Published in final edited form as:

Am J Dermatopathol. 2012 August ; 34(6): 592–596. doi:10.1097/DAD.0b013e31823e25d3.

Expression of CXCR4, E-Cadherin, Bcl-2, and Survivin in Merkel Cell Carcinoma: An Immunohistochemical Study Using a Tissue Microarray

Charles F. Knapp, MD*, Zena Sayegh, MD†, Michael J. Schell, PhD†, Bhupendra Rawal, MS†, Tatiana Ochoa, MD†, Vernon K. Sondak, MD†, and Jane L. Messina, MD‡

*Department of Dermatology, University of South Florida College of Medicine, Tampa, FL

†H. Lee Moffitt Cancer Center and Research Institute, Tampa, FL

‡Department of Pathology and Cell Biology, University of South Florida College of Medicine, Tampa, FL

Abstract

Merkel cell carcinoma (MCC) is a rare but highly aggressive cutaneous malignancy with a mortality rate exceeding that of melanoma. Although smaller studies of markers of progression have been performed, large-scale investigation has been difficult due to the rarity of this tumor. Investigation of 4 potential immunohistochemical progression markers using an MCC tissue microarray was performed. An immunohistochemical analysis of CXCR4, E-cadherin, Bcl-2, and Survivin was performed on a tissue microarray of two hundred twenty-seven 0.6-mm tumor cores—110 primary, 73 local/regional metastatic, and 44 distant metastatic—from 87 patients, 23 of which were sampled 2 or more times. There was a statistically significant increase in immunoreactivity to CXCR4 and Survivin in local/regional nodal MCC metastases compared with primary and distant metastatic lesions. No significant differences by disease location were found for either Bcl-2 or E-cadherin. These results suggest a potential role for CXCR4 and Survivin in MCC tumor progression. However, previous data from other studies suggesting a role for Bcl-2 and E-cadherin in MCC progression are not confirmed in this larger sample. Further discovery of additional markers are needed to better characterize this rare but deadly malignancy.

Keywords

Merkel cell carcinoma; immunohistochemistry; progression; markers; tissue microarray

INTRODUCTION

Merkel cell carcinoma (MCC) is a rare but highly aggressive cutaneous malignancy that principally occurs on the sun-exposed areas of older white men.¹ Its metastatic potential has been compared with that of melanoma, with an even higher mortality rate than the latter.¹

Reprints: Charles F. Knapp, MD, 12901 Bruce B Downs Boulevard, MDC 79, Tampa, FL 33612-4272 (cknapp@health.usf.edu).

The authors declare no conflicts of interest.

Currently, a multidisciplinary approach to treatment is recommended, consisting of wide local excision of the primary tumor, radiation therapy, lymph node dissection, and adjuvant chemotherapy.

Since its debut in the medical literature in 1980, numerous studies have been conducted to better understand the behavior and prognosis of this distinctive skin cancer. Yet, despite more than 1300 published reports on MCC, a great paucity of evidence still exists, and thus, the search for specific histologic progression markers continues. This study serves to further illuminate potential immunohistochemical progression markers for MCC through the use of a tissue microarray (TMA). The specific markers investigated included CXCR4, E-cadherin, Bcl-2, and Survivin. In this study, we examined 227 tissue samples of MCC from 87 patients in an attempt to discover reliable immunohistochemical progression markers for this tumor.

MATERIALS AND METHODS

A thorough review of the literature was initially conducted to determine which immunohistochemical markers possessed the greatest potential for significance in MCC progression. Candidate markers were selected based on quality of study, adequacy of sample size, and potential for further investigation, leading to a choice of 4 previously suggested MCC immunohistochemical progression markers, which possessed the highest potential for further investigation: CXCR4, E-cadherin, Bcl-2, and Survivin.

After institutional review board approval, a TMA was constructed using patients identified from our patient database, which includes all MCC cases at Moffitt Cancer Center from 1989 to 2008. Patients with available material in pathology archives were selected by searching for primary tumor, local and regional nodal disease, and distant metastatic disease. The microscopic slides were then analyzed by a Moffitt dermatopathologist for ideal core sectioning location. Tissue from these areas was randomly sampled from paraffin blocks. Two to three 0.6-mm cylindrical cores were collected from each donor block (with the exception of 2 blocks which had only enough tumor tissue present for a single core) using the MTA-I manual tissue arrayer (Beecher Instruments, Sun Prairie, WI). Cores were then arranged in a new paraffin block.

