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Abstract: Background: Antiangiogenic treatment has been explored in few patients with hemangioblastoma after failure of surgery and radiotherapy. **Case Report:** We present the first histopathological follow-up study of a bevacizumab-responsive hemangioblastoma that eventually progressed. For a period of 12 months, therapy with bevacizumab achieved a clinical response and radiological stabilization in a patient with progressive multifocal central nervous system (CNS) hemangioblastoma. Subsequently, selected tumor sites showed radiological progression, in particular, the formation of an intramedullary lesion of the initially predominantly leptomeningeal disease. Histology showed diffuse dural invasion by the hemangioblastoma accompanied with a relatively reduced cell density compared to the preserved vessel structures. **Conclusion:** The pattern of progression upon vascular endothelial growth factor (VEGF)-targeting antiangiogenic treatment in hemangioblastoma may involve increased tumor invasiveness. © 2014 S. Karger GmbH, Freiburg.

DOI: <https://doi.org/10.1159/000360780>

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ZORA URL: <https://doi.org/10.5167/uzh-95402>

Journal Article

Accepted Version

Originally published at:

Seystahl, K; Weller, M; Bozinov, O; Reimann, R; Rushing, E (2014). Neuropathological characteristics of progression after prolonged response to bevacizumab in multifocal hemangioblastoma. *Oncology Research and Treatment*, 37(4):209-212.

DOI: <https://doi.org/10.1159/000360780>

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**Neuropathologische Progressionsmuster nach protrahierter Tumorkontrolle durch
Bevacizumab bei multifokalem Hämangioblastom**

Short title: Patterns of progression after bevacizumab in hemangioblastoma

Kurztitel: Progressionsmuster nach Bevacizumab bei Hämangioblastom

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Neuropathologische Progressionsmuster nach protrahierter Tumorkontrolle durch Bevacizumab bei multifokalem Hämangioblastom

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Keywords: Bevacizumab, VEGF, Hemangioblastoma, Angiogenesis

Schlüsselwörter: Bevacizumab, VEGF, Hämangioblastom, Angiogenese

Summary

Background: Antiangiogenic treatment has been explored in few patients with hemangioblastoma after failure of surgery and radiotherapy.

Case report: We present the first histopathological follow-up study of a bevacizumab-responsive hemangioblastoma that eventually progressed. For a period of 12 months, therapy with bevacizumab achieved a clinical response and radiological stabilization in a patient with progressive multifocal CNS hemangioblastoma. Subsequently, selected tumor sites showed radiological progression, in particular, the formation of an intramedullary lesion of the initially predominantly leptomeningeal disease. Histology showed diffuse dural invasion by hemangioblastoma accompanied with a relatively reduced cell density compared to preserved vessel structures.

Conclusion: The pattern of progression upon VEGF-targeting antiangiogenic treatment in hemangioblastoma may involve increased tumor invasiveness.

Zusammenfassung

Hintergrund: Antiangiogene Therapieansätze werden vereinzelt bei Patienten mit Hämangioblastom nach Scheitern von operativen oder radiotherapeutischen Optionen verfolgt.

Kasuistik: Wir zeigen die erste histopathologische Vergleichsstudie bei zuvor auf Bevacizumab ansprechendem Hämangioblastom nach Tumorprogression. Während 12 Monaten wurde mit einer Therapie mit Bevacizumab ein klinisches Ansprechen und radiologische Stabilität bei einem Patienten mit einem multifokalen Hämangioblastom des ZNS erreicht. In der Folge zeigten einzelne Tumoranteile Zeichen radiologischer Progression, dabei fiel vor allem eine intramedulläre Läsion des initial primär leptomeningeal disseminierten Tumors auf. Histologisch fand sich eine diffuse Infiltration der Dura begleitet von einer relativ reduzierten Tumorzelldichte in Bezug auf erhaltene Gefäßstrukturen.

Schlussfolgerung: Die Progressionsmuster nach VEGF-basierter antiangiogener Therapie bei Hämangioblastomen weisen auf ein vermehrtes infiltratives Tumorwachstum hin.

Established facts:

Based on previous case studies, antiangiogenic treatment with bevacizumab represents a therapeutic option in patients with progressive and heavily pretreated hemangioblastoma.

Novel insights:

This is the first study presenting the patterns of progression in a patient with multifocal hemangioblastoma after prolonged response to bevacizumab. Radiological and histopathological findings point towards an increased tumor invasiveness on the basis of relatively reduced tumor cell density in contrast to a preserved vasculature.

