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Molecular cytogenetic characterization of epithelioid hemangioendothelioma

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Epithelioid hemangioendothelioma (EHE) is a rare vascular tumor whose pathological diagnosis can be difficult. In the literature two cases of EHE were found to harbor a balanced t(1;3)(p36.3;q25) translocation, suggesting a characteristic chromosomal rearrangement as cause for the development of EHE. In this study, 14 cases of EHE were investigated by interphase fluorescence in situ hybridization (FISH) directed against the translocation breakpoint 1p36.3. A subset of cases was also analyzed by comparative genomic hybridization (CGH) and image cytometry. Five out of eight cases that could be successfully analyzed by FISH harbored a chromosomal break in the 1p36.3 region. The break-apart signals were present in diploid nuclei, and less frequently also in tetraploid nuclei. In the latter, the chromosomal break was present twice, suggesting that polyploidy occurred after the chromosomal alteration. DNA cytometry confirmed that tetraploid cells were present in most examined cases with one case indicating almost equal amounts of diploid and tetraploid tumor cells. CGH revealed single chromosomal imbalances of unclear significance. We could confirm that EHE may harbor a recurrent mutation involving the 1p36.3 chromosomal region thus supporting the notion that the t(1;3)(p36.3;q25) translocation is a relevant genetic finding in this tumor entity.

Keywords Epithelioid hemangioendothelioma, vascular neoplasms, fluorescence in situ hybridization, comparative genomic hybridization, image cytometry

Epithelioid hemangioendotheliomas (EHEs) were first described 1982 by Weiss and Enzinger (1) as rare vascular tumors of intermediate or borderline malignancy (2). In the World Health Organization (WHO) classification of soft tissue, they are grouped with angiosarcomas in the category of malignant vascular tumors (3,7). However, they generally carry a better prognosis and biological behavior than aggressive sarcomas. Thus, epithelioid hemangioendothelioma must be separated from benign hemangioma and malignant angiosarcoma (1–3,7).

EHE represents only 1% of all vascular neoplasms. The entity may arise at any age and usually presents as a solitary mass in either superficial or deep soft tissues, also within organs. These neoplasms are characterized by nests or cords of epithelioid endothelial tumor cells, rounded or spindled, with specific cytoplasmic vacuoles, which are set in an atypical myxohyaline or collagenous matrix (4–6). The mitotic activity of the tumor is usually less than 10% (7,8). In the literature, the reported rates of systemic metastases are between 20 and 30%, the tumor related death of patients lies between 13 and 17% and a local recurrence rate is seen in up to 13% of all cases (7,9).

By cytogenetic analysis, two cases of EHE were found to harbor the balanced t(1;3)(p36.3;q25), suggesting a characteristic chromosomal rearrangement as molecular cause for the development of EHE (10). The present study aimed to confirm this translocation in primary tumors by interphase fluorescence in situ hybridization (FISH). In total, 14 cases of EHE were investigated by FISH for a possible breakpoint on 1p36.3. Since information on DNA ploidy and other chromosomal alterations of this tumor type is lacking or scarce, we additionally investigated a subset of cases by comparative genomic hybridization (CGH) and image DNA cytometry.

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Materials and methods

FISH analysis was performed on extracted whole nuclei and on thin tissue sections of 3 μm. Nuclei were extracted from formalin-fixed and paraffin-embedded tumor material. Isolated nuclei and sections from tonsil tissue were used as normal controls.

Materials

In total, 14 samples were available; of these, seven were from the archives of the Charité Berlin and seven from the reference center of soft-tissue tumors at the Institute of Pathology of the Jena University Hospital (Table 1). Expert pathologists (DK, IP) reviewed all tumor samples. In 12 cases, the samples were taken from women; only two tumor samples originated from male patients. Most tumors where found in the liver, but there were also ones located in the deep muscular tissue, the lungs, and the subcutaneous tissue. All samples were fixed with formalin and embedded in paraffin.

