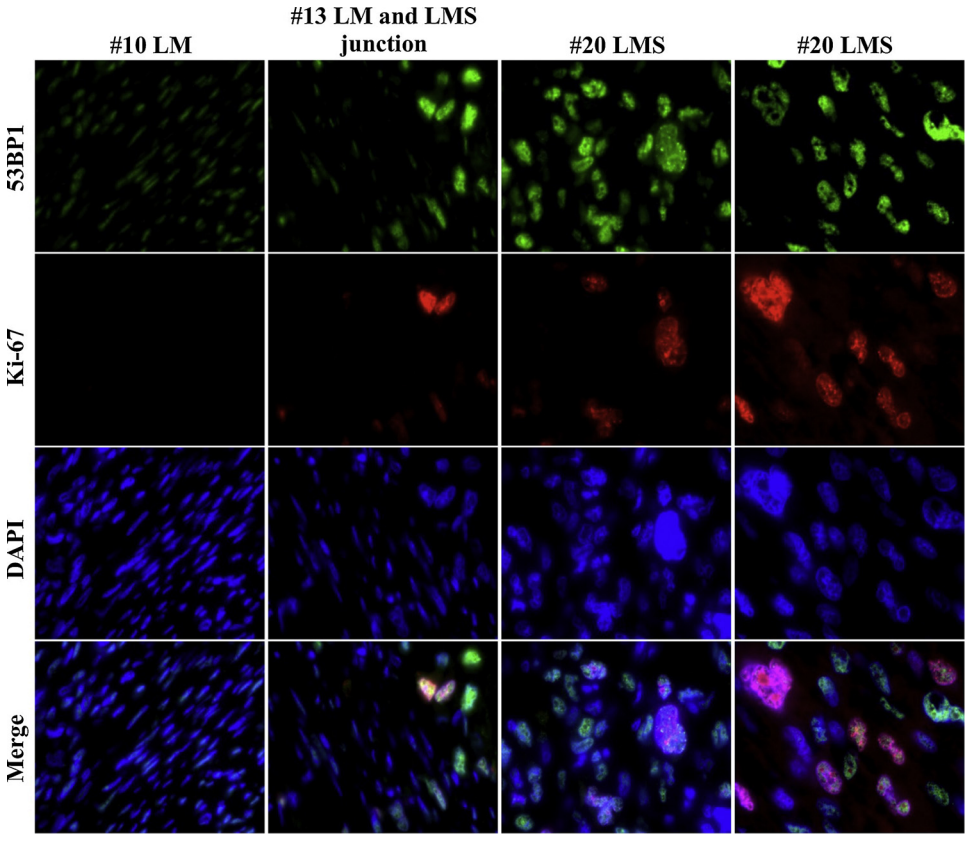

Fig. 5. Double immunofluorescence (IF) analysis of p53-binding protein 1(53BP1) and Ki-67 in the tumor.
IF showing co-localization of 53BP1 with Ki-67 in the LMS compornent. LM showed minimum expression of 53BP1 with Ki-67, whereas LMS section presented with nuclear expression of 53BP1, which was frequently co-localized with Ki-67; the expression was also detected in the junction area.

Table 1. A strong immunopositivity for both desmin (DakoCytomation, clone D33 ; IS606) and alpha-smooth muscle actin (αSMA) (DakoCytomation, clone 1A4 ; M0851) was demonstrated in the LM area; however, it was only focally positive in atypical spindle cells and negative in multinucleated giant cells in DLMS area, suggesting smooth muscle differentiation in DLMS tumor (Fig. 3). Immunoreactivity for progesterone receptor (Leica, clone 16; NCL-L-P-PGR-312) was found only in the LM component. In contrast, immunoreactivity for Ki-67 (DakoCytomation, clone MIB-1; M7240), p53 (DakoCytomation, clone DO-7; MA5-12557), and $p16^{INK4a}$ (Roche; 705-4793) were strong in atypical spindle cells and multinucleated giant cells in LMS area, indicating malignant potency (Fig. 3). Notably, $p16^{INK4a}$ was also positive in LM component.

4. Immunofluorescence analysis for the type of 53BP1 expression

In LMS, atypical spindle cells and multinucleated giant cells showed diffuse-type nuclear staining of 53BP1 (Bethyl Laboratories; A300-272A) expression, whereas no benign LM cells expressed 53BP1 immunoreactivity (Figs. 4, 5). Furthermore, we observed co-localization of 53BP1 and p53 /Ki-67 in the components of LMS (Fig. 4,5).

