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Genetic alterations in pulmonary epithelioid hemangioendothelioma and epithelioid angiosarcoma

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Summary. Epithelioid hemangioendothelioma (EHE) is a low-to-intermediate-grade vascular tumor that occurs in many organs, and epithelioid angiosarcoma (EA) is a subtype of angiosarcoma that is associated with high-grade malignancy. These two types of tumors have different forms of biological behavior. Pulmonary epithelioid hemangioendothelioma (PEH) and epithelioid angiosarcoma (PEA) are both very rare, and genetic studies on them are extremely limited. We examined and compared the cytogenetic characteristics of these two types of lung tumors in two patients utilizing the Array-Comparative Genomic Hybridization (Array-CGH) method.

Considerable differences in the cytogenetic characteristics were observed between the two types of tumors. Small fragment gains (<10 MB) were dominant in PEH, whereas large fragment gains and deletions (>10 MB) were dominant in PEA. Some large fragment alterations, such as gains in chromosomes 19q and 19p, and deletions in chromosomes 9p and 13q, involved over half of a chromosome arm.

PEH and PEA showed great cytogenetic differences; therefore, further genetic studies on these two types of tumors are warranted.

Key words: Epithelioid hemangioendothelioma, Epithelioid angiosarcoma, Array-CGH, Lung

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Introduction

Epithelia hemangioendothelioma (EHE) is a low-to--intermediate-grade vascular tumor that occurs in many organs, such as the skin, bone, lung, pleura, liver, peritoneum and lymph node (Yousem et al., 1987). High-grade vascular tumors are called epithelioid angiosarcomas (EA). Pulmonary epithelioid hemangioendothelioma (PEH) and epithelioid angiosarcoma (PEA) are both very rare. EHE represents 1% of all vascular neoplasms (Yousem and Hochholzer, 1987), and approximately 100 cases of PEH have been reported internationally. However, most of these reports were from the perspective of clinical pathology, and genetic studies of these types of tumors are extremely limited. Chromosome translocation of t(1;3) (p36.3;q25) has been reported in two cases of soft tissue EHE (Mendlick et al., 2001). Data on the cytogenetic features of EA are also scarce, and until now no characteristic aberrations have been demonstrated. To the best of our knowledge, cytogenetic abnormalities have not been reported in primary PEH and PEA. These two types of tumors have different forms of biological behavior; however, the potential differences in the genetic characteristics of the two types have not been studied. Based on our analyses of one patient with PEH and one with PEA, we describe the cytogenetic characteristics of these two types of tumors utilizing the Array-Comparative Genomic Hybridization (Array-CGH) method.

Materials and methods

Patients

Of the more than 9000 patients diagnosed with lung malignancy in our department over the past 10 years,

only 3 had PEH and 1 had PEA. Tissue specimens were available for genetic analyses from only one patient with PEH and one patient with PEA. The key clinical and pathological characteristics of the patients are presented in Table 1.

Cytogenetic analysis

Tumor cells were scraped and collected in 5-10 10-µm thick formalin-fixed paraffin-embedded (FFPE) sections for each histological lesion from the two cases. Genomic DNA (gDNA) was extracted from cells according to the G4410-90020_CGH_Protocol_FFPE1 from Agilent. Concentrations and purities were precisely measured using the NanoDrop ND-1000 UV-VIS Spectrophotometer.

Comparative genomic hybridization was performed according the manufacturer's protocol for the 1x185 K Agilent Oligo aCGH microarray. The tumor DNA and reference DNA were labeled with USL-Cy5 and USL-Cy3, respectively, using the Agilent Oligo aCGH Labeling Kit for FFPE Samples. A total of 2 µg of both DNA samples and human CotI DNA was dissolved in the hybridization mixture supplied with the Agilent Oligo array-CGH hybridization kit. After denaturation at 95°C for 3 minutes and incubation at 37°C for 30 minutes, the mixtures were slowly dispensed onto the gasket. A microarray slide was then placed onto the gasket slide. The samples were hybridized in a hybridization oven at 65°C and 20 rpm for 40 hours. The slides were scanned with an Agilent or GenePix Scanner in 5-µm sections, and the Feature Extraction Software was used for data extraction. CGH Analytics 4.0 from Agilent was used to analyze the data.

