Expression of erythropoietin receptor in human merkel cell carcinoma of the eyelid

Satoru Kase[1], Kazuhiko Yoshida[1], Mitsuhiko Osaki[2], Hironobu Adachi[2], Hisao Ito[2], and Shigeaki Ohno[1].

[1] Department of Ophthalmology and Visual Sciences, Hokkaido University Graduate School of Medicine, N15 W7, Kita-ku, Sapporo 060-8638, Japan.

2 Department of Microbiology and Pathology, Division of Organ Pathology, Tottori University, Faculty of Medicine, 86 Nishi-cho, Yonago, Tottori 683-8503, Japan.

Running title: Epo-R in merkel cell carcinoma

Key words: merkel cell carcinoma, erythropoietin receptor, histopathology
Word count: 933

Status: experimental study

Date submitted: September 23, 2006

Address correspondence to: Satoru Kase, MD, Ph.D.,
Department of Ophthalmology and Visual Sciences,
Hokkaido University Graduate School of Medicine,
N15 W7, Kita-ku, Sapporo, 060-8638 Japan.
e-mail: kaseron@med.hokudai.ac.jp
Fax: +81-11-736-0952
Tel: +81-11-706-5944

Abstract

Background: Merkel cell carcinoma (MCC) of the eyelid is a rare malignant solid tumor of elderly, which demonstrates a large, firm, reddish nodule mimicking an angiomatous lesion. In this study, we examined the expression of erythropoietin (Epo) and Epo receptor (EpoR) as well as vascular endothelial growth factor (VEGF), VEGF receptor (VEGFR) and basic fibroblast growth factor (bFGF) in human MCC tissues.

Materials and methods: Three patients diagnosed with MCC of the eyelid underwent surgical excision. Isolated tissues were fixed by 4% paraformaldehyde and then were examined using immunohistochemistry.

Results: Carcinoma cells were constituted of irregular tumor nests with linear stroma, and showed hypercellularity consisting of small round nuclei with several mitoses. While immunoreactivity of Epo was undetectable, increased expression of EpoR was noted in carcinoma cells. Cytoplasmic immunoreactivity for EpoR was detected in a variety of carcinoma cells including mitotic cells. VEGF, VEGF receptor, and bFGF, other angiogenic factors were not expressed in MCC tissues.

Conclusion: EpoR was highly expressed in tumor cells of MCC of the eyelid, suggesting that Epo-EpoR pathway plays an important role in the formation of MCC.

Introduction

Merkel cell carcinoma (MCC) of the eyelid is a rare malignant solid tumor of elderly. The tumor demonstrates a large, firm, reddish nodule that resembles an angiomatous lesion (1). The characteristic macroscopical findings are presumed to be suspicious clinical diagnosis with MCC in eyelid tumors. However, it is possible that primary MCC is misdiagnosed due to the inflammation-like appearance (2), suggesting that ocular surgeons have to pay attention to the diagnosis before the treatment. On the other hand, the histopathology of MCC is typically small round blue cells with medium-sized nuclei and sparse cytoplasm (3), while characteristic vascularity in the tumor tissue is still unknown. These discrepancies between macroscopical and histopathological findings indicate the fact that no ophthalmologists or ocular pathologists can explain why MCC demonstrates reddish appearance, mimicking angiomatous tumors.

Erythropoietin (Epo) is reported to regulate various human malignancies by its involvement in tumor growth, viability and angiogenesis (4). The hypoxia-dependent upregulation of Epo is a direct result of hypoxia inducible factor-1 activation, a transcription factor that binds a hypoxia responsive element in *Epo* gene. Erythropoietin receptor (EpoR) is a member of the type I cytokine receptor family that induces cellular transmembrane receptors for factors such as growth hormone and

interleukins (5). Functional EpoR expression has been documented in a variety of nonhematopoietic cell types such as neurons and retinal photoreceptor (6,7). Expression of Epo-EpoR as well as other angiogenic factors, however, has not been elucidated in MCC.

In this study, we immunohistochemically examined the expression of Epo and EpoR as well as vascular endothelial growth factor (VEGF), VEGF receptor (VEGFR) and basic fibroblast growth factor (bFGF) in human MCC tissues surgically removed.