1. Background

Hemangioblastoma represents a rare tumor of the central nervous system (CNS) occurring both as sporadic disease and especially in case of a spinal manifestation in association with von Hippel-Lindau disease (VHL) [1]. The tumor cell of origin and histogenesis of this entity remain controversial. The disease-associated inactivation of the VHL tumor suppressor gene leads to a stabilization of hypoxia-inducible factor (HIF)-1 α protein and thereby upregulation of associated target genes including vascular endothelial growth factor (VEGF) [2]. Hemangioblastomas are mostly encountered in the cerebellum and dorsal spinal cord, where they are usually superficial, centered on the pial surface and well-circumscribed. Two histopathologic subtypes related to tumor size are recognized, including the smaller “reticular or mesenchymal type” with a high ratio of vessels to tumor cells and the less common “cellular” type composed of large lobules of uniform cells surrounded by proliferating vessels [3]. In addition, the constituent tumor (stromal) cells of the reticular variant often show abundant vacuolated cytoplasm and are embedded in a dense reticulin network. The cellular variant shows less cytoplasmic vacuolation, sparse pericellular reticulin and glioma-like fibrillar areas in some cases. Since VEGF and VEGF receptors (VEGFR) are expressed in hemangioblastoma [4;5], antiangiogenic therapy is now being considered as a therapeutic option beyond surgery and radiotherapy. To date, few cases of progressive hemangioblastoma treated successfully with the VEGF antibody bevacizumab have been published [6;7]. In a single-arm phase II study with the VEGFR inhibitor sunitinib in patients with VHL disease, stable disease was reported in 9/11 patients with hemangioblastoma while 6/18 patients with other VHL-related lesions showed a partial response [8]. To better understand the patterns of response and therapeutic alterations, tissue analyses of patients before and after antiangiogenic therapy are highly desirable.

2. Patient and methods

The patient, specifically his response to bevacizumab with a follow-up of 4 months, was previously reported [6]. After written informed consent from the patient, treatment with bevacizumab at 10 mg/kg was administered intravenously every 14-21 days on the basis of compassionate use of the drug. Radiographic follow-up analyses were performed with a 1.5 Tesla magnetic resonance imaging (MRI) according to standard procedures. The tissue specimen from the surgery before treatment with bevacizumab consisted of a 0.4x0.2x0.01 cm soft, red-brown tissue fragment. At progression after bevacizumab, the tissue comprised multiple beige fragments measuring 8x7x2 mm in aggregate.

3. Case report

3.1 Clinical and radiological course

A 70-year-old man with a history of a solitary left cerebellar hemangioblastoma resected in 1994 was diagnosed with local tumor recurrence in 2009 and multiple leptomeningeal cerebral and spinal lesions in 2010. No other lesions suggestive of VHL were found as well as no germline mutation was identified upon analysis of exons 1-3 of the VHL gene. After rapid radiological and clinical progression, experimental treatment with bevacizumab was initiated.

The clinical course up to 4 months after start of treatment was previously described [6]. In brief, upon initiation of bevacizumab treatment, the patient's gait disorder and performance status improved remarkably and the formerly progressive lesions on imaging (fig. 1A,B) showed stable disease in the first follow-up after 2 months. The patient was maintained on bevacizumab and MRI imaging showed sustained stable disease up to 12 months after start of therapy (fig. 1C). Three months later, however, routine MRI scans revealed an intramedullary contrast-enhancing lesion, which showed further progression in the subsequent follow-up scan (fig. 1D, right). Later, progression of the extramedullary, intradural cystic lesions was found near the spinal cord at the level of C7 and T11 (fig. 1D, left). In line with the radiological course, ambulation difficulty was documented on examination. Another surgery was performed in order to prevent further compression of the spinal cord. Intraoperatively, the lower dorsal lesion appeared superficially infiltrative but exophytic and was completely resected. However, the upper lesion was ventrally incorporated in the spinal cord and therefore only biopsy could be safely performed to spare the motor tracts. The next follow-up scans showed further tumor progression of the spinal lesions warranting further treatment with limited evidence-based options.

3.2 Histologic analysis

The initial diagnosis of hemangioblastoma WHO grade I was rendered based on cerebellar lesions and multilocular disease was confirmed by a biopsy of spinal leptomeningeal lesions at the level of T10 and T11.

Before therapy with bevacizumab, the tumor was microscopically composed of moderately cellular lobules of densely packed tumor cells surrounded by a zone of proliferated capillary-caliber vessels and focal stromal fibrosis (fig. 2A,B). Cytoplasmic vacuolation was inconspicuous and reticulin staining was sparse. Mitotic activity or necrosis was not observed. Dural tissue was not present in the specimen. Immunohistochemical preparations revealed patchy inhibin alpha-positive tumor cells (fig. 2C). The differential diagnosis of a metastatic renal tumor was excluded by negative staining for pancytokeratin and renal cell carcinoma (RCC) antibody, and glial neoplasms by negative staining for glial fibrillary acidic protein (GFAP). The Ki-67 proliferation marker labeled around 3% (n = 860 cells) of the tumor cells (fig. 2D).