Results

Chromosome imbalances were observed frequently in both patients (Fig. 5). We observed that small-scale (<10 MB) gains in the DNA copy numbers of chromosomes 5q, 8q, 11q, 12p and others were dominant in PEH, whereas large-scale (>10 MB) gains in the DNA copy numbers of chromosomes 9q, 11q, 12q, 16p, 17q, 19p, 19q and 22q and deletions in chromosomes 2q, 3q, 4q, 5p, 6q, 9p, 11q, 13q, 14q and 20p were dominant in PEA. Moreover, the changes observed in the PEA patient were significantly larger than those in the PEH patient. Some large fragment alterations, such as gains in chromosomes 19q and 19p and deletions in chromosomes 9p and 13q, involved over half a

Table 1. Clinical and pathological characteristics of Patient 1 with PEH and Patient 2 with PEA.

	Patient 1	Patient 2
Diagnosis	PEH	PEA
Gender	Female	Male
Age (years) at diagnosis	56	45
Presentation/Symptoms	Chest pain and intermittent stimulatory cough for 4 months	Dyspnea and pleural effusion for 2 months
CT scan	Multiple nodules in the lung, liver and thoracic vertebrae (Fig. 1)	multiple nodules in both sides of the lung
Clinical diagnosis	Metastasis of advanced cancer	Mesothelioma
Thoracotomy	Multiple nodules were found in both lobes of the right lung, and part of the parietal pleura was shrunken.	Multiple nodules were found in both lobes of the right lung; the pleura and diaphragm revealed extensive adhesion.
Macroscopy	Tumors and tissues of the lung were resected, with dimensions of 6.5x2.5x1.5 cm. Multiple grayish white nodules were seen on the cut surface of lung and varied in diameter from 0.4 cm to 1.0 cm.	The lower lobe of the right lung was removed. Some parts of the visceral pleural were shrunken. A tumor with dimensions of 3x1.8x2.0 cm was found in the periphery of the lung with a grey-white cut surface; focal necrosis was observed.
Microscopy (Fig. 2)	Low power histological examination revealed that multiple tumor nodules of various sizes were disseminated in the parenchyma of the lung, which typically had a central sclerotic or mucinous, hypocellular zone, a cellular peripheral zone and slight nuclear dysplasia.	Tumor cells were arranged in bulks and nests, which were devoid of mucinous matrix. Obvious nuclear dysplasia and mitotic or necrotic tissue were seen.
Immunohistochemistry		
CD34 (Maixin Ltd.) (Fig. 3)	+	+
CD31 (Zhongshan Bio. Ltd.)	+	+
Factor VIII (Maixin Ltd.)	+	+
TTF-1; AE1/AE3 (Zhongshan Bio. Ltd.)	-	-
S-100 (DOKO Ltd.)	-	-
Ki-67 (Maixin Bio. Ltd.) (Fig. 4)	< 5% +	about 40% +

chromosome arm.

Discusion

EHE is mostly multifocal (Evans et al., 2003; Chirieac et al., 2006) and commonly involves the lung and liver (Celikel et al., 2007). Furthermore, EHE of the bone and soft tissue is equally likely to occur among males and females, whereas EHE of the lung and liver occurs primarily among females (Celikel et al., 2007). Patient 1 (female) is a typical case with EHE of the lung and liver. EHE is often misdiagnosed as an advanced tumor metastasis in the clinic because it involves many organs. No obvious evidence was found to indicate that the separate lesions were caused by metastases. However, EHE is very likely to show up as a multifocal disease because of its morphological appearance, such as less heteromorphism, no tumor embolus in vessel, long-term survival with tumor and a low 5-year mortality rate of less than 20% (Weiss et al., 1986; Deyrup et al., 2008).

