Establishment of Korean Merkel cell carcinoma patient cohort & Identification of the relationship between borderline microenvironment fibrosis and clinical outcomes

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Directed by Professor Mi Ryung Roh

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This certifies that the Master's Thesis of
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<ABSTRACT>

Establishment of Korean Merkel cell carcinoma patient cohort & Identification of the relationship between borderline microenvironment fibrosis and clinical outcomes

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Merkel cell carcinoma (MCC) is a rare and aggressive neuroendocrine carcinoma typically presenting as a flesh-colored subcutaneous nodule. Data on MCC in Korean patients have been limited to three case series, and there is little information about the characteristics of MCC in Korean patients. The tumor microenvironment is the environment around a tumor including the extracellular matrix, fibroblasts, surrounding vessels, infiltrating immune cells, and signaling molecules. Tumor microenvironments such as desmoplasia and tumor-infiltrating lymphocytes were previously identified as prognostic factors in some malignancies but were not well evaluated in MCC. Those tumor microenvironments have been evaluated with immunohistochemical staining and difficult to be routinely analyzed in clinics.

The objectives of this study were to establish a Korean patient cohort with MCC and to evaluate the relationship between clinical outcomes and borderline microenvironment fibrosis (bMF), one of the tumor microenvironment assessed via H&E staining.

We retrospectively reviewed 34 cases of MCC from two, tertiary hospitals from 2007 to 2020. All patients' clinicopathological data were analyzed and 20

MCC cases were histologically evaluated for analyses.

The median age of the 34 patients with MCC was 74 years (range, 32–97 years); of these, 13 were men, and 21 were women. The head and neck were the most common sites of the primary tumor (52.9%), followed by the lower extremities (17.6%), upper extremities (14.7%), and trunk (11.8%). Among the 33 patients who underwent surgery for primary MCC, 17 patients showed recurrence (local recurrences: n=13, distant metastases: n=4), and the average time to recurrence after surgery was 9.1 months. Of all patients, 11 patients died within 18 months of the median follow-up period (1.1–74.1 months).

In the univariate analysis to identify the prognostic factors, male sex, age at diagnosis, and presence of lung diseases were poor prognostic factors of overall survival, while male sex and involvement of lung diseases were found to be poor prognostic factors of progression-free survival. In the multivariate analysis, involvement of lung diseases was found to be an independent prognostic factor of progression-free survival. bMF was a reliable prognostic marker of progression-free survival (p=0.024) and associated with nodal involvement at last follow-up (p=0.04).

In Korean patients, MCC is an aggressive subcutaneous tumor that most commonly occurs in the head and neck, with a poor prognosis. bMF could be a novel prognostic marker of progression-free survival among patients with MCC. This study suggests that bMF may affect tumor growth and showed the possibility to be used as a significant prognostic biomarker in MCC.

Key Words : merkel cell carcinoma, korean, prognostic factors, tumor microenvironment, borderline microenvironment fibrosis

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I. INTRODUCTION

Merkel cell carcinoma (MCC) is a rare, aggressive, neuroendocrine tumor mostly presenting as an erythematous to violaceous, painless, indurated nodule[1]. Although MCC can be diagnosed at an early stage by biopsy, it has aggressive characteristics with a high risk of lymph node and distant metastases[2]. The incidence of MCC has currently steadily increased[3]**.** The incidence of MCC ranges from 0.1 to 1.6 cases per 100,000 individuals per year[2]. MCC is approximately 25 times more common in Caucasians than in other ethnic groups and tends to affect men more than women[1,2]. It commonly occurs on the head and neck in all populations[4,5].

Due to the rare incidence in non-white populations and racial differences in MCC, a large cohort in non-white MCC patients has not been established. Although no population-based data are available in Asia, the incidence of MCC in Asia is thought to be very low[6,7]. In the literature, there were only a total of 21

MCC cases in Korea in three studies[8-10]; thus, it was not enough to build a cohort of Korean MCC patients.