Nuclei isolation

We used an enzymatic pronase E (Serva, Heidelberg, Germany; dilution 1:200) treatment to isolate the nuclei from rehydrated and deparaffinized tissue sections. The tumor cells were dissolved with the enzyme, and the exposed nuclei were liberated by shaking and centrifugation of the pre-treated tissue. Resolved nuclei sediment was filtered and washed through a nylon mesh with a diameter of 50 μm (11). The nuclei were stored in Carnoy fixative. The prepared nuclei solution was dropped on slides, dehydrated in an alcohol series (70%, 95%, 99% alcohol), and dried for a minimum of 24 hours at room temperature. The success of the nuclear extraction was analyzed by phase contrast light microscopy.

Tissue sections

Sections of 3 μm thickness were sliced and transferred to coated slides (Superfrost Plus, Menzel, Braunschweig, Germany). The paraffin was removed by washing the slides three times with xylene. The tissue sections were then treated by an enzymatic digestion with proteinase K (Qiagen, Hilden, Germany, dilution 1:25) and afterward stored in formamide–sodium saline citrate solution (SSC) for at least 24 hours to denature the DNA in the nuclei (12).

Preparation of appropriated DNA probes

For in situ hybridization, a break-apart FISH technique was chosen. Suitable FISH probes located at the 1p36 breakpoint regions were identified based on a database search (http://genome.ucsc.edu/cgi-bin/hgGateway). All probes were purchased as bacterial artificial chromosome (BAC) clones from the human genotech BACPAC (Resources Center at Children's Hospital Oakland Research Institute, CHORI) and processed by plasmid isolation (Miniprep, Qiagen, Hilden, Germany) and degenerate oligonucleotide–primed polymerase chain reaction (DOP-PCR). The probe labelling was performed by direct nick translation. In total, 18 BAC clones were tested: RP11-145C4, RP11-285P3, RP11-484J7, RP11-69N18, RP11-109P21, RP11-452G11, RP11-945C1, RP11-188F7, RP11-740P5, RP11-83K22, RP11-799N13, RP11-82D16, RP11-156L15, RP11-659D23, RP11-798H13, RP11-722L19, RP11-659L19, and RP11-101J16. The exact localization of the individual BAC clones can be visualized by the above-mentioned web address as well as at http://www.ncbi.nlm.nih.gov/mapview.

FISH

First, the slides with tumor cells (isolated nuclei or tumor sections) were cleaned and prepared with a pepsin treatment

Table 1 Tumor collective and experimental results

Case	Sex	Age	Location	Pathological diagnosis	CGH + FISH	DNA-cytometry	FISH (1p36.3)[a]
1	F	54	Neck	EHE	n.a.	n.a.	pos. (25%)
2	F	67	Lung	EHE	n.a.	n.a.	neg.
3	F	42	Arteria femoralis	EHE	n.a.	n.a.	n.e.
4	F	57	Calf (muscle)	EHE	n.a.	n.a.	n.e.
5	F	74	Neck (muscle)	EHE	n.a.	n.a.	neg.
6	F	21	Subcutis, supraclavicular	EHE	n.a.	n.a.	pos. (22%)
7	F	73	Liver	EHE	n.a.	n.a.	pos. (12%)
8	M	23	Liver	Multicentric EHE (metastasis)	No gain or loss	Mainly diploid	pos. (21%)
9	F	65	Liver	Subcapsular haemangioendothelioma	−3p,−9p,−13q,+17	Mainly diploid	n.e.
10	F	31	Liver	Multicentric EHE	−6	n.e.	n.e.
11	F	57	Liver	Multicentric EHE (metastasis)	No gain or loss	Mainly diploid	pos. (9%)
12	F	58	Liver	EHE	No gain or loss	Diploid + tetraploid	neg.
13	M	61	Penis paraurethral	EHE	+6	n.e.	n.e.
14	F	62	Lung / pleura	EHE	−18	Mainly diploid	n.e.

Abbreviations: pos., positive; neg., negative; n.a., not analyzed; n.e., not evaluable.
[a] Percentage of the nuclei with chromosomal break.