EHE is a vascular tumor that behaves biologically between hemangioma and high-grade angiosarcoma, but possesses its own typical histological features. Tumors in EHE are typically composed of short strands, cords or solid nests of epithelioid endothelial cells. Relatively uniform epithelioid endothelial cells are embedded in a distinctive, sulfated acid-rich stroma, and intracytoplasmic lumen (vacuoles) are frequently observed (Fetsch et al., 2004), which is similar to the signet ring cells observed in poorly differentiated adenocarcinomas. PEH typically has nodules composed of reverse case hardening, with a zone of cytoreduction and exterior cytosis, but it seldom produces multicellular vascular channels. The tumor cells are less heteromorphic and have little or no mitotic activity. Necrosis is infrequently

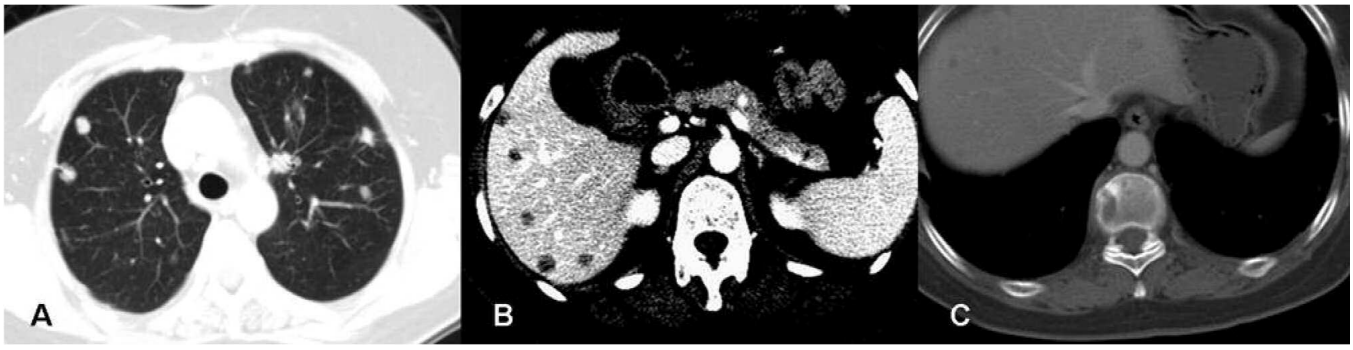

Fig. 1. CT scans of patient 1 show multiple bilateral nodules in the lung **(A)**, liver **(B)** and thoracic vertebrae **(C)**.

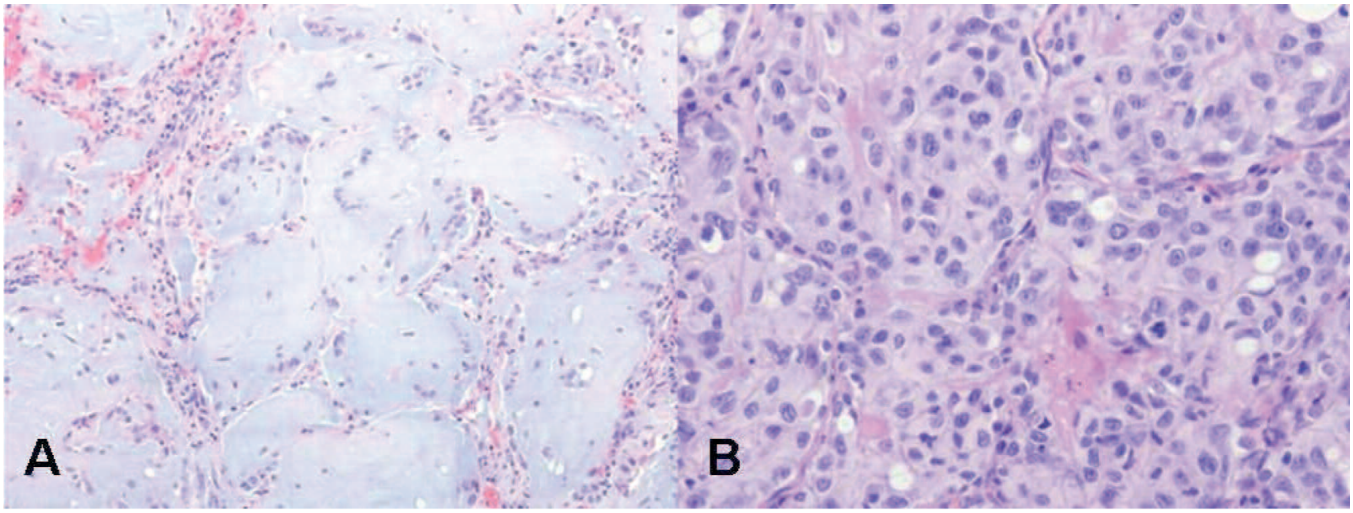

Fig. 2. A. PEH (patient 1) composed of round nodules with a mucinous hypocellular zone and a cellular peripheral zone. The nuclei of the tumor cells show slight nuclear dysplasia. No mitosis or necrosis is evident. **B.** PEA (patient 2) composed of tumor cells arranged in bulks and nests, which are devoid of mucinous matrix. Obvious nuclear dysplasia and mitoses are seen. H-E, x 200

