Molecular diagnosis in female genital tract tumors: Presentation of interesting cases

Shigeto Kawauchi, MD, PhD
Associate Professor of Pathology
Yamaguchi University Graduate School of Medicine,
Ube City, Yamaguchi 755-8505, JAPAN

Introduction

Recent developments in molecular cytogenetics and molecular cell biology have born fruits as new medical strategies. In the field of diagnostic surgical pathology, the introduction of so-called molecular diagnostics appears to be based on such trends. Although most female genital tract tumors have not fully been studied molecular-cytogenetically, the concept, nomenclature, and the therapy of them may be altered as a result of such a study.

In the present session, some molecular techniques including reverse-transcription polymerase chain reaction (RT-PCR), fluorescence in situ hybridization (FISH), and comparative genomic hybridization (CGH) were discussed with the diagnostic applications to diagnostically-problematic female genital tract tumors.

Case 1. Lt. ovarian tumor

We present a case of left ovarian tumor that belongs to the peripheral primitive neuroectodermal tumor (pPNET) / Ewing's sarcoma family[1] arising in a 29-year-old woman. Microscopically, the tumor was composed of solid nests and sheets of monotonous primitive small-rounded cells with a few abortive rosettes (fig. 1), making it difficult to distinguish from small cell carcinoma of the ovary, especially that of the pulmonary type. Immunohistochemically, the tumor cells exhibited cell-membranous immunoreactivity for CD99. A short-term cell culture and karyotypic analysis revealed the tumor to possess a balanced chromosomal translocation, t(11;22)(q24;q12), which is highly specific to the pPNET/Ewing's sarcoma family[2] (fig. 2. lt. side). The translocation has never been described in primitive neuroectodermal tumors of the central nervous system (cPNET). Furthermore, EWS-FLI1 chimeric mRNA originatimg from the specific translocation was detected by RT-PCR (fig. 2. rt. side). The results confirmed the diagnostic validity of the present tumor being pPNET arising in the ovary [3].

Fig. 1. pPNET of the ovary

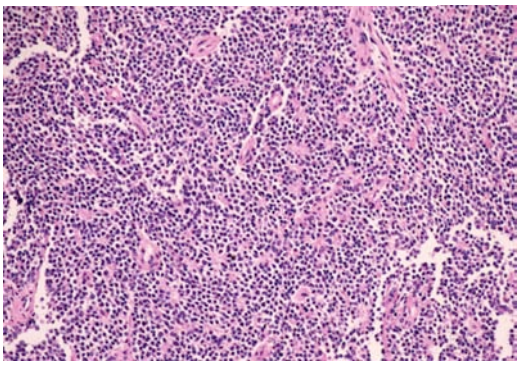
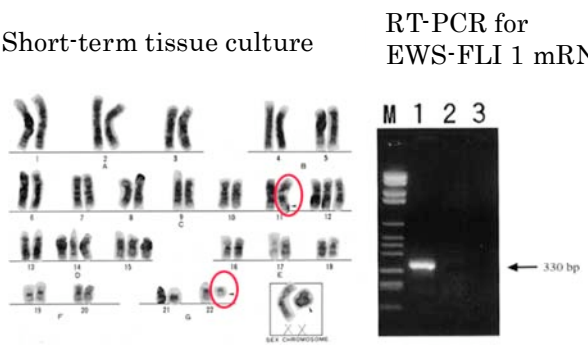


Fig. 2. Karyotypic analysis and RT-PCR



Case 2. Rt. vulvar tumor

Synovial sarcoma (SS) rarely arises in the female genital tract[4]. We present a case of SS occurring in the right vulva of a 21-year-old woman[5]. Microscopically, the tumor was composed of poorly differentiated rounded-cell regions, surrounded by spindle-shaped fibroblastic cell regions and intermingled myxoid regions (fig. 3). Immunohistochemically, the tumor cells were at least focally positive for cytokeratin, vimentin, CD99, Bcl-2, and NSE. The tumor was suspected, but difficult to confirmatively diagnose as SS based only on the light-microscopic and immunohistochemical findings. Although RT-PCR failed to detect SS-specific SYT-SSX fusion gene transcripts using the RNA sample extracted from the formalin-fixed, paraffin-embedded tumor tissue, SYT break-apart rearrangement fluorescence in situ hybridization (SYT bar-FISH) successfully confirmed the diagnosis of SS for the present tumor[5] (fig. 4). In using the archival paraffin-embedded tumor tissue specimen for the molecular diagnosis, SYT bar-FISH may be more suitable than RT-PCR[5,6].