Materials and methods

Operative specimens

Three patients diagnosed with MCC of the eyelid underwent surgical excision. Isolated tissues were fixed by 4% paraformaldehyde in phosphate-buffered saline (PBS, pH 7.4). Paraffin-embedded tissue sections were made and examined immunohistochemically by Sapporo General Pathology Laboratory Co., Ltd. (Sapporo, Japan). All studies conformed to the tenets of the Declaration of Helsinki.

Immunohistochemistry

The slides were dewaxed, rehydrated, and rinsed in PBS twice, incubated with normal goat serum, and then were assessed for Epo (dilution 1:100, R&D system), EpoR (1:100, Santa Cruz Bioetch, Inc,

California, USA), VEGF (1:200, Santa Cruz Bioetch, Inc, California, USA), bFGF (1:200, Transduction Laboratories, Lexington, USA), and fetal liver kinase-1 (Flk-1) (1:200, Santa Cruz Bioetch, Inc, California, USA), a VEGF receptors, by immunohistochemistry. Briefly, the sections were incubated with primary antibody for 24 hours at 4°C following preincubation in normal horse serum. Slides were washed three times in tween PBS and then incubated with fluorescent secondary antibody Alexa-546 (1:50) (Molecular Probes, Eugene, Oreg.) for an hour. Nuclei were then stained with YO-pro-1 for 5 minutes (8). The slides were examined by laser scanning confocal microscopy (MRC-1024; Bio-Rad, Richmond, CA; and LSM 510; Carl Zeiss, Oberkochen, Germany). To examine the specificity of immunostaining, the primary antibody was replaced with Tris-buffered saline. Control slides were invariably negative for immunostaining. As a positive control, endometrial carcinoma tissues of the uterus were examined, in which Epo and EpoR were expressed in the tumor cells as previously described (9).

Results

All the eyelid tumors represented reddish, round, elastic hard masses without pain. Histopathologically, atypical cells were constituted of irregular tumor nests with linear stroma (Fig.1 a). At high magnification, tumor cells showed hypercellularity consisting of small

round nuclei with several mitoses (Fig.2 a, arrow). Immunoreactivity for cytokeratin 20, neuron-specific enolase, epithelial membrane antigen, and chromogranin A were noted in tumor cells (data not shown). Based on these pathological data, the diagnosis of MCC of the eyelid was made. While immunoreactivity of Epo was undetectable (Fig.1 b), increased expression of EpoR was noted in carcinoma tissue (Fig.1 c). Cytoplasmic immunoreactivity for EpoR (Fig.2 b, c) was noted in a majority of carcinoma cells including mitotic cells (Fig.2 b, c, arrow). Intratumoral microvessels also showed cytoplasmic immunoreactivity for EpoR. In contrast, VEGF, VEGFR, and bFGF, other angiogenic factors, were not expressed in MCC cells (data not shown).

Discussion

Histopathological findings demonstrated that MCC cells showed hypercellularity consisting of round blue cells, and constituted irregular tumor nests with linear stroma (Fig.1 a). In contrast, obvious hypervascularity and angiomatous lesion were not noted in tumor tissue. This suggests that functional angiogenic factors might correlate with formation of macroscopical angiomatous lesion in MCC

Epo is reported to regulate various human malignancies by its involvement in tumor growth, viability and angiogenesis (4,5). The binding of Epo to EpoR leads to the activation of a transcriptional factor,

which then induces mitosis of the erythroid precursor cells (10). In this study, cytoplasmic immunoreactivity for EpoR was noted in a majority of MCC cells including mitotic cells, and stromal microvessels. In contrast, immunoreactivity of Epo was undetectable in MCC tissues. Taken together, downstream of EpoR might lead to progression to mitosis and proliferation of tumor and endothelial cells in MCC, whereas Epo is probably secreted by paracrine mechasnism (5).