5. Discussion

Although majority of the uterine LMS are thought to occur *de novo*, uterine LMS rarely develops from pre-existing LM [15]. Such cases tend to localize at the broad ligament [16–19], as observed in our case. The tumor in the present case showed a transition from benign LM to DLMS, presenting several highly atypical multinucleated giant cells via classical LMS components without multinucleated giant cells, thus providing us a valuable opportunity to explore the molecular alterations of uterine smooth muscle tumorigenesis.

Our analysis identified variation in 53BP1 expression within tumor components. The diffuse-type of 53BP1 was restricted to only the malignant components. However, $p16^{INK4a}$ expression was found in both LM and LMS components, and the expression was more intense in LMS components, consistent with a previous report [20]. Overexpression of $p16^{INK4a}$ protein is thought to be induced by replication stress to down-regulate cellular proliferation at the early phase of tumorigenesis [21]. We observed simultaneous overexpression of $p16^{INK4a}$ and Ki-67 in LMS components, suggesting a loss of tumor suppressive ability despite the presence of $p16^{INK4a}$ protein in LMS. Furthermore, frequent nuclear co-localization of 53BP1 expression and Ki-67 was observed in LMS, indicating a disrupted DNA damage response in malignancy. The diffuse-type of 53BP1 expression was frequently co-localized with p53 in the LMS component, including in multinucleated giant cells. The present study provides new evidence indicating an association between diffuse-type 53BP1 expression and mutations in the p53 tumor suppressor gene [22] in DLMS. Our supplemental analysis revealed that all components (LM, junction, and LMS) of the present case harbored wild-type *mediator complex subunit* 12 (*MED12*) *exon 2* (supplemental Fig. 1), which is frequently mutated in uterine LM but rarely in LMS [23,24]. Thus, oncogenic mutations in *MED12 exon 2* might not be critical for the malignant transformation of uterine smooth muscle tumors.

Genomic instability plays a crucial role in carcinogenic process and is involved in higher grade of diverse malignancy. Our published data showed that the diffuse-type of 53BP1 expression is frequently observed in anaplastic carcinoma of the thyroid [5], high-grade urothelial carcinoma of the bladder [11], and oxyphilic follicular tumor of the thyroid [7]. In addition, we have demonstrated that diffuse-type of 53BP1 is closely associated with molecular alterations indicating genomic instability in tumors, including p53 overexpression in anaplastic carcinoma, a high level of chromosomal instability by multicolored FISH, a loss of RAD51 expression (a key molecule for homologous recombination), p53 overexpression in high grade urothelial carcinoma, and a high level of chromosomal copy number aberrations by array comparative genomic hybridization in oxyphilic follicular tumor [5]. Therefore, we propose that the diffuse-type of 53BP1 expression is an indicator of genomic instability in carcinogenic processes. Chromosomal copy number aberrations are also well known in both uterine LMS [25] and dedifferentiated sarcoma [26,27].

Although further study is required with accumulation of similar cases, our study provides strong evidence that 53BP1 is an indicator of transitional enhancement of genomic instability from LM to LMS.

Ethics approval and consent to participate

The patient provided informed consent.

Consent for publication

Written informed consent was obtained from the patient for publication of this case report and any accompanying images. A copy of the consent form is available on request for review by the Editor of this journal.

This work was supported in part by the Atomic Bomb Disease Institute, Nagasaki University, Joint Research by Hiroshima University, Nagasaki University, and Fukushima Medical University Research Base for Radiation Accidents and Medical Science, and by a Grant-in-Aid for Scientific Research from the Japanese Ministry of Education, Science, Sports and Culture (No. 15K08380).

Declaration of Competing Interest

This work was supported in part by the Atomic Bomb Disease Institute, Nagasaki University, Joint Research by Hiroshima University, Nagasaki University, and Fukushima Medical University Research Base for Radiation Accidents and Medical Science, and by a Grant-in-Aid for Scientific Research from the Japanese Ministry of Education, Science, Sports and Culture (No. 15K08380).

Acknowledgement

We wish to thank Editage (www.editage.jp) for English language editing.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.prp.2019.152640.