Fig. 3. Synovial sarcoma of the vulva

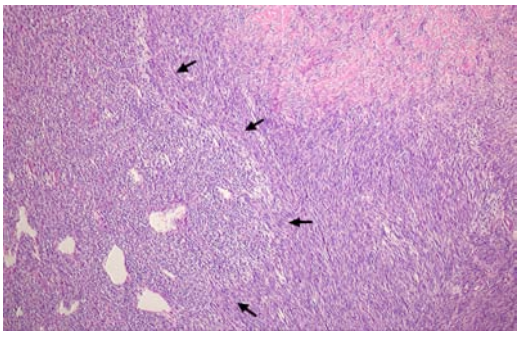


Fig. 4. SYT bar-FISH

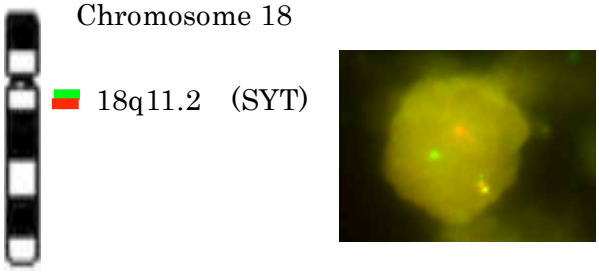


Fig. 4. LSI SYT break-apart rearrangement FISH. The first probe labeled with SpectrumOrange extends distally from the SYT gene (orange line and dot). The second probe labeled with SpectrumGreen lies proximal to the SYT gene (green line and dot). A synovial sarcoma cell carrying the SYT gene break usually demonstrates one orange, one green, and one fusion yellow signal pattern.

Case 3. Uterine corpus tumor

Angiomyolipoma is regarded as the representative tumor of the perivascular epithelioid cell tumor (PEComa) family[7,8]. In addition to the loss of heterozygosity (LOH) of 16p including TSC2 locus, the PEComa family tumors are characterized morphologically by the presence of perivascular epithelioid cell (PEC) and immunoreactivity for melanocytic and smooth muscle markers[7]. However, previously reported angiomyolipoma or angiomyolipoma-like tumors arising in the uterus were mostly negative in the immunohistochemistry of melanocytic markers[9]. Thus, angiomyolipoma has yet to accept as an established entity of the uterus.

We present a case of HMB-45-negative uterine corpus tumor, which was light-microscopically compatible with conventional angiomyolipoma, arising in a 24-year-old woman[10] (fig. 5). In the present tumor, comparative genomic hybridization (CGH) was used to estimate the diagnostic validity of the tumor being an angiomyolipoma. Although the tumor was immunohistochemically negative for a melanoma marker, HMB-45, the CGH analysis revealed that the present tumor shared a considerable number of non-random chromosomal imbalances with the PEComa family tumors reported previously[11], *that is*, gains of 2q, 3q, 5q, 12q and X, and losses of 1p, 16p and 19q (fig. 6). The molecular genetic evidences, as well as the morphologic features, confirmed that the present tumor was compatible with angiomyolipoma belonging to the PEComa family.

Fig. 5. Angiomyolipoma of the uterus

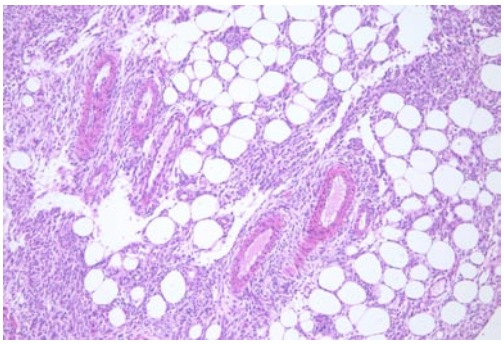
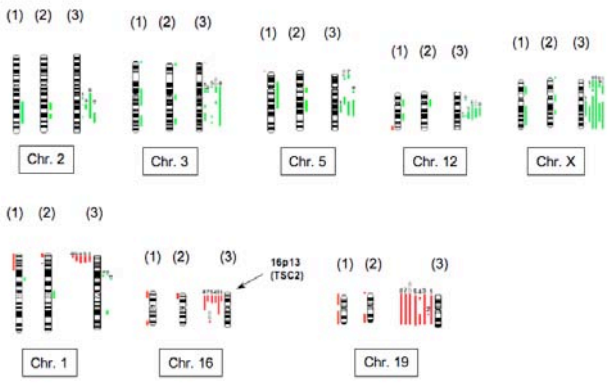


Fig. 6. CGH result



(1) Present case (3) Pan CC, et al. Hum Pathol 2006

Case 4. Uterine cervical tumor

In the WHO classification[12] 2003, cervical neuroendocrine tumors (NETs) divided into four categories, *that is*, carcinoid, atypical carcinoid, small cell carcinoma, and large cell neuroendocrine carcinoma (LCNEC) according the pulmonary counterparts. Although

pulmonary atypical carcinoid is regarded as an intermediate-grade carcinoma, it is not clear whether cervical atypical carcinoid is also an intermediate-grade carcinoma or not.