The tumor microenvironment is the environment surrounding the tumor comprising tumor cells, stroma, blood vessels, infiltrating immune cells, and various tissue cells[11]. It is thought to play an important role in tumor progression and is known to be closely related to prognosis via various mechanisms[12]. Desmoplasia, one of the tumor microenvironment, is the growth of fibrous or connective tissue causing fibrosis in the tumor matrix and has been identified as a prognostic marker in other malignancies such as breast cancer, colorectal cancer, and pancreatic cancer[13-15]. In MCC, researches of tumor microenvironments have rarely been conducted except for tumor-infiltrating lymphocytes[16,17]. Thus the tumor microenvironment including desmoplasia might provide novel biomarkers for MCC like for other malignancies.

Tumor microenvironments have been evaluated by microscopy with immunohistochemical staining because cell populations can be identified by their molecular characteristics. The methods to evaluate tumor microenvironments vary among researchers because of the heterogenous microenvironment, and it is complex and difficult to routinely implement the methods in clinics[18]. One previous study evaluated desmoplasia via using Hematoxylin and Eosin (H&E) staining in colorectal cancer, which was made possible only with a consultation from a pathologist. In a recent study, borderline microenvironment fibrosis (bMF) was first suggested as one of the tumor microenvironments, which can be relatively easily assessed with tumor borderline fibrosis by only H&E staining[18].

Together, this study aimed to establish a Korean patient cohort with MCC and evaluate the relationship between clinical outcomes and the tumor microenvironment by assessing bMF.

II. MATERIALS AND METHODS

1. Patients and methods

Patients with MCC treated at Severance Hospital and Gangnam Severance Hospital, Seoul, South Korea from January 2007 to May 2020 were retrospectively investigated. Patients without histopathological confirmation of MCC were excluded. This study was approved by the institutional review board of Severance Hospital (approval number: 4-2020-0578) and Gangnam Severance Hospital (approval number: 3-2020-0087).

In total, 34 patients with MCC were analyzed in our study. The electronic medical records of the patients were reviewed to assess baseline demographic information. Photographs obtained before surgery were also reviewed to assess information on the characteristics of the primary tumor. The reviewed characteristics included age at diagnosis, sex, tumor characteristics, tumor stage (T classification, positive lymph nodes, metastasis), type of surgery, type of additional treatment after surgery (radiotherapy, chemotherapy, and immunotherapy), recurrence, and prognosis. TNM staging was classified according to the criteria of the 8th edition of the American Joint Committee on Cancer[19]. Recurrence from the primary tumor to the regional lymph nodes was defined as regional recurrence, and that beyond the regional lymph nodes was referred to as distant metastasis.

2. Tissue preparation and assessment

Formalin-fixed, paraffin-embedded tissue samples were obtained from 20 patients with MCC and deparaffinized with xylene and hydrated using a graded ethanol series. The four-μm thick sections of MCC were stained with H&E and then assessed. The causes of failing to get 14 tissue samples were unstored tissue

samples, patients' refusal to enroll in this study, or failure to contact the patients. Epidermal change and tumor size were assessed in all sections.

bMF was defined by the presence of fibrosis in the borderline invasive margin of cancer as described previously[18]. The borderline stromal area within 100 μm from the invasive margin of MCC was evaluated at 200x magnification using a microscope. The status of bMF-positive was defined as a borderline stromal area occupied by fibrosis or fibroblasts over 50% and bMF-negative was defined as below 50%.

3. Statistical analysis

All analyses were conducted using the SPSS v25.0 software (SPSS, Chicago, IL, USA). Categorical variables were compared using the chi-square and Fisher's exact tests. The primary outcome measure was overall survival and progression-free survival. The Kaplan-Meier estimate was used to analyze the overall survival and progression-free survival and log-rank test was used to compare different survival stratified by clinicopathological factors. For univariate and multivariate analyses, the variables included gender, age at diagnosis, stage, tumor size, tumor locations, nodal involvements, metastasis, recurrence, and various comorbidities.

Comorbidities were grouped according to the presence or absence of hypertension, diabetes mellitus, lung diseases, other malignancies, cardiovascular diseases, and neurological diseases. Presence of lung diseases included asthma, chronic obstructive pulmonary disease, pulmonary tuberculosis, emphysema, and pneumonia.