The biopsy after bevacizumab treatment was performed at the level of C7 and Th3. Tissue specimens consisted of multiple fragments of dura infiltrated by tumor. The vessels appeared markedly hyalinized and thickened. Interestingly, the tumor cell density was strikingly decreased after treatment with bevacizumab (fig. 2E). The preserved vascular structures in relation to relatively reduced tumor cells was confirmed via CD31 staining (fig. 2F). The reticulin scaffolding appeared denser, likely reflecting increased vessel-associated reticulin fibers rather than stromal cell associated fibers. Similar to the initial biopsy, there was no evidence of mitotic activity or necrosis. Only minimal immunolabeling with neuron specific enolase (NSE, data not shown) and inhibin was seen (fig. 2G). Despite the relative paucity of stromal cells, the proliferative index at 20% (n=134 cells) was high (fig. 2H).

4. Discussion

This is the first study documenting the histologic findings in a patient with multifocal hemangioblastoma before and after antiangiogenic therapy. Recently, VEGF-based antiangiogenic therapy has entered clinical practice in selected cases of hemangioblastoma [6-8]. However, these studies mainly show disease stabilization, while radiologic response has been described in only one case of bevacizumab-treated hemangioblastoma [7]. Currently, there are no reports delineating the radiologic or histologic patterns of response after therapy. In our patient, despite an increased proliferation index, the tumor cell density was decreased after anti-VEGF treatment and vascular structures appeared predominant. Persistant tumor vasculature accompanied with a relatively reduced tumor cell density in a tissue sample obtained from radiologically progressive tumor could be interpreted as a sign of VEGF-independent angiogenesis. Escape mechanisms from antiangiogenic therapy via upregulation of non-VEGF proangiogenic signaling, e.g. via angiopoetins, fibroblast growth factors or placental growth factor have been proposed in preclinical and clinical models [9]. On the other hand, a diffuse infiltrative pattern of hemangioblastoma cells within the adjacent dura was seen after therapy. This observation may in part be dependent on the biopsy site and extent of sampling. However, the biopsy site of the second intervention was in a region of radiographic progression and therefore presumably represents bevacizumab-resistant tumor. Additionally, the radiologic pattern of progression in this patient with the development of an intramedullary lesion may be interpreted in the context of increased invasiveness of the tumor after antiangiogenic therapy. In other tumor entities, especially glioblastoma, increased tumor invasiveness is thought to represent a possible escape mechanism after antiangiogenic therapy [10,11].

In conclusion, VEGF-targeting antiangiogenic therapy in cases of hemangioblastoma may involve VEGF-independent angiogenesis via other proangiogenic pathways promoting tumor growth as well as tumor invasiveness. Further investigation is needed to evaluate the

underlying mechanisms of response and escape from antiangiogenic therapy in hemangioblastoma.

Acknowledgements:

We are grateful to the Institute of Radiology Lindberg in Winterthur, Switzerland, for providing MRI images.

Conflicts of interest:

K. Seystahl has received honoraria for advisory board participation and travel expenses from Roche. M. Weller has received research support and honoraria for advisory board participation from Roche. None of the other authors declare any potential conflicts of interest

Figure 1: Radiologic course before and after treatment with bevacizumab

Spinal (*left, A-D*) and cranial (*right, A-C*) MRI showing leptomeningeal contrast-enhancing lesions (A, arrows) that progressed over a period of 4 months (B). Durable stable disease was observed for up to 12 months after initial treatment with bevacizumab (C). The pattern of progression included progressive cystic leptomeningeal tumor (D, left, white arrow) as well as an intramedullary lesion (D, right, white arrow) and.

Figure 2: Histologic changes after therapy with bevacizumab

Hematoxylin-Eosin (HE) (A,E), CD31 (B,F), inhibin (C,G) and Ki-67 (D, H) stained tissue specimens at 200x magnification before (left) and after (right) bevacizumab therapy. The initial biopsy featured a compact mixture of generally uniform stromal tumor cells and capillary-caliber vessels (A,B). After bevacizumab therapy, the density of stromal tumor cells appears markedly reduced, while the vessels appear thickened and hyalinized, yet relatively preserved (E, F). Inhibin positive stromal cells are seen in the first biopsy (C) and within infiltrated dura in the tissue from the second surgery (G). Despite the high cellularity, the Ki-67 is low at 3% diagnosis (3%, n = 860 cells; D). The proliferative index in the second sample was high (20%, n=134 cells; H).

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A **B** **C** **D**