We present a case of cervical NET with a cytogenetic analysis by CGH[13,14]. The tumor, as well as the immunoreactivity for some neuroendocrine markers, showed moderately increased mitotic activity (8 to 14 mitotic figures per ten high-power fields) and focal areas of necrosis (fig. 7). These findings suggested atypical carcinoid as a diagnosis for the present tumor, although LCNEC should be ruled out. The CGH analysis failed to detect 11q loss that was reported to be highly specific to pulmonary atypical carcinoid[12] (fig. 8). Furthermore, remarkable 3q amplification, which has been detected frequently in pulmonary high-grade NETs including pulmonary small cell carcinoma and LCNEC, but not in pulmonary carcinoids, was the most remarkable chromosomal imbalance detected (fig. 8). Our CGH result indicated that the present cervical tumor was cytogenetically rather comparable to pulmonary high-grade NET than pulmonary atypical carcinoid. Our result also suggested that some cervical NETs that were diagnosed as atypical carcinoid might have included tumors cytogenetically compatible with high-grade NET.

Fig. 7. Atypical carcinoid of the cervix

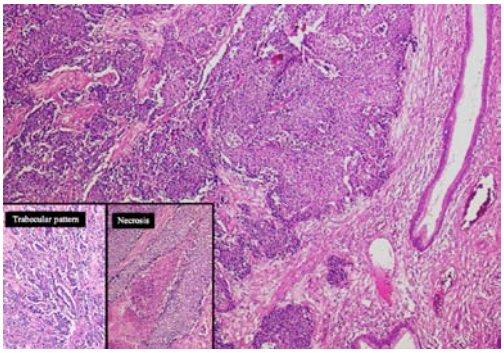
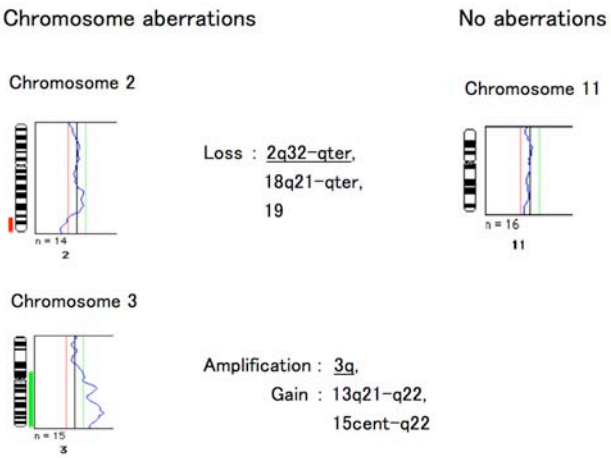


Fig. 8. CGH result



Discussion

The pPNET/Ewing's sarcoma family represents a group of small-round cell tumors of putative neuroectoderm origin and is the second most common sarcoma among children and young adults. Although the tumors may occur anywhere in the body and within any age group, they are most likely to occur in the bone and soft tissues. The fact potentially makes a diagnosis of pPNET difficult when the female genital tract is primary site. In the case 1,

RT-PCR detected a chimeric fusion gene transcript originating from the reciprocal chromosomal translocation t(11;22)(q24;q12) specific to the pPNET/Ewing's sarcoma family. Reported cases of pPNET of the ovary that were diagnosed by the molecular analysis have been extremely rare. Our result also raised the possibility that some pPNETs arising in the ovary might have been mistakenly diagnosed as small cell carcinoma of the ovary.

A diagnosis of SS may be challenging in diagnosing the monophasic fibrous or poorly differentiated types. Furthermore, SS typically arises in the deep soft tissue of the extremities and rarely arises in the female genital tract. In the case 2, although RT-PCR failed to detect SS-specific fusion gene transcripts with the RNA sample extracted from the formalin-fixed paraffin-embedded tumor tissue, SYT bar-FISH successfully confirmed the diagnostic validity of SS for the present tumor. In using the archival paraffin-embedded tissue specimen for the molecular diagnosis, SYT bar-FISH may be an alternative, more suitable tool.