Cox-regression analysis was used to identify the independent prognostic factors for overall survival and progression-free survival. Differences were considered statistically significant when the p-value was <0.05.

III. RESULTS

1. Baseline patient characteristics

The demographic characteristics of total 34 patients with MCC are described in Table 1. There were more female patients than male patients (male:female sex ratio = 13:21). The head and neck were the most common sites (18/34, 52.9%) followed by the lower extremities (6/34, 17.6%), upper extremities (5/34, 14.7%), trunk (4/34, 11.8%), and external ear canal (1/34, 2.9%). The median age of the patients was 73.5 years (mean: 70.9 years, range: 32–90 years). The median follow-up period was 23.9 months (mean: 18 months, range: 1.1–74.1 months).

Of the 34 patients, thirty-three patients underwent surgery of a primary tumor and one patient had palliative surgery for a metastatic tumor. The median duration time from symptom detection to the first visit of our clinics was 4 months (mean: 7.1 months, range 1–36 months). The primary lesion was a subcutaneous nodule (21/28), papule (2/28), plaque (2/28), nodule with surface erosion (2/28), and polyp (1/28). The percentage of the pathological tumor size over 2 cm was 33.3% (10/33), 1 to 2 cm was 42.4% (14/33), and under 1 cm was 27.3% (9/33). The pathological stage and tumor extension at diagnosis are shown in Table 1. Proportion of Stage I, II, IIIA, IIIB, and IV were 47.1% (16/34), 23.5% (8/34), 11.8% (4/34), 14.7% (5/34), and 2.9% (1/34), respectively. There were five patients with other malignancies including adrenal cancer, colon cancer, lung cancer, thyroid cancer, and pancreatic cancer. In the follow-up period, 11 patients died and the causes of death in the patients were the progression of MCC (n=7), infectious pneumonia (n=1), the progression of lung cancer (n=1), and unknown causes (n=2). Of 33 patients who underwent surgery of primary lesion, recurrence occurred in 17 patients (local recurrence 13/33 [39.4%] and distant metastasis 4/33 [12.1%]) within an average of 9.1 months.

Table 1. Demographics of the patient cohort with Merkel cell carcinoma.

Variable	Total, n=34
Age at diagnosis (years)	
Mean	70.9
Median (range)	73.5 (32–90)
Sex, n (%)	
Male	13 (38.2)
Female	21 (61.8)
Tumor site, n (%)	
Head and neck	18 (52.9)
Lower extremities	6 (17.6)
Upper extremities	5 (14.7)
Trunk	4 (11.8)
External ear canal	1 (2.9)
Follow-up time (months)	
Mean	23.9
Median (range)	18 (1.1–74.1)
Pathological stage at diagnosis, n (%)*	
I	16 (47.1)
II	8 (23.5)
IIIA	4 (11.8)
IIIB	5 (14.7)
IV	1 (2.9)
Tumor extension at diagnosis, n (%)	
Restricted primary tumor	24 (70.6)
Regional lymph node involvement	6 (17.6)
In-transit metastasis	3 (8.8)
Distant metastasis	1 (2.9)
Death, n (%)	
Yes	11 (32.4)
No	23 (67.6)
Cause of death, n (%)	
Merkel cell carcinoma progression	7 (63.6)
Infectious pneumonia	1 (9.1)
Other malignancy	1 (9.1)
Unknown causes	2 (18.2)
Recurrence after surgery, n (%)	Total, n=33**
Local recurrence	13 (39.4)
Distant metastasis	4 (12.1)

*American Joint Committee on Cancer staging 8th edition
**One patient who underwent palliative surgery was excluded

2. Pathological characteristics of the tumor

In the analysis of a total of 20 cases of histopathology, epidermal changes, including epidermal thinning (2/20, 10%), hyperpigmentation (3/20, 15%), reactive epidermal hyperplasia (3/20, 15%), intraepidermal spread of tumor (3/20, 15%), Bowen's disease (1/20, 5%), basal vacuolization (1/20, 5%) and Pautrier-like microcollection formation (1/20, 5%), were observed in some cases.

Table 2. Histopathological features of Merkel cell carcinoma.

