Extranodal marginal zone lymphoma of the uterine cervix with concomitant copy number gains of the MALT1 and BCL2 genes: A case report

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Abstract. Extranodal marginal zone lymphoma (EMZL) of mucosa-associated lymphoid tissue (MALT) of the uterus is rare, and the etiology, pathophysiology and cytogenetic features remain unknown at present. The present study reports a case of a 71-year-old female with EMZL of the uterine cervix that was 80 mm in diameter and invaded directly into the rectal serosa. Complete remission was successfully induced by 6 courses of immunochemotherapy with rituximab plus cyclophosphamide, doxorubicin, vincristine and prednisolone. Although the metaphase spread of the tumor cells was unavailable for whole cytogenetic analysis, fluorescence in situ hybridization (FISH) detected triple signals for MALT1 and B-cell lymphoma 2, located at chromosome 18q21, and the centromere of chromosome 18, which was suggestive of trisomy 18, and in combination with previous studies, suggested a possible association between trisomy 18 and the large tumor at initial presentation in the present patient. In addition, FISH examination detected immunoglobulin heavy chain gene rearrangement, although the translocation partner was unconfirmed. A total of 18 previously-studied patients with EMZL of the uterus, including that of the present study, were reviewed with respect to their clinical features and treatment and cytogenetic abnormality. In the evaluation of the English scientific literature, this is the first reported patient with EMZL of the uterus with partly determined cytogenetic abnormalities.

Introduction

Extranodal marginal zone lymphoma (EMZL) of mucosa-associated lymphoid tissue (MALT) is a subtype of indolent non-Hodgkin lymphoma that develops in the extranodal organs, such as the stomach, salivary glands, ocular adrenexa and the thyroid (1). Repeated cytogenetic alterations include reciprocal chromosomal translocations such as t(11;18) (q21;q21) in Helicobacter pylori infection-unassociated gastric EMZL (2), t(14;18)(q32;q21) in ocular adnexa EMZL, t(1;4) (p22;q32) in intestinal and pulmonary EMZL and numerical abnormalities such as trisomy of chromosome 3 or chromosome 18 (3,4). These alterations are valuable as diagnostic markers and for understanding the molecular pathophysiology of the lymphomagenesis of EMZL (5). The aforementioned chromosomal translocations are usually mutually exclusive, and their frequencies vary widely depending on the primary tumor site. Furthermore, these chromosomal translocations and numerical abnormalities frequently co-exist in tumor cells from individual patients.

Primary lymphoid neoplasms of the uterus are rare, accounting for ~2.0% of extranodal lymphomas and for <0.5% of gynecologic cancer (6). In addition, the majority of primary uterus lymphomas are high-grade subtypes, such as diffuse large B-cell lymphoma (DLBCL) (7), whilst the occurrence of EMZL of the uterus is rare, with only 17 previously reported cases in the English literature (8-21), and their cytogenetic/genetic characteristics remain unknown. The present study reports a patient with primary uterine cervical EMZL with the concomitant copy number gains of MALT1 and B-cell lymphoma 2 (BCL2) genes, which are located at chromosome 18q21. As the tumor cells also harbored triple centromeres of chromosome 18, the lymphoma cells in the patient of the present study were suggestive of trisomy 18. In addition, the clinical features of previously reported cases of EMZL of the uterus were reviewed. In this examination of the English literature, the present study is the first case of uterine cervical EMZL in which cytogenetic abnormalities were at least partly determined.

