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Stromal Cells of Cerebellar Hemangioblastomas Express Carbonic Anhydrase IX

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Abstract: *Hemangioblastomas, which tend to arise in the central nervous system, especially in the cerebellum, are benign brain tumors consisting histologically of stromal cells and a large number of blood vessels. We examined the expression of carbonic anhydrase IX (CA9) in hemangioblastomas. Thirteen hemangioblastomas from 13 patients were examined in this study. All hemangioblastomas were sporadic cases, and no patients with von Hippel-Lindau disease (VHD) were included in the study. CA9 was clearly expressed in all investigated hemangioblastomas, especially in stromal cells, but not in endothelial cells. These results are consistent with previously reported data demonstrating that stromal cells, but not endothelial cells, show VHD gene mutations in sporadic hemangioblastomas, which suggests that stromal cells form the actual tumorigenic cell component in hemangioblastomas.*

Keywords: *hemangioblastoma, stromal cell, carbonic anhydrase IX (CA9), von Hippel-Lindau disease (VHD)*

1. INTRODUCTION

Hemangioblastomas are benign vascular neoplasms consisting of stromal cells and a well-developed capillary network (1). Hemangioblastomas usually arise in the central nervous system (CNS), especially in the cerebellum (1), and represent 1.5%-2.5% of all intracranial neoplasms, and 7%-12% of all posterior fossa tumors (1). It is well known that hemangioblastomas tend to arise in patients with von Hippel-Lindau disease (VHD) (2). Patients with sporadic hemangioblastomas have been also reported, these patients outmumbering those with hemangioblastomas associated with VHD.

It has been reported that VHD is inherited in an autosomal dominant fashion (3) and that the defective gene is located on the short arm of chromosome 3 (4). VHD is associated with the development of tumors and tumor-like lesions including not only hemangioblastomas but also renal cell carcinomas, pheochromocytomas, cysts, or cystadenomas of the kidneys, liver, pancreas, or epididymis, and well-differentiated but locally aggressive papillary tumors of the inner ear/temporal bone (5).

Carbonic anhydrase IX (CA9) was first identified in the cervical cancer cell line HeLa in 1992 (6). It has been reported that the expression of CA9 is regulated by the hypoxia inducible factor (HIF) transcriptional complex in aberrant oxygen states and acidic conditions (7, 8). Moreover, it has been reported that CA9 is overexpressed in many solid tumors; in particular, CA9 is strongly expressed in

most clear cell renal cell carcinomas (RCCs) without equivalent expression in the corresponding normal kidney tissues (9).

In this paper, we demonstrate that even sporadic hemangioblastomas in the cerebellum express CA9 and that stromal cells of hemangioblastomas, in particular, express CA9, suggesting that stromal cells represent the main tumorigenic unit of hemangioblastomas.

2. MATERIALS AND METHODS

2.1. Data Collection

The records of Kansai Medical University and Toyooka Hospital were screened for patients with hemangioblastomas in the years between 2000 and 2014. In cases with available paraffin blocks, immunohistochemical staining for CA9 was conducted. Five cases of pilocytic astrocytoma, 5 cases of paraganglioma, 4 cases of angiomatous meningioma and 19 cases of clear cell RCC were prepared as control tumors from the stock in Kansai Medical University. Samples of clear cell RCC were prepared as positive controls for CA9.

2.2. Hematoxylin and Eosin (H&E) Staining and Immunohistochemistry

Sections (3-μm) were prepared from paraffin blocks of the tumors. H&E and immunohistochemical staining of the sections was carried out. Further, immunohistochemical staining for CA9 was carried out using anti-CA9 antibody (mouse monoclonal antibody, clone TH22, 1:100, Ventana BenchMark XT, Roche Diagnostics, Mannheim, Germany). Staining was performed using a DAKO Autostainer (DAKO, Glostrup, Denmark), following the manufacturer's instructions.

3. RESULTS

3.1. Clinical Findings

Thirteen hemangioblastomas from 13 patients were examined in this study (table). All studied hemangioblastomas were sporadic cases, and no patients with VHD were included in the study. All examined tumors had developed in the cerebellum. The ages of the patients at presentation ranged from 14 to 79 years, and the median age was 60 years. The mean and standard deviation of the ages was 56.2±18.8 years. The male-to-female ratio was 7:6.

Table. *Expression of CA9 in sporadic hemangioblastoma*

Case No	Age	Sex	Expression Of CA9	
			Stromal Cells	Endothelial Cells
1	14	F	+	-
2	35	M	+	-
3	38	M	+	-
4	46	M	+	-
5	51	F	+	-
6	60	M	+	-
7	60	M	+	-
8	64	F	+	-
9	65	M	+	-
10	71	M	+	-
11	72	F	+	-
12	76	F	+	-
13	79	F	+	-

3.2. Stromal Cells, but not Endothelial Cells, Express CA9 in Hemangioblastomas

First, we histologically re-examined all hemangioblastomas examined in this study. The hemangioblastomas showed typical histological findings in the H&E-stained sections: the tumors were found to consist of both stromal cells and an abundance of blood vessels (Fig. A and B). After

histological examination, we examined the expression of CA9 in the hemangioblastomas by using immunohistochemistry, as described in the "Materials and Methods". As shown in the table and Fig. C and D, all hemangioblastomas (13/13) expressed CA9. In these hemangioblastomas, CA9 was expressed in the cell membrane and in the cytoplasm of stromal cells, but not in endothelial cells, as shown in Fig. C and D

