Expression of Erythropoietin Receptor in Human Merkel Cell Carcinoma of the Eyelid

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Abstract. Background: Merkel cell carcinoma (MCC) of the eyelid is a rare malignant solid tumor of the elderly, which demonstrates a large, firm, reddish nodule mimicking an angiomatous lesion. 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) were examined 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: The carcinoma cells consisted of irregular tumor nests with linear stroma and showed hypercellularity indicated by small round nuclei with several mitoses. While immunoreactivity of Epo was undetectable, an increased expression of EpoR was noted in the carcinoma cells. Cytoplasmic immunoreactivity for EpoR was detected in a variety of carcinoma cells, including mitotic cells. VEGF, VEGFR, and bFGF, other angiogenic factors were not expressed in the MCC tissues. Conclusion: EpoR was highly expressed in MCC of the eyelid, suggesting that the Epo-EpoR pathway plays an important role in the formation of MCC.

Merkel cell carcinoma (MCC) of the eyelid is a rare malignant solid tumor, which primarily affects the elderly. The tumor appears as a large, firm, reddish nodule that resembles an angiomatous lesion (1). The characteristic macroscopic 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 ophthalmologists 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 macroscopic and histopathological findings explain misinterpreting of the reddish appearance (mimicking angiomatous tumors) of MCC and, thus, rendering diagnosis difficult.

Erythropoietin (Epo) was reported to regulate various human malignancies through 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 the Epo gene. Epo 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 non-hematopoietic cell types, such as neurons and retinal photoreceptor (6, 7). The expression of Epo-EpoR, as well as of others angiogenic factors, however, has not been elucidated in MCC.

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

Materials and Methods

Operative specimens. Three patients diagnosed with MCC of the eyelid underwent surgical excision. The 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 the 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, rinsed in PBS twice, incubated with normal goat serum and then assessed for Epo (dilution 1:100, R&D system), EpoR (1:100, Santa Cruz Biotech, Inc), VEGF (1:200, Santa Cruz Biotech, Inc), bFGF (1:200, Transduction Laboratories, Lexington, USA) and fetal liver kinase-1 (Flk-1) (1:200, Santa Cruz Biotech, Inc), a VEGF receptor, by immunohistochemistry. Briefly, the sections were incubated with primary antibody for 24 h at 4°C following pre-incubation in normal horse serum. The slides were washed three times in tween PBS and then incubated with fluorescent secondary antibody Alexa-546 (1:50) (Molecular Probes, Eugene, OR, USA) for 1 h. Nuclei were then stained with YO-pro-1 for 5 min (8). The slides were examined by laser scanning confocal microscopy (MRC-1024: Bio-Rad, Richmond, CA, USA; and LSM 510: Carl Zeiss, Oberkochen, Germany). To examine the immunostaining specificity, 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 three eyelid tumors were reddish, round, elastic hard masses and caused no pain to the patients. Histopathologically, atypical cells were composed of irregular tumor nests with linear stroma (Figure 1a). At high magnification, the tumor cells showed hypercellularity and consisted of small round nuclei with several mitoses (Figure 2a, arrow). Immunoreactivity for cytokeratin 20, neuron-specific enolase, epithelial membrane antigen and chromogranin A were noted in the 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 (Figure 1b), increased expression of EpoR was noted in carcinoma tissue (Figure 1c). Immunoreactivity for cytokeratin 20, neuron-specific enolase, epithelial membrane antigen and chromogranin A were noted in the 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 (Figure 1b), increased expression of EpoR was noted in carcinoma tissue (Figure 1c). Immunoreactivity for cytokeratin 20, neuron-specific enolase, epithelial membrane antigen and chromogranin A were noted in the 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 (Figure 1b), increased expression of EpoR was noted in carcinoma tissue (Figure 1c). Immunoreactivity for cytokeratin 20, neuron-specific enolase, epithelial membrane antigen and chromogranin A were noted in the tumor cells (data not shown).
Discussion

The histopathological findings demonstrated that the MCC cells showed hypercellularity consisting of round blue cells, as well as irregular tumor nests with linear stroma (Figure 1a). In contrast, obvious hypervascularity and angiomatous lesions were not noted in the tumor tissue, suggesting that functional angiogenic factors might correlate with the formation of macroscopical angiomatous lesions in MCC.

Epo has been reported to regulate various human malignancies through 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, Epo immunoreactivity was undetectable in MCC tissues. Taken together, these observations indicated that expression of EpoR might lead to mitosis and proliferation of tumor and endothelial cells in MCC, whereas Epo is probably secreted by a paracrine mechanism (5).

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

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Received September 29, 2006
Accepted October 19, 2006