Variables	Total, N = 20
Epidermal changes, n (%)	
Epidermal thinning	2 (10)
Hyperpigmentation	3 (15)
Reactive epidermal hyperplasia	3 (15)
Intraepidermal spread of tumor	3 (15)
Bowen's disease	1 (5)
Basal vacuolization	1 (5)
Pautrier-like microcollection formation	1 (5)

To assess bMF. we analyzed interstitial lesions within 100 μm of the tumor margin, bMF was observed in 11 cases (55%) and the other 9 cases showed bMF-negative (45%). We identified two patterns of interstitial area around the tumor. One pattern was fibroblast and/or fibrosis enrichment (Figure 1A). On the other hand, the other pattern lessly contained fibroblast and/or fibrosis and more enriched with lymphocytes (Figure 1B). All of the bMF-negative status corresponded to the pattern with enriched lymphocytes.

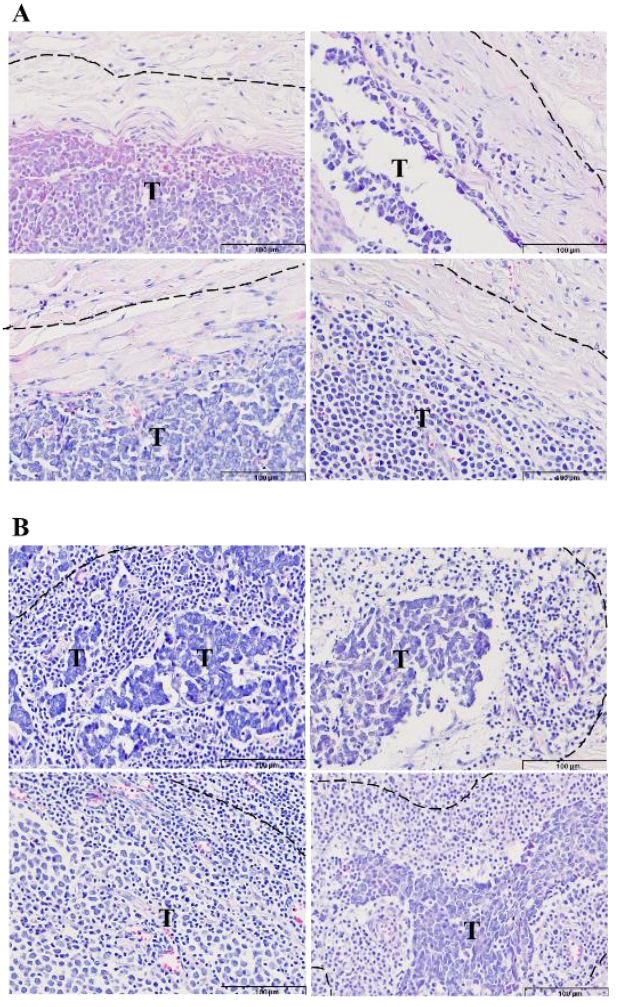

Figure 1. Two different status of bMF: bMF-positive and bMF-negative status

A: Representative images of bMF-positive cases (n=11). B: Representative images of bMF-negative cases (n=9). Magnification, 200x. Dotted line, 100 μm from the borderline of cancer; ′T′, tumor region; bMF, borderline microenvironment fibrosis

We analyzed the association between bMF-positive status and other clinicopathological factors including gender, age at diagnosis, tumor size, nodal involvement, metastasis, and tumor stage. Nodal involvement at the last follow-up was statistically related to bMF (p=0.04), but other factors were not significantly related to bMF (Table 3).