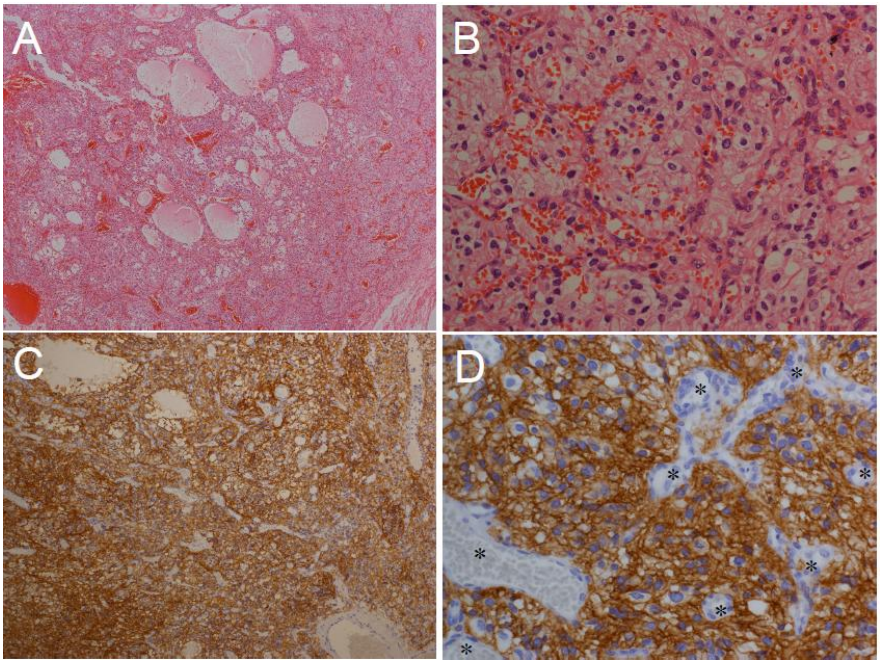

Fig. *Stromal cells of hemangioblastomas express CA9. H&E staining of hemangioblastoma section is shown in a low-power field (A) and a high-power field (B). Immunohistological staining using an anti-CA9 antibody is shown in a low-power field (C) and a high-power field (D). "∗" indicates the blood vessels. Magnifications of object lens in A, B, C and D are 2x, 40x, 4x and 60x, respectively.*

3.3. All Clear Cell RCCs were Found to Express CA9, While Pilocytic Astrocytomas, Paragangliomas, and Angiomatous Meningiomas did not Express CA9

Since clear cell RCCs have been previously reported to express CA9 (2), we examined CA9 expression in clear cell RCCs. All examined clear cell RCCs were found to express CA9. Since pilocytic astrocytomas, angiomatous meningiomas and paragangliomas are representative tumors considered in differential diagnoses of hemangioblastomas, we examined CA9 expression in these tumors, as described in the "Materials and Methods". The pilocytic astrocytomas, angiomatous meningiomas or paragangliomas examined were not found to express CA9 (data not shown).

4. DISCUSSION

In this study, we demonstrated that sporadic hemangioblastomas in the cerebellum express CA9, and that stromal cells, but not endothelial cells, express CA9 in these hemangioblastomas.

In human tissue, CA9 is strongly expressed on the basolateral surface of proliferating enterocytes in the crypts of the duodenum, jejunum and ileal mucosa (10). Furthermore, CA9 is overexpressed in many cancers such as those in the lung (11), colon (12), breast (13), cervix (14), urinary bladder (15), ovary (16) and kidney (7, 8). It is surmised that CA9 regulates intracellular pH in the tumor cells, resulting in the induction of tumor cell growth and survival (17). In our study, all the hemangioblastomas examined strongly expressed CA9, suggesting that CA9 could play an important role in the growth of hemangioblastomas.

It has been reported that CA9 transcription is induced by transcription factors, hypoxia-inducible factors 1 and 2 (HIF-1 and HIF-2) (18). Although HIF-1α is rapidly degraded via the ubiquitin-proteasome pathway in the physiological microenvironment (19), HIF-1α is stabilized and accumulated under hypoxic conditions, resulting in the induction of CA9 production (20). The loss of

von Hippel-Lindau (VHL) function contributes to the post-transcriptional stabilization of HIF-1α and HIF-1β, resulting in tumorigenicity (21). Under normal conditions, VHL protein induces the degradation of HIF-1α via the ubiquitin-proteasome system. Therefore, VHD is associated with the development of tumors, as described in the "Introduction". In particular, clear cell RCCs, most of which have been reported to express CA9, tend to develop in patients with VHD (2). Moreover, mutations of the VHD gene have been reported even in sporadic hemangioblastomas, suggesting that the loss of VHL-function induces CA9 expression in hemangioblastomas as well (22). Recently, Schaller et al. showed that hemangioblastomas express CA9 both in patients with VHL as well as in patients with sporadic hemangioblastomas (23). However, they did not specify which cells of hemangioblastomas express CA9. Our data demonstrate that stromal cells, but not endothelial cells, express CA9. It has been reported that stromal cells could represent the neoplastic component of hemangioblastomas, since somatic mutations of the VHL gene have been found in stromal cells, but not in endothelial cells, of sporadic hemangioblastomas (24). Moreover, it has been reported that some VHL-related proteins are upregulated in these stromal cells (25). Our findings, which indicate that only stromal cells express CA9 in hemangioblastomas, are consistent with these data, supporting the theory that stromal cells are the actual tumor cells in hemangioblastomas, and that endothelial cells proliferate due to the reactivity to cytokines from the stromal cells.

In this paper, we have demonstrated that stromal cells of hemangioblastomas express CA9. These data not only identify a new marker in hemangioblastomas but also support previous findings that stromal cells are the tumorigenic unit of hemangioblastomas.

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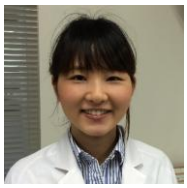

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