observed. Case 1 in our study belongs to the PEH tumor type with low proliferative activity in Ki-67 expression (less than 5%), while Ki-67 expression is about 40% in PEA. It has been reported that PEH may demonstrate angiosarcoma-like characteristics in only a portion or foci of the specimens with marked pleomorphic cells and irregular, poorly formed vascular spaces (Evans et al., 2003; Hisaoka et al., 2005). Moreover, it has been reported that approximately one-third of cases show atypical histological features called angiosarcoma-like features, which are associated with more aggressive behavior. These features include marked nuclear atypia, cellular mitotic spindle activity and necrosis (Weiss and Bridge, 2002). Some cases even present the morphological continuum of EA. From our perspective, tumors with these histological features could be diagnosed as EA, similar to EHE with a major area of angiosarcoma-like features. The cytogenetic characteristics of these tumors may in this sense be more similar to EA.

Unfortunately, few studies have described the cytogenetic changes of EHE. One study demonstrated

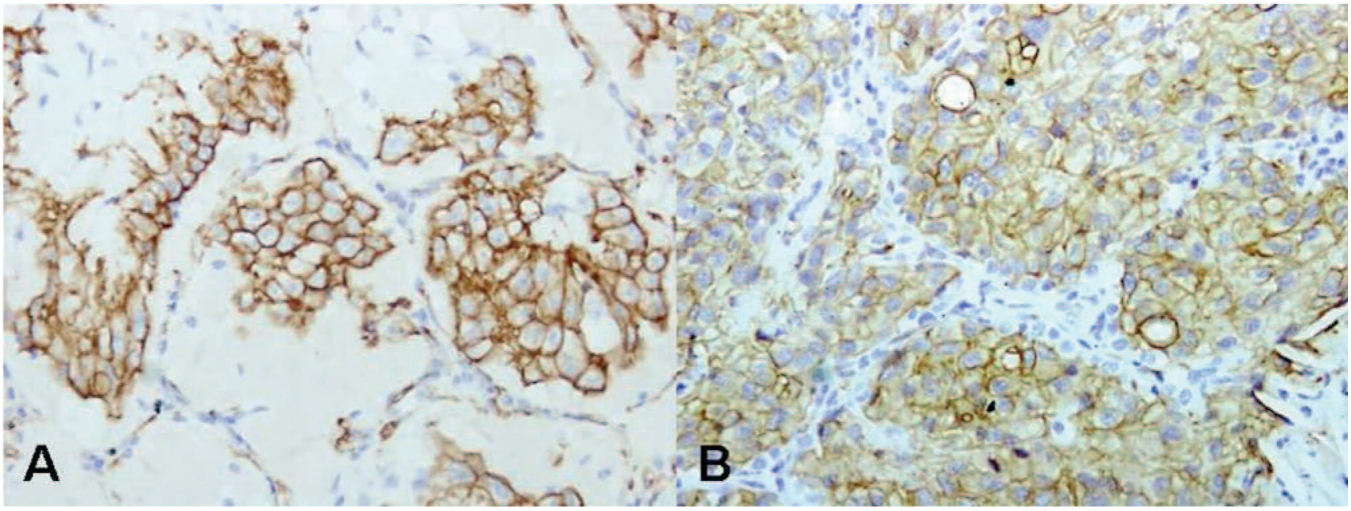

Fig. 3. CD34 immunohistochemistry stain at x 200 magnification shows the immunoreactivity of the neoplastic cells in the (**A**) PEH (patient 1) and (**B**) PEA (patient 2) samples. x 200