References

[1] C. Lengauer, K.W. Kinzler, B. Vogelstein, Genetic instabilities in human cancers, Nature 396 (1998) 643–649, https://doi.org/10.1038/25292.
[2] W.B. Coleman, G.J. Tsongalis, The role of genomic instability in human carcinogenesis, Anticancer Res. 19 (1999) 4645–4664.
[3] I.M. Ward, K. Minn, K.G. Jorda, J. Chen, Accumulation of checkpoint protein 53BP1 at DNA breaks involves its binding to phosphorylated histone H2AX, J. Biol. Chem. 278 (2003) 19579–19582, https://doi.org/10.1074/jbc.C300117200.
[4] L.B. Schultz, N.H. Chehab, A. Malikzay, T.D. Halazonetis, p53 binding protein 1 (53BP1) is an early participant in the cellular response to DNA double-strand breaks, J. Cell Biol. 151 (2000) 1381–1390, https://doi.org/10.1083/jcb.151.7.1381.
[5] M. Nakashima, K. Suzuki, S. Meirmanov, Y. Naruke, M. Matsuu-Matsuyama, K. Shichijo, V. Saenko, H. Kondo, T. Hayashi, M. Ito, S. Yamashita, I. Sekine, Foci formation of p53-binding protein 1 in thyroid tumors: activation of genomic instability during thyroid carcinogenesis, Int. J. Cancer 122 (2008) 1082–1088, https://doi.org/10.1002/ijc.23223.
[6] Z. Mussazhanova, K. Matsuda, Y. Naruke, N. Mitsutake, B. Stanojevic, T. Rougounovitch, V. Saenko, K. Suzuki, E. Nishihara, M. Hiirokawa, M. Ito, M. Nakashima, Significance of p53-binding protein 1 (53BP1) expression in thyroid papillary microcarcinoma: Association with brafv600e mutation status, Histopathology 63 (2013) 726–734, https://doi.org/10.1111/his.12233.
[7] Z. Mussazhanova, Y. Akazawa, K. Matsuda, K. Shichijo, S. Miura, R. Otsubo, M. Oikawa, K. Yoshiura, N. Mitsutake, T. Rogounovitch, V. Saenko, Z. Kozykenova, B. Zhetpisbaev, D. Shabdarbaeva, N. Sayakenov, B. Amantayev, H. Kondo, M. Ito,