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Case report

Patient. A 71-year-old female was referred to University Hospital of Kyoto Prefectural University of Medicine (Kyoto, Japan), complaining of abnormal vaginal bleeding. She exhibited no B symptoms, such as pyrexia, night sweating or body weight loss at presentation. Vaginal examination identified abnormal thickening of the vaginal wall (Fig. 1A), and transvaginal ultrasound sonography detected a large mass, 80 mm in diameter, at the uterine cervix (Fig. 1B). T2-weighted magnetic resonance imaging detected a slightly high-intensity tumor at the uterus cervix that invaded directly to the rectal serosa (Fig. 1C and D). Positron emission tomography with 2-deoxy-2-(fluorine-18) fluoro-D-glucose (FDG) integrated with computed tomography also detected the presence of enlarged FDG-avid lymph nodes in the pelvis, whilst other lesions were intact (Fig. 2). The serum soluble interleukin-2 receptor level was elevated to 1230 U/ml, normal range; 122-496 U/ml, whilst other laboratory tests were normal, including blood cell counts, lactate dehydrogenase, C-reactive protein and albumin. The serum antibody test for Chlamydia trachomatis was negative. Histological examination of the biopsied specimen of the tumor revealed infiltration of small and round-shaped abnormal lymphoid cells with oval or convoluted nuclei that were positive for cluster of differentiation (CD)20, CD79a and BCL2 markers (Fig. 3A-D), but were negative for CD5, CD10, cyclin D1, BCL6, or pan-cytokeratin markers (data not shown). These results were consistent with the results obtained by flow cytometric analysis, which detected the presence of CD19-phycocerythrin (PE)-cyanin 5.1 (Beckman Coulter, Inc., Brea, CA, USA) and CD20-fluorescein isothiocyanate (FITC), but the absence of CD5-FITC or CD10-PE (BD Biosciences, San Jose, CA, USA). Additionally, flow cytometry also revealed the expression of surface immunoglobulin (Ig) λ chain of tumor cells (data not shown). Collectively, the patient was diagnosed with EMZL, stage II according to the Ann Arbor staging system (22). The patient was treated with 6 courses of rituximab plus cyclophosphamide, doxorubicin, vincristine and prednisolone (R-CHOP): Rituximab 375 mg/m² on day 1, cyclophosphamide 750 mg/m² on day 2, doxorubicin 50 mg/m² on day 2, vincristine 1.4 mg/m² on day 2, prednisolone 100 mg/body on days 2-6, and attained complete response (CR). She has maintained CR for 9 months at the time of writing. Informed consent was obtained from the patient.

Procedure and result by the interphase fluorescence in situ hybridization (FISH) analysis. Single- and double-color FISH in single cell preparations of patient-derived lymphoma cells were performed as previously described (23). In addition, FISH was performed on paraffin-embedded tissue sections, tissue-FISH, according to previously described methods (24). The LSI Dual Color sets for the IGH Break Apart Rearrangement Probe (Abbott Molecular Inc., catalog no. 8L63-20), a mixture of 2 probes that hybridize to opposite sides of the joining gene segment through constant regions of the Immunoglobulin heavy chain (IgH) locus, were used to detect chromosomal breakage at the IgH gene. The LSI Dual Color set for the MALT1 Break Apart Rearrangement Probe was utilized to detect the gene rearrangement of MALT1. The LSI Dual Color sets for the fusion genes of MALT1/API2, MALT1/IgH, BCL2/IgH, or MYC/IgH (Abbott Molecular Inc., Des Plaines, Ill., USA) were also utilized in the present study. Vysis Chromosomes Enumeration Probe 18 (CEP18) (catalog no. 05J08-028; Abbott Molecular Inc.) was used to detect the centromeric region of chromosome 18.

As the metaphase spreads were unavailable due to the lack of dividing cells in the biopsied specimens, the cytogenetic studies were performed using interphase FISH. Although the FISH probes that specified chromosomal translocations...
Table I. List of cases of extranodal marginal zone lymphoma of mucosa-associated lymphoid tissue of the uterus.

| Age | Clinical presentation | Site | Gross appearance | Stage | Treatment | FISH | Other | (Refs.) |
|-----|----------------------|------|------------------|-------|-----------|------|--------|---------|
| 1   | 80 Vaginal prolapse   | Corpus | Normal, Polyp, Tumor | II | Hysterectomy | Neg. | Neg. | Neg. | - | (7) |
| 2   | 58 Incidental         | Corpus | Normal, Tumor, Stage | I | Hysterectomy | Neg. | Neg. | Neg. | - | (8) |
| 3   | 46 Bleeding           | Corpus | Normal, Tumor | I | Hysterectomy | Neg. | Neg. | Neg. | - | (8) |
| 4   | 59 Bleeding           | Corpus | Normal, Tumor | I | Hysterectomy | Neg. | Neg. | Neg. | - | (8) |
| 5   | 72 Bleeding           | Corpus | Normal, Tumor | I | None | Neg. | Neg. | Neg. | - | (8) |
| 6   | 61 Incidental         | Corpus | Normal, Tumor | I | Hysterectomy | - | Neg. | - | - | (9) |
| 7   | 43 Bleeding           | Corpus | Normal, Tumor, Stage | II | TAH-BSO+LN | - | - | - | - | (10) |
| 8   | 47 Dysmenorrhea       | Corpus | Normal, Tumor, Stage | I | TAH-BSO | - | - | - | - | (11) |
| 9   | 52 Bleeding           | Corpus | Normal, Tumor, Stage | IV | TAH-BSO | - | - | - | - | (12) |
| 10  | 77 Incidental         | Corpus | Normal, Tumor, Stage | I | Hysterectomy | - | - | - | - | (13) |
| 11  | 81 Incidental         | Corpus | Normal, Tumor, Stage | I | - | - | - | - | - | (14) |
| 12  | 55 Bleeding           | Corpus | Normal, Tumor, Stage | I | TAH-BSO | - | - | - | - | (15) |
| 13  | 65 Bleeding           | Corpus | Normal, Tumor, Stage | I | TAH-BSO | - | - | - | - | (16) |
| 14  | 72 Dysurea            | Corpus | Normal, Tumor, Stage | II | TAH+RT | - | - | - | - | (17) |
| 15  | 46 Bleeding           | Cervix | Normal, Tumor, Stage | IV | ProMACE/cytaBOM hysterectomy+RT+Rit | - | - | - | - | (18) |
| 16  | 56 Vaginal spotting   | Cervix | Normal, Tumor, Stage | I | Hysterectomy with bilateral salpingo-oopherectomy | - | - | - | - | (19) |
| 17  | 53 Cervical dysplasia | Cervix | Normal, Tumor, Stage | I | Conization | - | - | - | - | (20) |
| 18  | 71 Bleeding           | Cervix, Vagina | Normal, Tumor, Stage | II | R-CHOP | Neg. | Neg. | Neg. | Trisomy 18 Present study | (18) |