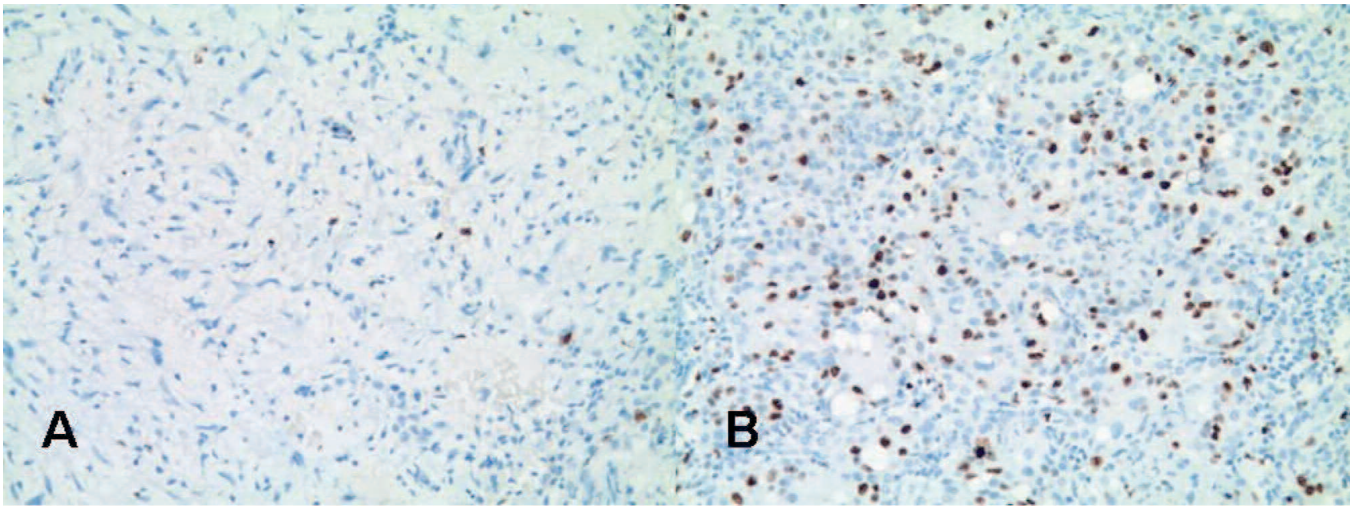

Fig. 4. Ki-67 immunohistochemistry stain at x100 magnification shows the proliferative index of the (**A**) PEH (patient 1) and (**B**) PEA (patient 2) samples. x 100