Although RT-PCR and FISH are popular in the field of molecular diagnostics as a molecular diagnosis tool, CGH has rarely been used as such. The cases 3 and 4 represented our attempts to expand the application of CGH into tumor diagnosis. Although the uterine tumor mimicking angiomyolipoma was negative in the HMB45 immunohistochemistry (a diagnostic marker of the PEComa family), the present tumor demonstrated chromosome aberrations very similar to those of the PEComas reported. Therefore, based on the morphologic and molecular-cytogenetic evidences, we diagnosed the HMB45-negative uterine corpus tumor as angiomyolipoma.

In the case 4, the cervical tumor was considered as atypical carcinoid according to the WHO classification 2003. However, the characteristic chromosomal aberrations detected by CGH were comparable to those of pulmonary high-grade NET, *that is*, LCNEC and small cell carcinoma, rather than those of pulmonary intermediate-grade NETs, *that is*, typical and atypical carcinoids. The CGH result thus suggested that the present cervical atypical carcinoid might be a high-grade tumor. Because uterine cervical NETs are rare tumors in comparing to the pulmonary counterparts, it is not clear whether cervical atypical carcinoids are intermediate-grade tumors or not. Further studies will be necessary to fully elucidate the diagnostic significance of CGH in the classification of cervical NETs.

We herein presented the applications of some molecular techniques including RT-PCR, FISH, and CGH to the diagnoses of problematic female genital tract tumors.

References

1. Dehner LP. Primitive neuroectodermal tumor and Ewing's sarcoma. *Am J Surg Pathol* 1993; 17: 1-13.
2. Kawauchi S, et al. Peripheral primitive neuroectodermal tumor of the ovary confirmed by CD99 immunostaining, karyotypic analysis, and RT-PCR for EWS/FLI-1 chimeric mRNA. *Am J Surg Pathol* 1998; 22: 1417-1422.
3. Nogales H, et al. Germ cell tumours, p. 272-277 In: Tavassoli FA, Devilee P, eds. *World Health Organization Classification of Tumours: Pathology and Genetics of Tumours of the Breast and Female Genital Organs*. IARC Press, Lyon, 2003.
4. Fisher C, et al. Synovial sarcoma, p. 200-204 In: Fletcher CDM, Unni KK, Mertens F, eds. *World Health Organization Classification of Tumours: Pathology and Genetics of Tumours of Soft Tissue and Bone*. IARC Press, Lyon, 2002.
5. Kawauchi S, et al. Synovial sarcoma arising in the vulva cytogenetically confirmed by SYT break-apart rearrangement fluorescence in situ hybridization: a case report and discussion of diagnostic methods. *Oncol Lett* 2012; 4: 955-959.
6. Sin B, et al. The diagnostic value of SYT-SSX detected by reverse transcriptase-polymerase chain reaction (RT-PCR) and fluorescence in situ hybridization (FISH) for synovial sarcoma: a review and prospective study of 255 cases. *Cancer Sci* 2008; 99: 1355-1361.
7. Hornick JL, Fletcher CDM. PEComa: what do we know so far? *Histopathology* 2006; 48: 75-82.
8. Folpe AL, Kwiatkowski DJ. Perivascular epithelioid cell neoplasms: pathology and pathogenesis. *Hum Pathol* 2010; 41: 1-15.
9. Yaegashi H, et al. Uterine angiomyolipoma: case report and review of the literature. *Pathol Int* 2001; 51: 896-901.
10. Kawauchi S, et al. Chromosomal imbalances detected by comparative genomic hybridization provide evidence that HMB-45-negative uterine angiomyolipomas belong to the PEComa family. *Histopathology* 2010; 56: 974-977.
11. Pan CC, et al. Comparative genomic hybridization study of perivascular epithelioid cell tumor: molecular genetic evidence of perivascular epithelioid cell tumor as a distinctive neoplasm. *Hum Pathol* 2006; 37: 606-612.
12. Wells M, et al. Epithelial tumors. p. 279. In: Fletcher CDM, Unni KK, Mertens F, eds. *World Health Organization Classification of Tumours: pathology and genetics of tumors*

of the breast and female genital organs. IARC Press, Lyon, 2003.

13. Kawauchi S, et al. Large cell neuroendocrine carcinoma of the uterine cervix with cytogenetic analysis by comparative genomic hybridization: a case study. *Hum Pathol* 2005; 36: 1096-1100.
14. Kawauchi S, Sasaki K. Comparative genomic hybridization for DNA extracted from cytological smear. *Hum Pathol* 2005; 36: 1241-1242.