Primary uterine broad ligament ependymoma with CHEK2 p.H371Y germline mutation: A CARE-compliant case report
uterine broad ligament ependymoma

Jiaxin Yin1, Min Yao2, Hongdi Lu3, Xiaofeng Cong1, Pengfei Cui1 and Ziling Liu1
1Department of Oncology, The First Hospital of Jilin University, Jilin, China
2Department of Pathology, The Second Hospital of Jilin University, Jilin, China
3Department of Oncology, Jilin Cancer Hospital, Jilin, China

Abstract
Ependymomas arise from ependymal cells lining the ventricles and central canal of the spinal cord and can occur throughout the whole neuraxis. The lesion rarely occurs in extracranial or extraspinal regions, particularly in the uterine broad ligament. Thus, for the pathogenesis of nonsacral extra-central nervous system (CNS) ependymomas remains elusive. Here, we describe a rare case of primary uterine broad ligament ependymoma with cell-cycle-checkpoint kinase 2 (CHEK2) p.H371Y germline mutation. A 45-year-old woman presented with a uterine mass. The transvagal sonographic examination confirmed a 4.4 cm × 3.7 cm, cystic and solid, mass located on the right side uterine wall near isthmus. First, laparoscopy with the neoplasm resection was carried out. Based on morphological and immunohistochemical characteristics of tumor cells that expressed glial fibrillary acidic protein (GFAP), S-100, and vimentin, the tumor was diagnosed as an ependymoma. After that, she underwent a laparotomic total hysterectomy, bilateral salpingo-oophorectomy, and lymphadenectomy. Furthermore, we performed next-generation sequencing (NGS) of the patient’s resected tumor tissue and peripheral blood and identified a novel CHEK2 p.H371Y germline mutation. Following surgery, the patient received oral tamoxifen (10 mg 2/day) and followed by letrozole (2.5 mg/day) for 6 months. The patient remained disease-free after 4 years of follow-up. Conceivably, CHEK2 p.H371Y is a driving gene for the development of extra-CNS ependymoma.

Key words: CHEK2 p.H371Y, ependymoma, next-generation sequencing, uterine broad ligament.

Introduction
Ependymomas predominantly develop from neuroectodermal organs. Primary extraneural ependymomas are rare and typically defined as a sacrococcygeal, pelvic, or extrapelvic ependymoma based on tumor location. The most common site for extraneural ependymomas remains the sacrococcygeal region, with more than 50 reported cases. However, thus far, the pathogenesis of nonsacral extra-central nervous system (CNS) ependymomas remains elusive.

Here, we report a rare case of primary uterine broad ligament ependymoma with cell-cycle-checkpoint kinase 2 (CHEK2, also known as CHK2) p.H371Y germline mutation. The patient was treated with surgery, followed by endocrine therapy.

Case Report
A 45-year-old woman presented with chronic multilevel spinal pain. Transvaginal sonographic
examination revealed a 4.4 cm x 3.7 cm, cystic and solid, mass located in the right side of the uterine wall near isthmus. She underwent laparoscopy with the neoplasm resection. At the first time of surgery, a uterine mass of approximately 4.0 cm x 4.0 cm was seen protruding toward the right broad ligament, and the degree of bulging did not exceed the right round ligament. Grossly, tumor presented as a cystic and solid, gray section, crisp, and rotting meat-like lesion (Figure S1). Based on both histopathological and immunohistochemical examination of the mass, a diagnosis of ependymoma was made. Subsequently, after 7 days, she underwent a laparotomic total hysterectomy, bilateral salpingo-oophorectomy, and pelvic lymphadenectomy. Furthermore, gross pathological findings revealed that tumor exhibited infiltrated right myometrium and parametrial tissue, right fallopian tube wall, and surrounding tissue, and there were two metastatic obturator lymph nodes. The other pelvic organs and abdomen appeared normal. At high-power magnification of the specimen, tumor cells were consistent in size and arranged densely. Tumor cells exhibited uniform oval to the spindle, hyperchromatic nuclei, and lightly stained cytoplasm. Furthermore, tumor specimens revealed perivascular pseudorosettes and ependymal rosettes, with a central lumen radially surrounded by tumor cells, a structure similar to the medullary central canal (Figure 1). Immunohistochemical staining revealed that the tumor cells were diffusely immunoreactive to GFAP, vimentin, PAX-8, S-100 protein, P16, epithelial membrane antigen, and AE1/AE3. Among them, GFAP, S-100, vimentin expression is characteristic of the tumor (Figure 2). Immunostaining for hormone receptors showed positive staining for progesterone receptor (PR) and estrogen receptor (ER) in many tumor cells. Negative staining for P53, calciretinin, α-inhibin, cytokeratins 7 and CD10 was observed. Postoperative positron emission tomography and computed tomography (PET/CT) scans confirmed no residual lesions and distant metastasis. Furthermore, using PCR and NGS, a novel CHEK2 p.H371Y germline mutation, which specifically referred to H371Y hybrid germline mutation caused by the single base substitution of exon 11 of CHEK2 gene, was detected in the DNA obtained from tumor tissue and DNA peripheral blood (Figure S2). Hormonal therapy with an aromatase inhibitor or chemotherapy was considered most effective and the patient opted for endocrine therapy. She received oral tamoxifen (10 mg twice/day) followed by letrozole (2.5 mg/day) for 6 months. Follow-up CT scans of the chest, abdomen, and pelvis showed no evidence of disease. She remained asymptomatic and disease-free during the follow-up of 4 years.