FISH, fluorescence in situ hybridization; BSO, bilateral salpingo-oopherectomy; TAH, total abdominal hysterectomy; LN, lymph node; proMACE/cytaBOM, cyclophosphamide, epirubicin, etoposide, prednisone, cytarabine, vincristine, bleomycin and methotrexate; RT, radiation therapy; Rit; rituximab; Neg., negative; R-CHOP, rituximab, cyclophosphamide, doxorubicin, vincristine, prednisolone.

Figure 3. Histological findings. Hematoxylin & eosin staining of a biopsied specimen from the uterine cervical tumor revealed infiltration of small and round-shaped abnormal lymphoid cells with oval or convoluted nuclei in the uterine cervix, leading to the diagnosis of extranodal marginal zone lymphoma at (A) magnification, x100 and (B) magnification, x400 (light microscope). Immunohistochemical staining using the Ventana VIEW DAB Universal Kit (Ventana Medical Systems, Inc., Oro Valley, AZ, USA) revealed that the abnormal lymphoid cells expressed (C) cluster of differentiation 20 stained with anti-CD20 antibody (L26) (Roche Diagnostics, Branchburg, NJ, USA) at magnification, x100 and (D) B-cell lymphoma 2 stained with anti-BCL2 antibody (clone 124) (Dako; Agilent Technologies, Inc., Santa Clara, CA, USA) at magnification, x100.

Figure 4. FISH analyses. (A-C) Whilst double-color FISH analyses for the API2/MALT1, IgH/MALT1, and IgH/BCL2 fusion genes were negative, these examinations revealed that the tumor cells harbored triple copies of (A, B) MALT1 (arrows) and (C) BCL2 (arrowheads). The tumor cells harbored (D) three centromeres of chromosome 18 (arrow), whilst (E) the MALT1 gene was not rearranged (arrowheads). (F) FISH evaluation also identified the IgH split signal, indicating the presence of IgH gene rearrangement (arrow). FISH, fluorescence in situ hybridization; CEP, chromosome enumeration probe; MALT1, mucosa associated lymphoid tissue lymphoma translocation gene 1; IgH, immunoglobulin heavy locus; BCL2, B-cell lymphoma 2.
tumor invaded directly to the rectal serosa at presentation, and radiotherapy was considered high risk for rectal penetration or rupture. It was also anticipated that the wide field irradiation for the large tumor may lead to unwanted adverse events in the pelvic viscera. Secondly, surgical resection was also excluded, as complete resection of the large tumor and the additionally involved lymph nodes required pelvic evisceration. To avoid those invasive therapy-associated complications, systemic immunochemotherapy was selected. However, EMZL is generally a clinically indolent disease with a 5-year overall survival ranging from 86 to 100% (28,29). The optimal approach for the management of uterine EMZL has not yet been established, and the treatment choice should be carried in consideration of the tumor site, disease stage and clinical manifestations on an individual basis. In conclusion, the present study reports the first case of uterine cervical EMZL with trisomy 18 and \( IgH \) translocation with an unknown partner, as detected by FISH analyses.

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