Expression of Epidermal Growth Factor Receptor: Risk Factor in Uveal Melanoma

H. Monique H. Hurks,1 Jessica A. W. Metzelaar-Blok,1 Ed R. Barthen,1 Aeikko H. Zwinderman,2 Didi De Wolff-Rouendaal,1 Jan E. E. Keunen,1 and Martine J. Jager1

PURPOSE. To investigate the prognostic significance of the expression of epidermal growth factor receptor (EGFR) in uveal melanoma. EGFR is a transmembrane glycoprotein, and its expression has been correlated with the development of metastases in various malignancies.

METHODS. Frozen sections from 22 primary uveal melanomas were examined for EGFR expression by a three-step immunoperoxidase staining, using a mouse anti-human EGFR IgG2b monoclonal antibody. The results were compared with patient survival and clinical and histopathologic parameters.

RESULTS. EGFR expression could not be determined on one tumor due to excessive pigmentation. Two patients died of causes unrelated to melanoma, and two patients were lost to follow-up. Out of 21 tumors, six tumors showed immunoreactivity for EGFR. Five of these six patients (83%) died due to metastases, compared with 2 (17%) of 12 patients with no EGFR expression (Kaplan–Meier analysis \( P = 0.0004 \)). EGFR-positive tumors tended to have a greater tumor prominence and a higher mitotic rate.

CONCLUSIONS. The expression of EGFR was significantly correlated with death due to metastatic disease and therefore can be regarded as an important prognostic factor in human uveal melanoma. (Invest Ophthalmol Vis Sci. 2000;41:2023–2027)

With an annual incidence of 6 to 8 cases per million persons in white populations, uveal melanoma is the most common primary malignant intraocular tumor in adults. The high mortality rate is related to the development of metastases with a strong preference for the liver.1,2 No effective therapy for the treatment or prevention of these metastases is available. Several prognostic factors have been identified for uveal melanoma, including histopathologic factors (e.g., cell type, largest tumor diameter, SD of nucleolar area), genetic factors (e.g., aneuploidy, chromosome 3 monosomy), and immunologic factors (e.g., expression of human leukocyte antigens, T cell infiltration).3

Recently, Ma and Niederkorn4 observed in murine studies that expression of epidermal growth factor receptor (EGFR) by uveal melanoma cells was significantly correlated with the development of liver metastases and decreased survival. EGFR is a 170-kDa transmembrane glycoprotein that is expressed on a wide variety of normal and malignant cells. Binding of EGFR by epidermal growth factor (EGF), transforming growth factor (TGF)-\( \alpha \), or hepatocyte growth factor, can induce cell differentiation, proliferation, and the expression of oncogenes such as fos and jun.5 Enhanced EGFR expression has been shown to be an indicator of poor prognosis in various human malignancies.6–9

The purpose of the present study was to investigate the prognostic significance of EGFR expression in human uveal melanoma. Therefore, we determined the EGFR expression on frozen sections of 22 primary uveal melanomas and compared these data with survival and various clinical and histopathologic parameters. The results showed that EGFR expression was a significant marker of poor prognosis.

MATERIALS AND METHODS

Patients and Tumors
Between 1987 and 1992, tumor specimens were obtained from 22 primary uveal melanomas immediately after enucleation. The patients were not treated with radio- or thermotherapy. The research protocol followed the tenets of the Declaration of Helsinki. A part of the tumor was snap-frozen in liquid isopentane and stored at \(-70^\circ\text{C}\). The remainder of each specimen was processed for histopathologic examination by an ocular pathologist. Of the 22 patients studied, 10 were women and 12 were men. Age range was 38 to 91 years, with a mean age at diagnosis of 66 years. Seven patients died of a tumor-related cause, 2 died of other causes, 11 were still alive, and 2 patients were lost to follow-up at the time of the study.

Histopathology
The tumors were histologically classified according to cell type. Five (23%) tumors consisted of spindle cells, 3 (13%) tumors of epithelioid cells, and 14 (64%) tumors of both cell types. All tumors were localized in the choroid, with the

From the 1Departments of Ophthalmology and 2Medical Statistics, Leiden University Medical Center, The Netherlands.

Supported in part by the Dutch Cancer Foundation (KWF).

Submitted for publication September 14, 1999; revised January 24, 2000; accepted February 2, 2000.

Commercial relationships policy: N.