M. Nakashima, Association between p53-binding protein 1 expression and genomic instability in oncocytic follicular adenoma of the thyroid, Endocr. J. 63 (2016) 457–467, https://doi.org/10.1507/endocrj.EJ15-0629.
[8] R. Otsubo, K. Matsuda, Z. Mussazhanova, A. Sato, M. Matsumoto, H. Yano, M. Oikawa, H. Kondo, M. Ito, A. Miyauchi, M. Hirokawa, T. Nagayasu, M. Nakashima, A novel diagnostic method for thyroid follicular tumors based on immunofluorescence analysis of p53-binding protein 1 expression: detection of genomic instability, Thyroid 29 (2019) 657–665, https://doi.org/10.1089/thy.2018.0548.
[9] Y. Naruke, M. Nakashima, K. Suzuki, M. Matsuu-Matsuyama, K. Shichijo, H. Kondo, I. Sekine, Alteration of p53-binding protein 1 expression during skin carcinogenesis: association with genomic instability, Cancer Sci. 99 (2008) 946–951, https://doi.org/10.1111/j.1349-7006.2008.00786.x.
[10] K. Matsuda, S. Miura, T. Kurashige, K. Suzuki, H. Kondo, M. Ihara, M. Nakajima, H. Masuzaki, M. Nakashima, Significance of p53-binding protein 1 nuclear foci in uterine cervical lesions: endogenous DNA double strand breaks and genomic instability during carcinogenesis, Histopathology 59 (2011) 441–451, https://doi.org/10.1111/j.1365-2559.2011.03963.x.
[11] K. Matsuda, T. Kawasaki, Y. Akazawa, Y. Hasegawa, H. Kondo, K. Suzuki, M. Iseki, M. Nakashima, Expression pattern of p53-binding protein 1 as a new molecular indicator of genomic instability in bladder urothelial carcinoma, Sci. Rep. 8 (2018) 15477, https://doi.org/10.1038/s41598-018-33761-9.
[12] K.R. Rawish, O. Fadare, Dedifferentiated leiomyosarcoma of the uterus with heterologous elements: A potential diagnostic pitfall, Case Rep. Obstet. Gynecol. 2012 (2012) 534634, , https://doi.org/10.1155/2012/534634.
[13] K.R. Mittal, F. Chen, J.J. Wei, K. Rijhvani, R. Kurvathi, D. Streck, J. Dermody, G.A. Toruner, Molecular and immunohistochemical evidence for the origin of uterine leiomyosarcomas from associated leiomyoma and symplastic leiomyoma-like areas, Mod. Pathol. 22 (2009) 1303–1311, https://doi.org/10.1038/modpathol.2009.96.
[14] H.J. Grote, R. Schneider-Stock, W. Neumann, A. Roessner, Mutation of p53 with loss of heterozygosity in the osteosarcomatous component of a dedifferentiated chondrosarcoma, Virchows Arch. 436 (2000) 494–497.
[15] S.G. Silverberg, R.J. Kurman, Smooth muscle and other mesenchymal tumors, Tumors of the Uterine Corpus and Gestational Trophoblastic Disease (3rd Series), Armed Forces Institute of Pathology, Washington, DC, 1992.
[16] D.M. Lowell, J. Karsh, Leiomyosarcoma of the broad ligament. A case report, Obstet. Gynecol. 32 (1968) 107–110.
[17] A.G. McDonald, P. Dal Cin, A. Ganguly, S. Campbell, Y. Imai, A.E. Rosenberg, E. Oliva, Liposarcoma arising in uterine lipoleiomyoma: A report of 3 cases and review of the literature, Am. J. Surg. Pathol. 35 (2011) 221–227, https://doi.org/10.1097/PAS.0b013e31820414f7.
[18] M. Kefeli, S. Baris, O. Aydin, L. Yildiz, S. Yamak, B. Kandemir, An unusual case of an osteosarcoma arising in a leiomyoma of the uterus, Ann. Saudi Med. 32 (2012) 544–546, https://doi.org/10.5144/0256-4947.2012.23.5.1111.
[19] D.W. Collins, P.A. Menon, J.V. Carr, W.G. McCluggage, Carcinosarcoma arising in a uterine leiomyoma: Report of a unique neoplasm, Int. J. Gynecol. Pathol. 30 (2011) 576–580, https://doi.org/10.1097/PGP.0b013e31821bbb5a.
[20] B. Bodner-Adler, K. Bodner, K. Czerwenka, O. Kimberger, S. Leodolter, K. Mayerhofer, Expression of p16 protein in patients with uterine smooth muscle tumors: An immunohistochemical analysis, Gynecol. Oncol. 96 (2005) 62–66, https://doi.org/10.1016/j.ygyno.2004.09.026.
[21] M. Ishikawa, T. Fujii, M. Saito, I. Nindl, A. Ono, K. Kubushiro, K. Tsukazaki, M. Mukai, S. Nozawa, Overexpression of p16 ink4a as an indicator for human papillomavirus oncogenic activity in cervical squamous neoplasia, Int. J. Gynecol. Cancer 16 (2006) 347–353, https://doi.org/10.1111/j.1525-1438.2006.00355.x.
[22] M. Oren, P53: the ultimate tumor suppressor gene? FASEB J. 8 (1992) 3169–3176, https://doi.org/10.1096/fasebj.6.13.1397838.
[23] N. Mäkinen, M. Mehine, J. Tolvanen, E. Kaasinen, Y. Li, H.J. Lettonen, M. Gentile, J. Yan, M. Enge, M. Taipale, M. Aavikko, R. Katainen, E. Virolainen, T. Böhling, T.A. Koski, V. Launonen, J. Sjöberg, J. Taipale, P. Vahteristo, L.A. Aaltonen, MED12, the mediator complex subunint 11 gene, is mutated at high frequency in uterine leiomyomas, Science 334 (2011) 252–255, https://doi.org/10.1126/science.1208930.
[24] S. Croce, F. Chibon, MED12 and uterine smooth muscle oncogenesis: state of the art and perspectives, Eur. J. Cancer 51 (2015) 1603–1610, https://doi.org/10.1016/j.ejca.2015.04.023.
[25] Y.L. Cho, S. Bae, M.S. Koo, K.M. Kim, H.J. Chun, C.K. Kim, D.Y. Ro, J.H. Kim, C.H. Lee, Y.W. Kim, W.S. WS, Array comparative genomic hybridization analysis of uterine leiomyosarcoma, Gynecol. Oncol. 99 (2005) 545–551, https://doi.org/10.1016/j.ygyno.2005.07.017.
[26] J.V. Bovee, A.M. Cleton-Jansen, C. Rosenberg, A.H. Taminiau, C.J. Cornelisse, P.C. Hogendoorn, Molecular genetic characterization of both components of a dedifferentiated chondrosarcoma, with implications for its histogenesis, J. Pathol. 189 (1999) 454–462, https://doi.org/10.1002/(SICI)1096-9896(199912)189:4<454::AID-PATH467>3.0.CO;2-N.
[27] M. Hameed, C. Ulger, D. Yasar, N. Limaye, R. Kurvathi, D. Streck, J. Benevenia, F. Patterson, J.J. Dermody, G.A. Toruner, Genome profiling of chondrosarcoma using oligonucleotide array-based comparative genomic hybridization, Cancer Genet. Cytogenet. 192 (2009) 56–59, https://doi.org/10.1016/j.cancergencyto.2009.03.009.