In conclusion, we confirmed that only EpoR was highly expressed in tumor cells as well as stromal microvessels of MCC of the eyelid, suggesting that Epo-EpoR pathway plays an important role in the formation of MCC. Even though our observation disclosed a part of angiogenic pathology, these results might contribute to reply to the query why MCCs of the eyelid demonstrate reddish nodule. Refractive surgeons and ophthalmologists should recognize again that MCCs of the eyelid represent reddish mass, possibly involved by angiogenic factors, which might complement clinical diagnosis in eyelid tumors.

Acknowledgements

This study was supported by a grant for Research on Sensory and Communicative Disorders from The Ministry of Health, Labor, and Welfare, and by Grants-in-Aid for Scientific Research from The Ministry of Education, Culture, Sports, Science, and Technology (MEXT), Japan.

References

1. Yanoff M, Fine B. *Skin, In: Ocular pathology: a text and atlas*. 3rd ed ed. Philadelphia: JB Lippincott; 1989.
2. Pawar R, Vijayalakshmy AR, Khan S, al Lawati FA. Primary neuroendocrine carcinoma (Merkel's cell carcinoma) of the vulva mimicking as a Bartholin's gland abscess. *Ann Saudi Med*. 2005;25:161-4.
3. Koljonen VS. Merkel cell carcinoma. *World J Surg Oncol*. 2006;4:7.
4. Yasuda Y, Fujita Y, Matsuo T, Koinuma S, Hara S, Tazaki A, et al. Erythropoietin regulates tumour growth of human malignancies. *Carcinogenesis*. 2003;24:1021-9.
5. Hardee ME, Arcasoy MO, Blackwell KL, Kirkpatrick JP, Dewhirst MW. Erythropoietin biology in cancer. *Clin Cancer Res*. 2006;12:332-9.
6. Nagai A, Nakagawa E, Choi HB, Hatori K, Kobayashi S, Kim SU. Erythropoietin and erythropoietin receptors in human CNS neurons, astrocytes, microglia, and oligodendrocytes grown in culture. *J Neuropathol Exp Neurol*. 2001;60:386-92.
7. Grimm C, Wenzel A, Groszer M, Mayser H, Seeliger M, Samardzija M, et al. HIF-1-induced erythropoietin in the hypoxic retina protects against light-induced retinal degeneration. *Nat Med*. 2002;8:718-24.

8. Suzuki T, Fujikura K, Higashiyama T, Takata K. DNA staining for fluorescence and laser confocal microscopy. *J Histochem Cytochem*. 1997;45:49-53.

9. Acs G, Zhang PJ, Rebbeck TR, Acs P, Verma A. Immunohistochemical expression of erythropoietin and erythropoietin receptor in breast carcinoma. *Cancer*. 2002;95:969-81.

10. Youssoufian H, Lodish HF. Transcriptional inhibition of the murine erythropoietin receptor gene by an upstream repetitive element. *Mol Cell Biol*. 1993;13:98-104.

Figure legend

Figure 1

Hematoxylin & eosin staining (a), and immunodetection of erythropoietin (Epo) (b) and erythropoietin receptor (EpoR) (c) in human merkel cell carcinoma (MCC) of the eyelid.

Carcinoma cells constitute irregular tumor nests with linear stroma (a). Immunoreactivity of Epo is undetectable in tumor cells (b). In contrast, increased expression is noted in MCC tissue (c).

Figure 2.

YO-PRO-1 nuclear staining (a, c: green) and immunoreactivity for erythropoietin receptor (EpoR) (b, c: red) in human merkel cell carcinoma of the eyelid at high magnification.

Tumor cells show small round nuclei with several mitoses (a, arrow). Cytoplasmic immunoreactivity for EpoR (b, c) is noted in carcinoma cells including mitotic cells (b, c, arrow).

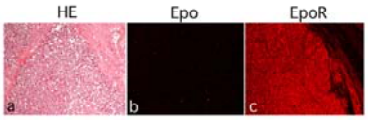

Figure 1

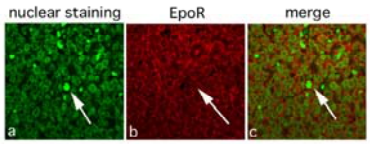

Figure 2