Corresponding author: H. Monique H. Hurks, Department of Ophthalmology, J3-S, Leiden University Medical Center, PO Box 9600, 2300 RC Leiden, The Netherlands. mhurks@div3.azl.nl
exception of patient 2, in whom the tumor was based in the ciliary body. The tumor of patient 13 was localized in both the choroid and the ciliary body. Largest tumor diameter and prominence of the tumor were measured in millimeters, and the number of mitoses was counted in 15 high-power fields with a magnification of 320 (Table 1).

Immunohistochemistry

Frozen sections, mounted on glass slides coated with aminopropyltriethoxysilane (APES; Sigma, St. Louis, MO), were fixed in acetone at 4°C for 10 minutes. After they were washed in phosphate-buffered saline (PBS), the slides were preincubated with 40% human serum in PBS with 3% bovine serum albumin (BSA) for 20 minutes. The slides were washed again in PBS and incubated for 30 minutes with a mouse anti-human EGFR IgG2b monoclonal antibody (R-1; Santa Cruz Biotechnology, Santa Cruz, CA) at a dilution of 1:20 or with 1% BSA-PBS as negative control. After they were washed, the slides were then incubated with biotinylated antimouse immunoglobulins (LSAB-2 kit from Dako, Glostrup, Denmark) for 10 minutes, washed again and incubated in methanol with 0.3% H2O2 for 30 minutes. After another washing, the slides were incubated with horseradish peroxidase–conjugated streptavidin (Dako) for 10 minutes. The peroxidase reaction was developed using 5% 3-amino-9-ethyl-carbazole (Sigma) in 0.1 M sodium acetate buffer (pH 5) containing 0.05% H2O2. The sections were counterstained with Mayer’s hematoxylin and finally embedded in Kaiser’s glycerin. Sections of colon carcinoma were used as positive control for the EGFR expression.

Statistical Analysis

The correlation between EGFR expression and melanoma-related death and clinical and histologic parameters was assessed using the Kaplan–Meier survival analysis, the log rank test, and Cox proportional hazards regression model. The relationship between EGFR expression and clinical and histopathologic parameters was determined with the χ² test or the nonpaired, two-sided Student’s t-test, as appropriate.

RESULTS

Immunohistochemistry

EGFR expression was determined on frozen sections of 22 primary uveal melanomas by a three-step immunoperoxidase staining. One tumor (tumor 4, Table 1) could not be evaluated due to excessive pigmentation. Six of the remaining 21 tumors showed immunoreactivity for EGFR (Table 1). The staining pattern in these tumors was diffuse, with EGFR-positive as well as EGFR-negative areas (Fig. 1). Although one EGFR-positive tumor was amelanotic, the EGFR-positive fields in the other tumors were more often found in the pigmented parts of the tumor. In addition, EGFR-positive cells were frequently observed near blood vessels (Fig. 1).

EGFR Expression and Survival

In the 22 patients included in the study, the 5-year tumor-related survival rate was 67%. Of the six patients with EGFR expression in the tumor, five patients (83%) died within 5 years of uveal melanoma metastases. One EGFR-positive patient was still alive 9 years after enucleation. Of the remaining 15 patients with EGFR-negative tumors, two patients were lost to follow-up, and one patient died of a cause unrelated to the tumor. Of the remaining 12 patients, 2 (17%) died of metastatic disease, 5 and 7 years after enucleation. The median follow-up time of the 10 patients still alive was 8.9 ± 0.4 years (SE). The Kaplan–Meier survival curves (Fig. 2) show a clear significant difference (P = 0.0004) between the survival rates of patients with EGFR-

<table>
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<th>Tumor Prominence (mm)</th>
<th>Mitotic Rate†</th>
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* D-T, death due to tumor; A, still alive; D-O, other cause of death; NT, not tested; –, lost to follow-up.
† Number of mitoses per 15 fields of microscopic examination at a magnification of ×320.

Table 1. Clinical and Histopathologic Data

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IOVS, July 2000, Vol. 41, No. 8

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positive and EGFR-negative tumors. The relative risk was 12.2, with a 95% confidence interval (CI) of 2.17–68.1 (Cox proportional hazards regression model). These data indicate that EGFR expression is an important prognostic factor.

EGFR Expression and Clinical and Histopathologic Parameters

Various histopathologic data were compared between EGFR-positive and EGFR-negative tumors (Fig. 3). Although the differences were not significant, EGFR-positive tumors had a greater tumor prominence (average, 7.3 mm versus 5.8 mm, \( P = 0.19 \)) and a higher mitotic rate (13.8 versus 9.3 mitoses per 15 high-power fields, \( P = 0.23 \)). In addition, patients with EGFR-positive tumors were older (average, 70 years versus 64 years, \( P = 0.33 \)). No associations were found between EGFR expression and other clinical parameters.
expression and tumor diameter, cell type, pigmentation, or gender. In contrast to the EGFR-negative group, Bruch’s membrane was broken in all EGFR-positive tumors (no significance).