Informed written consent was obtained from the patient for publication of this case report and accompanying images.

Discussion

Primary uterine broad ligament ependymoma is an extremely rare extra CNS neoplasm with only seven previously reported cases2–7 in the literature worldwide. Of these, four tumors originated in broad ligament,2–4 one in uterosacral ligament,5 one in mesovarium right ovary,6 and one in endometrium7 (Table 1). Ependymomas predominantly affect women of relatively younger age (mean age, 38 years, 13–61 years). The extra-CNS ependymoma is usually large, partially cystic, and solid masses (mean size, 9 cm, 1–14 cm) that cause patients to experience abdominal pain. The solid mass was mostly associated with hemorrhage and necrosis. Tumors were located mostly at the right (six of eight cases) compared with the left (one of eight cases). Histopathologically, only two ependymomas exhibited myxopapillary, and the others presented the classical morphology. Immunohistochemically, the diagnosis of
A typical primary extra-CNS ependymoma was supported by the presence of ependymal rosettes, perivascular pseudorosettes, and GFAP immune-positivity. In most cases, extra-CNS ependymoma is histologically classified as low-grade ependymomas; however, they can be potentially fatal. Notably, of histologically classified low malignant tumors, nearly 50% of tumors have been recognized to exhibit a metastasis at initial treatment. Furthermore, ependymoma cells metastasize to and recur mostly in the peritoneum. Perhaps, as there is no
blood-brain barrier impeding dissemination, the tumor cells can easily access to the bloodstream and lymphatic system. The findings were consistent with that of the previously reported cases, where the subcutaneous sacrococcygeal ependymoma is as high as 20% with distant metastasis.  

Because of the rarity of this neoplasm, no standard or overall effective treatment or chemotherapy has been identified. Previously, most of the patients underwent surgery at initial treatment, followed by adjuvant therapy after surgery, including chemotherapy, radiotherapy, chemoradiotherapy, or endocrine therapy. The patient exhibited recurrence at 11 and 24 years, postoperatively. Nevertheless, surgical resection may be most effective when the recurrent tumor is detected. Although data regarding survival remains limited, a few of the cases exhibited prolonged survival and slow growth of the tumor, even in the presence of metastasis.

While the female gender biases of sacrococcygeal tumors are similar to that of CNS ependymomas, cases of extra-axial ependymomas arising elsewhere appear to occur exclusively in women. Ependymomas arise throughout the neuraxis and have an intimate relationship to ependymal cells or their remnants. However, the pathogenesis of nonsacral extra-CNS ependymomas remains largely unclear. Ependymomas in the ovary, the uterine ligaments, or the mediastinum have been described very rarely. This can be attributed to the fact that ependymomas in the ovary, broad ligament, and mediastinum might originate from neuroectodermal teratoma tissue. Besides, according to another theory, under the influence of female hormones, misdirected primordial germ cells transform to ependymal cells, and this could possibly explain the female predominance of such tumors. It might also explain why extra-CNS ependymomas stain strongly and diffusely positive for estrogen and progesterone receptors.

NGS of DNA obtained from peripheral blood and tumor tissues samples revealed that \textit{CHEK2} p.H371Y germline mutations caused by single base permutation in exon 11. This finding is unprecedented. \textit{CHEK2} is located on the long (q) arm of chromosome 22. \textit{CHEK2} is a tumor suppressor gene that encodes a multifunctional kinase that is activated essentially by the ataxia telangiectasia-mutated (ATM) protein in response to DNA double-strand breaks. Activated \textit{CHEK2}, in turn, phosphorylates several critical cell-cycle proteins, including p53, Cdc25, and \textit{BRCA1}, which trigger cell-cycle arrest, apoptosis, and the activation of DNA repair. \textit{CHEK2} p.H371Y mutation is located within the activation loop of the \textit{CHEK2} protein kinase domain.
which is crucial for the activation of CHEK2 in response to DNA damage. Further functional analysis revealed that the CHEK2 p.H371Y mutation causes a dramatic decline in CHEK2 activity and is a pathogenic mutation. CHEK2 p.H371Y confers a 2.43-fold increase in breast cancer risk among Chinese women. Thus, CHEK2 p.H371Y mutation may share some similarity to BRCA1 mutation; therefore, carriers of CHEK2 H371Y mutation may be considered potential candidates for treatment with poly ADP-ribose polymerase-1 inhibitors.