Table 3. Relationship between bMF and clinicopathologic factors of Merkel cell carcinoma.

	bMF		*p* value*
	Positive (n=11)	Negative (n=9)	
Gender			
Male	5 (45.5%)	3 (33.3%)	0.465
Female	6 (54.5%)	6 (66.7%)	
Age at diagnosis			
≥75	7 (63.6%)	5 (55.6%)	0.535
<75	4 (36.4%)	4 (44.4%)	
Stage at the diagnosis			
1, 2	8 (72.7%)	7 (77.8%)	0.604
3, 4	3 (27.3%)	2 (22.2%)	
Tumor size			
≥2cm	3 (27.3%)	4 (44.4%)	0.37
<2cm	8 (72.7%)	5 (%)	
Nodal involvement at diagnosis			
Yes	3 (27.3%)	2 (22.2%)	0.604
No	8 (72.7%)	7 (77.8%)	
Nodal involvement at last follow-up			
Yes	9 (81.8%)	3 (33.3%)	0.04
No	2 (18.2%)	6 (66.7%)	
Metastasis at last follow-up			
Yes	2 (18.2%)	1 (11.1%)	0.579
No	9 (81.8%)	8 (88.9%)	

bMF, borderline microenvironment fibrosis
*The Fisher's exact test was used to assess the relationship

3. Treatment

Of the 33 patients who underwent surgical removal of the primary lesion, Mohs micrographic surgery was conducted in 15 patients (45.6%), wide excision in 10

patients (30.3%), and excision with free margin in 8 patients (24.2%). Among these patients, concurrent regional lymph nodes dissection was performed in 14 patients (42.4%). Sentinel lymph node biopsy was performed in 6 patients (18.2%) at the time of first evaluation. Twenty-two patients received adjuvant radiotherapy (22/33, 66%) which included 11 patients with radiation monotherapy (33%), 6 patients with concurrent chemoradiotherapy (18.2%), and 5 patients with immunotherapy with radiotherapy (15.2%). In the group treated with immunotherapy, pembrolizumab was used in four patients and avelumab in one patient.

4. Prognostic factors

There were no statistical differences in prognosis depending on the surgical methods in overall survival (p=0.981) and progression-free survival (p=0.823). We next evaluated various clinicopathological factors related to prognosis (Table 4). In the univariate analysis, the predictors of overall survival were male sex, age at diagnosis, presence of lung diseases, recurrence after surgery, and metastasis at the last follow-up (Table 4). A multivariate analysis was implemented for three risk factors including male sex, age at diagnosis, and presence of lung diseases; however, no independent prognostic factor was found in overall survival (Table 4).

Table 4. Univariate and multivariate analyses of the overall survival of Korean patients with Merkel cell carcinoma.

Variables	No. of patients (%)	No. of deaths (%)	Univariate hazard ratio (95% CI)	*p value*	Multivariate hazard ratio (95% CI)	*p value*
Sex			3.82 (1.14–12.80)	0.030	1.68 (0.42–6.72)	0.463
Male	13 (38.2%)	6 (46.2%)				
Female	21 (61.8%)	5 (23.8%)				

Age at diagnosis			1.15 (1.04–1.28)	0.009	1.11 (0.99–1.25)	0.073
Age			3.54 (0.90–13.88)	0.069		
≥75 years	15 (44.1%)	5 (33.3%)				
<75 years	19 (55.9%)	6 (31.6%)				
Stage						
I	16 (47.1%)	3 (18.8%)	1			
II	8 (23.5%)	3 (37.5%)	1.38 (0.28–6.88)	0.693		
III	9 (26.5%)	5 (55.6%)	3.72 (0.88–15.72)	0.074		
IV	1 (3.0%)	0 (0.0%)	0	0.990		
Tumor size			0.64 (0.16–2.50)	0.522		
≥2 cm	10 (31.3%)	3 (30.0%)				
<2 cm	22 (68.8%)	7 (31.8%)				
Tumor location						
Head and neck	18 (52.9%)	5 (27.8%)	1			
Extremities	11 (32.4%)	3 (27.3%)	1.16 (0.25–5.29)	0.851		
Trunk	4 (11.8%)	3 (75.0%)	0.57 (0.13–2.47)	0.448		
Other	1 (2.9%)	0 (0.0%)	0	0.991		
Nodal involvements at diagnosis			2.88 (0.87–9.57)	0.085		
Yes	10 (29.4%)	5 (50.0%)				
No	24 (70.6%)	5 (20.8%)				
Nodal involvements at the last follow-up			39.34 (0.21–7409.14)	0.169		
Yes	21 (61.8%)	11 (52.4%)				
No	13 (38.2%)	0 (0.0%)				
Metastasis at the last follow-up			4.44 (1.27–15.47)	0.019		
Yes	8 (23.5%)	5 (62.5%)				
No	26 (76.5%)	6 (23.1%)				
Recurrence after surgery			10.39 (1.33–81.35)	0.026		
Yes	17 (50.0%)	10 (58.8%)				
No	17 (50.0%)	1 (5.9%)				
Hypertension			1.67 (0.49–5.74)	0.417		
Yes	19 (55.9%)	7 (36.8%)				
No	15 (44.1%)	4 (36.4%)				
Diabetes mellitus			2.04 (0.58–7.09)	0.264		
Yes	9 (26.5%)	4 (44.4%)				
No	25 (73.5%)	7 (28.0%)				
Lung diseases			4.69 (1.03–21.34)	0.045	2.39 (0.43–13.34)	0.323
Yes	7 (20.6%)	3 (42.9%)				
No	27 (79.4%)	8 (29.6%)				