TMA slides were stained using a Ventana Discovery XT automated system (Ventana Medical Systems, Tucson, AZ) as directed by the manufacturer's protocol. Slides were deparaffinized using EZ Prep Solution (Ventana) and subsequently subjected to a heat-induced antigen retrieval in Cell Conditioning 1 (Ventana). Each TMA slide was stained with one of the following primary antibodies: anti-Bcl-2 mouse monoclonal antibody (Cell Marque, Rocklin, CA), anti-CXCR4 mouse monoclonal antibody (R & D Systems, Minneapolis, MN), anti-E-cadherin mouse monoclonal antibody (Ventana), and anti-Survivin rabbit polyclonal antibody (Novus Biologicals, Littleton, CO). Staining was then visualized using the Ventana OmniMap detection system kit, counterstained with hematoxylin, dehydrated, and cover slipped. Staining was manually scored in a range of 0 to 3 based on intensity and percentage of cells stained—0 signified no staining; 1, weak

staining in 0%–50% of cells; 2, moderate to strong staining in 50%–75% of cells; and 3, strong staining in >75% of cells.

The TMA slides were also scanned and converted into an image format using the Aperio ScanScope XT Automatic Slide Scanning System (Aperio Technologies, Vista, CA) at $\times 20$ magnification using linear array technology. Image analysis was performed with Spectrum Information Manager software (Aperio Technologies) using a positive pixel count algorithm (Fig. 1).

To construct aggregate expression level of MCC TMAs from their 2% positivity (%Pos) scores, we used the raw data for CXCR4 cell data, a square root transformation for Bcl-2 and Survivin cell data, and a quarter root transformation for E-cadherin to approximately normalize these scores, which were then averaged. The Tukey studentized range test was used to detect difference among primary (P), regional nodal metastatic (LN), and distant metastatic (M) tissues at $\alpha = 0.05$. Descriptive statistics were presented using frequency, percentage, mean, median, range, and interquartile range. All statistical analyses were performed using SAS (version 9.2; SAS Institute Inc., Cary, NC).

RESULTS

The 87 patients had a mean age of 73.9 years (median age, 77 years; range, 39–94 years); 66 (76%) were male, and 21 (24%) were female; 13 (15%), 72 (83%), and 1 (1%) patients were stage I, II, and III MCC disease, respectively, at initial diagnosis, while 1 (1%) patient had unknown disease stage.

Manual scoring revealed that strong Bcl-2 staining was present in the majority of primary and regionally metastatic tumors (69% and 73%, respectively), whereas it was slightly lower in distant metastatic disease (40%). E-cadherin was found in a low percentage of all types of tumors (14%, 9%, and 17%). CXCR4 stained virtually all specimens. Nuclear Survivin was found to be strongly positive more often in primary (65%) and regional recurrent tumors (70%) than distant metastatic tumors (47%) (Table 1).

When analyzing imaged data, however, subtle differences were highlighted. A positive correlation (Spearman correlation coefficient ranging from 0.55 to 0.84) was observed between 2 %Pos scores for 2 of the 4 stains. Tukey studentized range tests revealed that LN tissue had statistically significantly ($P = 0.0041$) higher %Pos than P and M for CXCR4; M tissue had statistically significantly ($P = 0.0043$) lower %Pos than P and LN for Survivin; no differences were significant for Bcl-2 ($P = 0.22$) or E-cadherin ($P = 0.64$). The distribution of aggregate positivity scores of each of the 4 MCC TMAs, measured on different sites, is presented in box plots (Fig. 1). Descriptives (N, Md, interquartile range, and range) of biomarkers by tumor type are presented in Table 2.

DISCUSSION

Despite recent research into the behavior and pathogenesis of MCC, there exists a paucity of evidence for potential immunohistochemical progression markers in this tumor. Attempts have been made to discover correlation between various assessments of protein expression

and likelihood of tumor evolution or clinical outcome. Unfortunately, the literature on immunohistochemical progression markers in MCC is composed of small studies that rarely account for all or even most other potential confounding factors. A brief review of the findings of many of these studies is presented.