The finding of CHEK2 p.H371Y mutation in extra-CNS is novel to the molecular profile of CNS ependymomas. However, due to limited tissue availability and lack of funding for more in-depth molecular testing, whether CHEK2 p.H371Y is a pathogenic gene for the development of extra-CNS ependymoma is currently uninvestigated. Thus, future studies are needed to confirm the gene expression profile of extra-CNS ependymomas, which may provide insight into the cellular origin and molecular drivers of these rare tumors. In summary, primary uterine broad ligament ependymomas are extremely rare kinds of extra central nervous system ependymomas and there is no one unified standard treatment for this. Thus, to better understand the molecular pathogenesis of this rare neoplasm, further molecular profiling studies are needed to guide clinical therapy. The finding of CHEK2 p.H371Y might conceivably become a major breakthrough for the treatment of extra-CNS ependymomas.

Conflict of Interest
None declared.

Author Contributions
Jiaxin Yin was mainly responsible for drafting of the manuscript and revising. Min Yao was responsible for supporting pathology images. Xiaofeng Cong was responsible for acquisition of data and follow up. Hongdi Lu contributed analysis and Interpretation of data. Pengfei Cui contributed supervision. Ziling Liu contributed conception and design.

Data Availability Statement
The data that support the findings of this study are available from the corresponding author upon reasonable request.

References
1. Hwang HJ, Sohn JH, Han SJ, et al. Multi-disciplinary treatment of a rare pelvic cavity ependymoma. Yonsei Med J. 2007;48:719–22.
2. Matsuyama A, Hisaoka M, Yamamoto I, et al. Extraspinal ependymoma of the broad ligament. Pathol Int. 2010;60:241–4.
3. Whittemore DE, Grondahl RE, Wong K. Primary extraneuronal myxopapillary ependymoma of the broad ligament. Arch Pathol Lab Med. 2005;129:1338–42.
4. Bell DA, Woodruff JM, Scully RE. Ependymoma of the broad ligament. A report of two cases. Am J Surg Pathol. 1984;8:203–9.
5. Duggan MA, Hugh J, Nation JG, et al. Ependymoma of the uterosacral ligament. Cancer. 1989;64:2565–71.
6. Grody WW, Nieberg RK, Bhuta S. Ependymoma-like tumor of the mesovarium. Arch Pathol Lab Med. 1985;109:291–3.
7. Schultd M, Retamera JA, Bergeron C, et al. Papillary ependymoma of the endometrium. Histopathology, 2014;65:923–5.
8. Ma YT, Ramachandra P, Spooner D. Case report: primary subcutaneous sacrococcygeal ependymoma: a case report and review of the literature. Br J Radiol. 2006;79:445–7.
9. Maeda S, Takahashi S, Koike K, et al. Primary ependymoma in the posterior mediastinum. Ann Thorac Cardiovasc Surg. 2011;17:494–7.
10. Idowu MO, Rosenblum MK, Wei XJ, et al. Ependymomas of the central nervous system and adult extra-axial ependymomas are morphologically and immunohistochemically distinct—a comparative study with assessment of ovarian carcinomas for expression of glial fibrillary acidic protein. Am J Surg Pathol. 2008;32:710–8.
11. Jemal A, Bray F, Center MM, et al. Global cancer statistics. CA Cancer J Clin. 2011;61:69–90.
12. Falck J, Mailand N, Syljuåsen RG, Bartek J, Lukas J. The ATM-Chk2-Cdc25A checkpoint pathway guards against radioresistant DNA synthesis. Nature, 2001;410(6830):842–9. https://doi.org/10.1038/35071124
13. Zhang J, Willers H, Feng Z, et al. Chk2 phosphorylation of BRCA1 regulates DNA double-strand break repair. Mol Cell Biol. 2004;24:708–18.
14. Hirao A, Kong YY, Matsuoka S, et al. DNA damage-induced activation of p53 by the checkpoint kinase Chk2. Science. 2000;287:1824–7.
15. Liu Y, Liao J, Xu Y, et al. A recurrent CHEK2 p.H371Y mutation is associated with breast cancer risk in Chinese women. Hum Mutat. 2011;32:1000–3.
16. Liu Y, Xu Y, Ouyang T, et al. Association between CHEK2 H371Y mutation and response to neoadjuvant chemotherapy in women with breast cancer. BMC Cancer. 2015;15:194.

Supporting information
Additional Supporting Information may be found in the online version of this article at the publisher’s web-site:
Figure S1 Macroscopic photo of this neoplasm
Figure S2 Cell-cycle-checkpoint kinase 2 (CHEK2) is located on the long (q) arm of chromosome 22. p. H371Y germline mutation is caused by single base permutation in exon 11 of the CHEK2 gene.

© 2021 The Authors. Journal of Obstetrics and Gynaecology Research published by John Wiley & Sons Australia, Ltd on behalf of Japan Society of Obstetrics and Gynecology.