Other malignancies			0.70 (0.15–3.32)	0.657
Yes	5 (14.7%)	2 (40.0%)		
No	29 (85.3%)	9 (31.0%)		
Cardiovascular diseases			0.607 (0.148–2.484)	0.487
Yes	8 (23.5%)	3 (37.5%)		
No	26 (76.5%)	8 (30.8%)		
Neurological diseases			1.43 (0.17–11.87)	0.743
Yes	4 (11.8%)	1 (25.0%)		
No	30 (88.2%)	10 (33.3%)		

Similarly, the predictors of progression-free survival were male sex, presence of lung diseases, nodal involvement at the last follow-up, and metastasis at the last follow-up in the univariate analysis (Table 5). Meanwhile, the independent poor prognostic factor affecting progression-free survival was involvement of lung diseases (hazard ratio [95% confidence interval], 4.29 [1.37–13.38]; p=0.012) in the multivariate analysis (Table 5).

Table 5. Univariate and multivariate analyses of the progression-free survival of Korean patients with Merkel cell carcinoma.

Variables	No. of patients (%)	No. of progression (%)	Univariate hazard ratio (95% CI)	*p value*	Multivariate hazard ratio (95% CI)	*p value*
Sex			2.85 (1.12–7.27)	0.028	1.63 (0.60–4.38)	0.337
Male	13 (38.2%)	9 (69.2%)				
Female	21 (61.8%)	9 (42.9%)				
Age at diagnosis			1.05 (1.00–1.11)	0.065		
Age			2.46 (0.94–6.45)	0.068		
≥75 years	15 (44.1%)	9 (60.0%)				
<75 years	19 (55.9%)	9 (47.4%)				
Stage						
I	16 (47.1%)	6 (37.5%)	1			
II	8 (23.5%)	6 (75.0%)	1.72 (0.55–5.36)	0.353		
III	9 (26.5%)	6 (66.6%)	2.43 (0.78–7.57)	0.127		
IV	1 (3.0%)	0 (0.0%)	0	0.987		