In 1993, Micali et al² analyzed 8 patients with MCC and concluded that immunohistology did not correlate with outcome. This conclusion was also reached by Kennedy et al³ after staining 15 cases of MCC for Bcl-2 (associated with resistance to apoptosis) and p53 (a critical cell cycle regulatory protein), even after finding moderate/strong staining for p53 in 5 cases, and moderate/strong staining for Bcl-2 in 10 cases. In 1999, Feinmesser et al⁴ looked at the expression of bax (associated with resistance to apoptosis), Bcl-2, and p53 in MCC. Although they could not establish a correlation between staining intensity and treatment response or survival, Feinmesser et al⁴ did suggest the possibility of tumor cell resistance to apoptosis-inducing agents due to their significant Bcl-2 positivity (76%).

CXCR4 is a chemokine receptor perhaps most recognized for its facilitation of HIV infection of T-helper lymphocytes. However, the role of CXCR4 in carcinogenesis, namely its effect on directing migration of metastatic cancer cells, has recently been elucidated. Tucci et al⁵ looked at the staining patterns of CXCR4, Ki67, Bcl-2, p53, p16, and Survivin (an oncogene which inhibits apoptosis) in 12 cases of primary MCC. They found that Bcl-2 correlated strongly with poor prognosis, as did Ki67 and Survivin, albeit insignificantly. No expression of CXCR4 was found in the samples studied. Kim and McNiff⁶ tested 19 cases of MCC (18 primary tumors and 1 metastasis) with antibodies to Survivin and found that all cases demonstrated strong immunoreactivity, with predominantly nuclear staining in 8 cases, cytoplasmic staining in 4, and mixed staining in 3. Seven of the 8 patients with predominantly nuclear staining developed metastatic disease or died, whereas all 4 cases with predominately cytoplasmic staining were disease free.

E-cadherin is a calcium-dependent intercellular adhesion protein normally expressed in skin. Loss of this protein has been recognized in several malignant tumors and is thought to be associated with a higher rate of tumor invasion due to loss of adhesion to adjacent cells. Han et al⁷ found that E-cadherin was the most frequently expressed cadherin in MCC, although interestingly, this expression was largely localized to the nucleus rather than the cytoplasm. Tanaka et al⁸ and Panelos et al,⁹ however, although confirming reduced cytoplasmic E-cadherin expression in MCC, found nuclear localization of E-cadherin in only a small minority of cases (4 of 31, and 3 of 26, respectively).

Using TMAs of 227 samples of MCC from 87 patients, we investigated the potential relationship between MCC progression and tissue immunoreactivity to each of 4 markers—CXCR4, Bcl-2, Survivin, and E-cadherin. In our study, we exhibited positive staining for each of these markers, in contrast to previous reports of negative CXCR4 expression in MCC. Expression of E-cadherin in MCC tumor cells was observed to be of low frequency, generally weak, and cytoplasmic. We found that Survivin demonstrated a nuclear staining pattern with almost universal expression in MCC of all tumor stages, but significantly lower percent positivity in distantly metastatic tumors than in primary tumors or regional nodal metastases (Fig. 2). A potential explanation for Survivin loss in distant MCC metastases

may indicate independence from normal antiapoptotic mechanisms in advanced disease. Additionally, CXCR4 stained with a significantly higher percent positivity in regional metastatic disease than in primary or distant metastatic tumors (Fig. 3). The increased expression of CXCR4 in local and regional nodal tumors may suggest a role for this protein in directing local and regional metastases in MCC, but adoption of alternate “homing” pathways in advanced metastatic disease. Notably, no statistically significant difference in staining patterns was observed for Bcl-2 or E-cadherin. This lack of differential staining in various tumor types suggests no role for Bcl-2 or E-cadherin in MCC progression.

The strengths of this study are the large sample size and lack of exclusion criteria, which allows for broader application of our results. Perhaps the largest limitation of this study was the lack of complete patient follow-up data, which would have allowed us the opportunity to correlate our results with clinical outcome and further evaluate both progression and prognosis, as opposed to only the former. In light of this, our study is best understood as an objective report of data, and not an extrapolation of prognosis. Another limitation of this study was the lack of inpatient standardization (very few patients had multiple stages of tumor available for inclusion in the TMA).