In addition to EGFR, survival was significantly associated with age ($P = 0.038$, relative risk $= 1.07$ per year; 95% CI, 1.00–1.14). After adjustment for age, EGFR was still a significant predictor of tumor-related survival ($P = 0.01$, multiple Cox regression model). No correlations were found between survival and the other histopathologic characteristics.

**DISCUSSION**

EGFR expression has been correlated with the growth and development of metastases of various neoplasms. In a number of cancers, including breast cancer, gliomas, squamous carcinoma, and laryngeal cancer, EGFR has been shown to be a marker of poor survival. In other cases, such as cutaneous melanoma, there is controversy about whether EGFR expression is related to tumor progression. In the present study, a clearly significant difference was found between the survival rates of patients with EGFR-positive and EGFR-negative tumors, indicating that, in uveal melanoma, EGFR expression is an important prognostic factor.

Tumor expression of EGFR may enhance uveal melanoma metastasis by different mechanisms. Binding of EGFR (e.g., by EGF or TGF-α, both present in the eye) results in the activation of several pathways that may lead to increased cell growth, DNA synthesis, motility, chemotaxis, secretion of proteases, cell adhesion, and the expression of oncogenes. These effects all play an important role in the metastatic cascade. An interesting question is whether EGFR is involved in the preference of uveal melanoma cells to metastasize to the liver. In human breast carcinoma, EGFR expression was correlated with lymph node metastases, whereas in studies with human cutaneous melanoma cell lines, a positive correlation was found between EGFR expression and the potential of lung metastases to develop after subcutaneous inoculation of human tumor cells in nude mice. In colon carcinoma, EGFR expression was, as in uveal melanoma, correlated with the development of liver metastases. In the liver, high levels of hepatocyte growth factor and TGF-α which can bind to EGFR, are present. As also has been suggested by Ma and Niederkorn, the presence of these EGFR ligands in the liver may be important for the metastasis of uveal melanoma cells to metastasize to the liver. However, ligands for EGFR (e.g., TGF-α and EGF) have been found in many other tissues and body fluids. In the nude mice studies of Ma and Niederkorn, intravenously injected human uveal melanoma cells were shown to accumulate predominantly (70%) in the liver. Blocking EGFR with neutralizing antibodies reduced liver metastasis and prolonged host survival. Of interest, anti-EGFR antibody treatment reduced the number of EGFR-positive melanoma cells in the liver, but did not affect the liver homing of EGFR-negative tumor cells. Finally, EGFR has been shown to protect tumor cells against the cytolytic effects of tumor necrosis factor-α and against killing by interferleukin-2-activated peripheral blood mononuclear cells. Prevention of tumor lysis may be an additional mechanism by which EGFR contributes to tumor progression.

In the present study, tumor EGFR expression tended to be correlated with tumor prominence and mitotic activity. In several cancers, a positive correlation between EGFR expression and proliferation has been demonstrated. In our study, tumor diameter, cell type, and mitotic rate, which are established prognostic factors in uveal melanoma, were not correlated with survival. The relatively small number of patients and the selection for large tumors (tumor material was obtained from patients for whom eye-salvaging therapies were inappropriate) may explain our findings.

The clear association between EGFR expression and increased risk of metastatic disease offers the possibility of using EGFR for the development of new therapeutic strategies in uveal melanoma. Ma and Niederkorn have shown in a murine model that treatment with anti-EGFR antibodies prolongs host survival. In phase I trials of lung carcinoma and gliomas, EGFR-blocking antibodies significantly inhibited tumor growth. Ligands for EGFR can also be used as carriers of cytotoxins or radioactive isotopes. In addition, because tyrosine kinase is important in the signal transduction pathway of EGFR, the use of tyrosine kinase inhibitors may be a promising strategy. These and other approaches with EGFR as a therapeutic target are reviewed by Voldborg et al.

To summarize, in agreement with the murine studies of Ma and Niederkorn, the present study demonstrated that in humans, EGFR expression was also correlated with death due to uveal melanoma metastases. Most likely, the presence of EGFR-binding growth factors in the liver provide the most optimal environment for the embedding of uveal melanoma cells in this organ. Expression of EGFR may be used as a prognostic factor for survival and for the development of new therapeutic strategies in patients with uveal melanoma.

**References**


