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Original contribution

Cellular and reticular variants of hemangioblastoma differ in their cytogenetic profiles

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Summary Capillary hemangioblastomas of the central nervous system are benign tumors and occur either sporadically or as a manifestation of von Hippel-Lindau disease. A rarer cellular and a more common reticular variant can be distinguished on the basis of the abundance of the stromal cell component, with the cellular variant being significantly associated with a greater probability of recurrence. To investigate whether these subtypes differ in their cytogenetic profile, a comparative genomic hybridization analysis of 10 cellular and 10 reticular hemangioblastomas was undertaken. Comparative genomic hybridization revealed DNA copy number changes in 14 of 20 cases (8 of 10 cellular and 6 of 10 reticular hemangioblastomas). The most common changes overall were losses of chromosomes 19 (35%), 6 (30%), and 22q (15%), whereas loss of 3 and gain of 4 were encountered in one case each (5%). The cellular variant showed losses of chromosomes 6 (60%), 22q and 19 (20% each), as well as gain of 4 (10%), whereas the reticular variant presented with losses of chromosomes 19 (50%), 22q and 3 (10% each). Loss of chromosome 6 was significantly associated with the cellular subtype ($P < .005$), whereas loss of 19/19p was found more frequently in the reticular variant, albeit not significantly ($P = .16$). In conclusion, our data may point toward different genetic pathways in the pathogenesis of the 2 histologic subtypes of capillary hemangioblastoma.

1. Introduction

Capillary hemangioblastomas of the central nervous system are benign tumors and occur either sporadically or in 25% of cases as a manifestation of von Hippel-Lindau (VHL) disease [1], with VHL alleles reported to be inactivated in up to 50% of sporadic and 100% in VHL-associated hemangioblastomas [2,3]. They are characterized histologically by the 2 main components of large vacuolated stromal cells and a rich capillary network [1] and can be subclassified into 2 variants on the basis of the abundance of the stromal cell component: the rarer cellular hemangioblastomas defined by zellballen-like cellular clusters of uniform tumor cells, and the more common reticular subtype showing abundant capillaries and stromal cells [4,5]. A recent large clinicopathologic study of 88 hemangioblastomas found the cellular variant to comprise 11.4% of all hemangioblastomas and to be significantly associated with a greater probability of recurrence and a higher MIB-1

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proliferation index [4]. To investigate whether these 2 subtypes of capillary hemangioblastomas also differ in their cytogenetic profile and may thus provide biologically and diagnostically useful information, we carried out a comparative genomic hybridization (CGH) analysis of 10 cellular and 10 reticular hemangioblastomas.

2. Materials and methods

2.1. Patients and tumors

Formalin-fixed and paraffin-embedded biopsy specimens of 20 primary hemangioblastomas (10 men, 10 women; mean age, 50 years; range, 11-73 years) were investigated (Table 1). These consisted of 10 cases of the cellular variant (3 men, 7 women; mean age, 40 years; range, 11-68 years) and 10 cases of the reticular variant (7 men, 3 women; mean age, 60 years; range, 38-73 years). There were 18 tumors that were located infratentorially, 1 in a supratentorial site, and 1 in a spinal site.

Histologic criteria for subclassifying a hemangioblastoma as cellular or reticular were those used by Hasselblatt et al [4]. Briefly, cellular hemangioblastomas were characterized by zellballen-like cellular clusters of uniform tumor cells with finely granular eosinophilic cytoplasm and endothelial hyperplasia, whereas the reticular subtype showed abundant capillaries and scattered stromal cells with highly vacuolated or clear cytoplasms (Fig. 1).

Routine hematoxylin-eosin and reticulin staining as well as immunohistochemistry using an avidin-biotin complex technique and antibodies against epithelial membrane antigen (1:1000) and MIB-1/Ki67 were performed (1:100; all Dako, Glostrup, Denmark). Independent diagnoses were made by 3 neuropathologists (C. H. R., M. H., W. P.) who were in agreement regarding the subclassification of each tumor. Statistical analysis of chromosomal changes was performed using Fisher exact test. Recurrence data were evaluated with Kaplan-Meier analysis and log-rank test.

2.2. CGH analysis

DNA was isolated by phenol-chloroform extraction according to standard protocols. CGH analysis was performed as described previously [6]. Briefly, tumor DNA was labeled with biotin-16-dUTP (Boehringer Mannheim, Mannheim, Germany) and reference DNA from a healthy male donor with digoxigenin-11-dUTP (Boehringer Mannheim) in a standard nick translation reaction. The DNAse concentration in the labeling reaction was adjusted to reveal an average fragment size of 200 to 500 base pairs. Labeled DNA fragments were purified from remaining nucleotides by column chromatography.

For CGH, 500 ng of tumor DNA, 300 ng of reference DNA, and 30 μg of human Cot1 DNA for blocking repetitive DNA sequences of the centromer regions (Gibco, Karlsruhe, Germany) were coprecipitated and redissolved

Table 1 Summary of demographic, clinical, and CGH data of hemangioblastomas

Case	VHL	Age (y)	Sex	Loc	OP	Rec	Death	PFS (mo)	OS (mo)	Gains	Losses
Cellular variant (10 cases)											
1	–	11	F	st	STR	+	–	56	88	–	6
2	+	17	M	it	GTR	+	–	28	32	–	–
3	–	30	F	it	GTR	–	–	44	44	4	6
4	–	32	M	it	GTR	+	–	17	17	–	6, 22q
5	–	33	M	it	GTR	–	–	27	27	–	6
6	–	41	F	it	GTR	+	–	137	137	–	6
7	–	44	F	it	GTR	–	–	27	27	–	–
8	–	60	F	it	GTR	–	–	66	66	–	19
9	–	64	F	it	GTR	–	–	13	13	–	19,22q
10	–	68	F	it	GTR	–	+[a]	1	1	–	6
Reticular variant (10 cases)											
11	–	38	M	it	GTR	–	–	68	68	–	19
12	–	43	F	it	GTR	–	–	166	166	–	–
13	–	43	F	it	GTR	ND	ND	ND	ND	–	–
14	–	62	M	sp	GTR	–	–	23	23	–	19p
15	–	65	F	it	GTR	–	–	130	130	–	22q
16	–	66	M	it	GTR	–	–	39	39	–	–
17	–	67	M	it	GTR	–	–	36	36	–	–
18	–	68	M	it	GTR	–	–	3	3	–	3, 19p
19	–	71	M	it	GTR	–	–	50	50	–	19p
20	–	73	M	it	GTR	–	–	5	5	–	19p

Abbreviations: M, male; F, female; Loc, localization; it, infratentorial; sp, spinal; st, supratentorial; OP, operation; GTR, gross total resection; STR, subtotal resection; Rec, recurrence; PFS, progression-free survival; OS, overall survival; ND, no data available; +, yes/present; –, no/lacking.

[a] Cause of death—postoperative pneumonia, sepsis.