In conclusion, although the role of CXCR4 and Survivin in the progression of MCC is not fully elucidated, their differential expression in more advanced stages of MCC may suggest independence from more typical antiapoptotic and cell localization pathways. Certainly, the body of research involving pathologic and immunohistochemical progression and prognosis of MCC is relatively sparse due to the rarity of this tumor and larger and more controlled studies must still be conducted.

References

1. Akhtar S, Oza KK, Wright J. Merkel cell carcinoma: report of 10 cases and review of the literature. *J Am Acad Dermatol*. 2000; 43:755. [PubMed: 11050578]
2. Micali G, Ferrau F, Innocenzi D. Primary Merkel cell tumor: a clinical analysis of eight cases. *Int J Dermatol*. 1993; 32:345. [PubMed: 8505160]
3. Kennedy MM, Blessing K, King G, et al. Expression of Bcl-2 and p53 in Merkel cell carcinoma. An immunohistochemical study. *Am J Dermatopathol*. 1996; 18:273. [PubMed: 8806961]
4. Feinmesser M, Halpern M, Fenig E, et al. Expression of the apoptosis-related oncogenes Bcl-2, bax, and p53 in Merkel cell carcinoma: can they predict treatment response and clinical outcome? *Hum Pathol*. 1999; 30:1367. [PubMed: 10571519]
5. Tucci MG, Lucarini G, Giangiacomi M, et al. Immunohistochemical study of apoptosis markers and involvement of chemokine CXCR4 in skin Merkel cell carcinoma. *J Eur Acad Dermatol Venereol*. 2006; 20:1220. [PubMed: 17062035]
6. Kim J, McNiff JM. Nuclear expression of Survivin portends a poor prognosis in Merkel cell carcinoma. *Mod Pathol*. 2008; 21:764. [PubMed: 18425079]
7. Han AC, Soler AP, Tang CK, et al. Nuclear localization of E-cadherin expression in Merkel cell carcinoma. *Arch Pathol Lab Med*. 2000; 124:1147. [PubMed: 10923074]
8. Tanaka Y, Sano T, Qian ZR, et al. Expression of adhesion molecules and cytokeratin 20 in Merkel cell carcinomas. *Endocr Pathol*. 2004; 15:117. [PubMed: 15299198]
9. Panelos J, Batistatou A, Paglierani M, et al. Expression of Notch-1 and alteration of the E-cadherin/beta-catenin cell adhesion complex are observed in primary cutaneous neuroendocrine carcinoma (Merkel cell carcinoma). *Mod Pathol*. 2009; 22:959. [PubMed: 19396152]

FIGURE 1.
Average pixel counts of TMAs (Bcl-2, CXCR4, Survivin, and E-cadherin) by image analysis.

FIGURE 2. Survivin immunostaining, indicating lower nuclear expression in distant metastatic (C) tumors compared with primary (A) and local/regional nodal (B) tumors.

FIGURE 3.
CXCR4 immunostaining, indicating increased expression in local/regional nodal (B) tumors compared with primary (A) and distant metastatic (C) tumors.

TABLE 1

Manual Scoring of Immunohistochemical Markers in Merkel Cell Carcinoma

Marker	Primary Tumor Positive (%)	Lymph Node Positive (%)	Metastatic Positive (%)
Bcl-2	69	73	40
E-cadherin	14	9	17
CXCR4	96	100	100
Nuclear Survivin	65	70	47

TABLE 2**Biomarker Scores by Tumor Type**

Tumor Type	Bel-2			CXCR4			Survivin			E-Cadherin		
	N	Md	IQR	Range	N	Md	IQR	Range	N	Md	IQR	Range
Primary	52	0.19	0.03–0.36	0–0.73	51	0.61	0.45–0.68	0.02–0.77	51	0.07	0.02–0.10	0–0.30
Local/regional metastatic	35	0.15	0.09–0.39	0–0.84	35	0.67	0.59–0.75	0.29–0.84	35	0.08	0.04–0.11	0.01–0.24
Distant metastatic	21	0.08	0.01–0.31	0–0.55	21	0.52	0.38–0.64	0.26–0.78	21	0.03	0.01–0.05	0–0.15
									20	0.0	0–0.03	0–0.16

N, sample size used; Md, median; IQR, interquartile range.