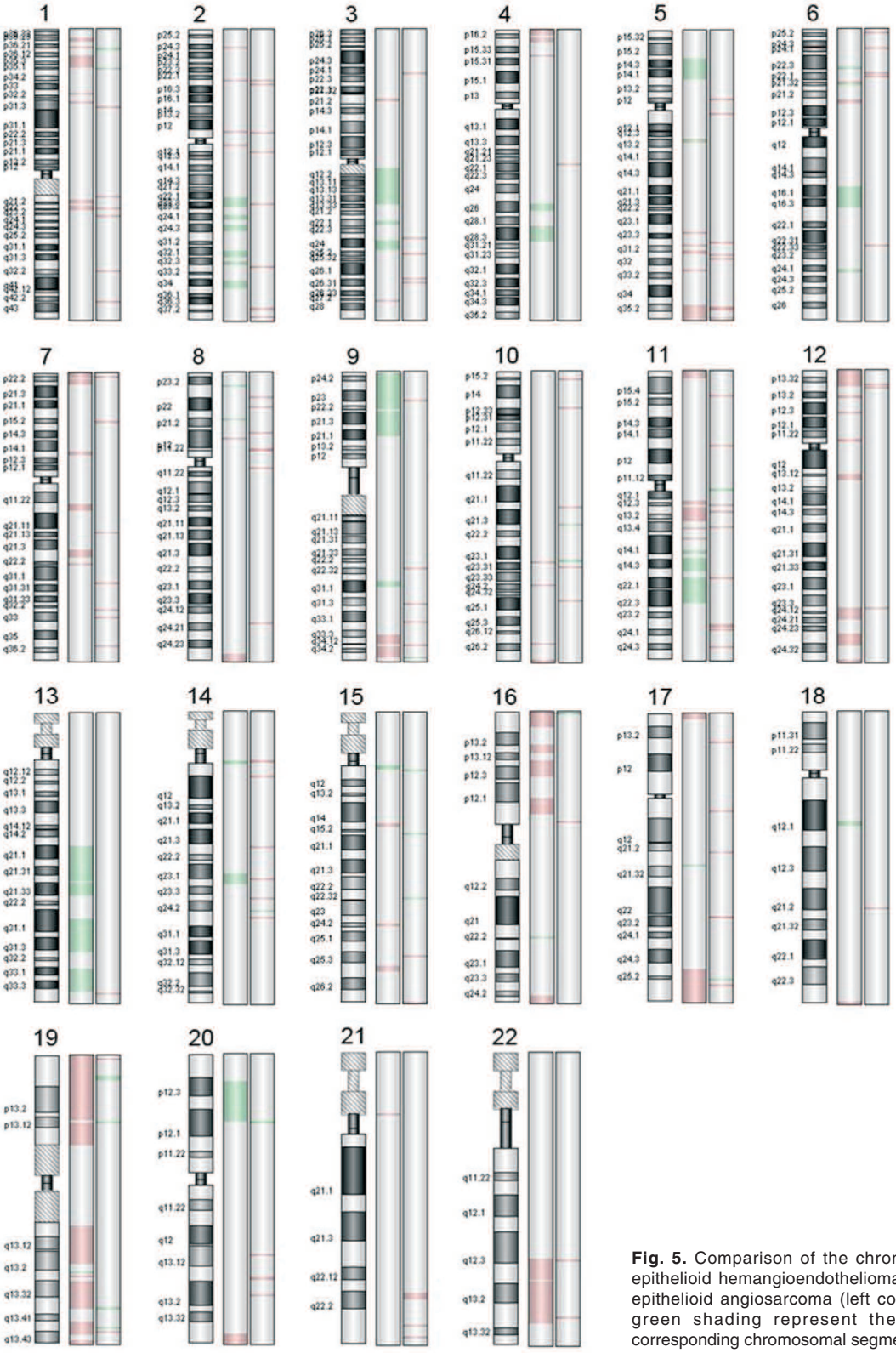

Fig. 5. Comparison of the chromosome imbalances between epithelioid hemangioendothelioma (right column, patient 1) and epithelioid angiosarcoma (left column, patient 2). The red and green shading represent the gain and deletion of the corresponding chromosomal segments, respectively.

that EHE was associated with t(1;3) (p36.3;q25) (Mendlick et al., 2001), and another showed gains on chromosomes 11 (q13-q14) and 12 (q11-q21) and deletions on chromosome 11 (q21-qter) (Tsarouha et al., 2006). However, there have not been any comprehensive comparative studies on the cytogenetic changes of EHE and EA.

We used the Array-Comparative Genomic Hybridization (Array-CGH) method to study the cytogenetic characteristics of tumor cells from two patients. Many different alterations were detected in these two types of tumors, which indicate that they may be two distinct tumors. Compared with PEA, PEH is a borderline or low-grade malignant tumor in terms of DNA copy number. Previously, effective tumor markers that could differentiate between benign soft tissue tumors and malignant tumors had not been identified at the protein level. However, at the genetic level, sarcomas often display abnormalities, whereas cytogenetic alterations are seldom detected in benign soft tissue tumors. Parente et al. (1999) used CGH to detect chromosomal alterations in a set of 90 soft tissue tumors and found that only 2 Schwannomas among 20 benign tumors showed slight genetic changes. Cho et al. (2005) reported four benign tumor cases of uterine leiomyomas that lacked genetic alterations. These findings, combined with the obvious differences between the PEH and PEA cases found in this study, indicate that the detection of cytogenetic alterations should become an important method for the molecular classification of tumors.

In summary, our study revealed that in addition to histological differences, there are also cytogenetic differences between PEH and PEA. These differences reveal two distinct tumors. In particular, PEH shows low levels of malignancy, whereas PEA shows high-grade malignancy in terms of DNA copy number. Although these results were based on only two patients, our study is one of the first attempts to compare the cytogenetic differences between these two tumor types. Further studies are needed to determine whether our findings are robust or representative of large samples. Investigation of the genetic features of EHE and EA may provide additional clues for the further exploration of the etiopathogenesis and molecular classification of these tumors.

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