Tumor size			1.58 (0.60–4.16)	0.352		
≥2 cm	10 (31.3%)	7 (70.0%)				
<2 cm	22 (68.8%)	10 (45.5%)				
Tumor location						
Head and neck	18 (52.9%)	8 (44.4%)	1			
Extremities	11 (32.4%)	8 (72.7%)	0.91 (0.19–4.30)	0.903		
Trunk	4 (11.8%)	2 (50.0%)	1.55 (0.58–4.18)	0.383		
Other	1 (2.9%)	0 (0.0%)	0	0.987		
Nodal involvements at diagnosis			1.58 (0.59–4.21)	0.365		
Yes	10 (29.4%)	6 (60.0%)				
No	24 (70.6%)	12 (50.0%)				
Nodal involvements at the last follow-up			14.41 (1.91–108.59)	0.010		
Yes	21 (61.8%)	17 (81.0%)				
No	13 (38.2%)	1 (7.7%)				
Metastasis at the last follow-up			3.03 (1.15–8.00)	0.025		
Yes	8 (23.5%)	7 (87.5%)				
No	26 (76.5%)	11 (42.3%)				
Hypertension			1.20 (0.46–3.09)	0.712		
Yes	19 (55.9%)	11 (57.9%)				
No	15 (44.1%)	7 (44.7%)				
Diabetes mellitus			1.72 (0.67–4.44)	0.264		
Yes	9 (26.5%)	7 (77.8%)				
No	25 (73.5%)	11 (44.0%)				
Lung diseases			4.01 (1.44–11.21)	0.008	4.29 (1.37–13.38)	0.012
Yes	7 (20.6%)	6 (85.7%)				
No	27 (79.4%)	12 (44.4%)				
Other malignancies			0.56 (0.13–2.46)	0.444		
Yes	5 (14.7%)	2 (40.0%)				
No	29 (85.3%)	16 (55.2%)				
Cardiovascular diseases			1.14 (0.40–3.24)	0.808		
Yes	8 (23.5%)	5 (62.5%)				
No	26 (76.5%)	13 (50.0%)				
Neurological diseases			1.98 (0.57–6.96)	0.285		
Yes	4 (11.8%)	3 (75.0%)				
No	30 (88.2%)	15 (50.0%)				

In histopathological analyses of 20 cases, the status of bMF-positive was not related to overall survival (p=0.253) (Figure 2A); however, it was statistically related to progression-free survival in MCC (p=0.024) (Figure 2B). It may suggest bMF may be a novel prognostic marker in progression-free survival.

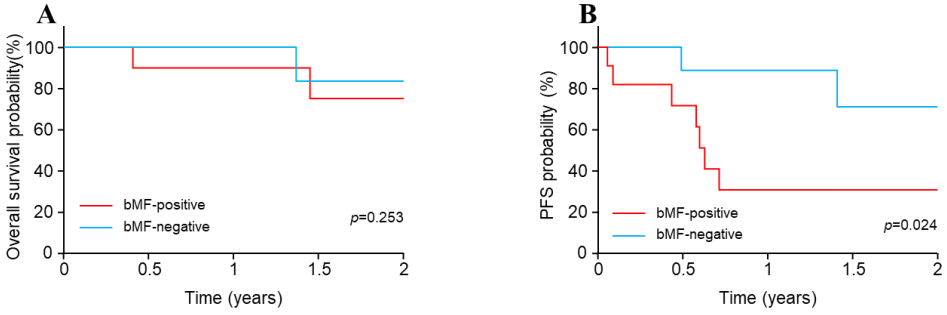

Figure 2. Overall survival and progression-free survival of the patients with Merkel cell carcinoma charted by bMF

A: Overall survival of bMF-positive and bMF-negative cases (p=0.253). B: PFS of bMF-positive and bMF-negative cases (p=0.024). bMF, borderline microenvironment fibrosis; PFS, progression-free survival

IV. DISCUSSION

To date, there have been no publication-based data regarding MCC in Korea. Only a few small case series have been published, which are summarized in Table 6[8-10]. In addition to the previous Korean patients with our cohort, Korean MCC was predominantly located on the head and neck (52.7%, 29/55), followed by the lower extremities (20.0%, 11/55), upper extremities (18.2%, 10/55), and trunk (7.3%, 4/55) (Table 6). The head and neck were the most common site of MCC in Korea, which is consistent with the fact that it accounts for 50% of cases; however, the trunk was a rare site in Korea, which was estimated at 30% worldwide[20-22]. The trunk was the common site in patients younger than 65 years in the previous literature[4], but the head and neck (41.2%, 7/17) were the most common sites of MCC among the Koreans under 65-year-old. Merkel cell polyomavirus (MCPyV) and DNA damage by ultraviolet radiation are known to be the major pathogenic causes of MCC[23], and MCPyV-positive tumors were more frequently located on the extremities than MCPyV-negative tumors in a large-scale previous study[24]. Unfortunately, we didn't examine tests to detect MCPyV, but thirteen Korean cases in total fourteen (92.9%) showed MCPyV-positive status in polymerase chain reaction in previous Korean MCC cases[8,9]. Together, the high frequency of MCPyV-positive in Korean patients with MCC may be associated with the affected tumor locations.

Table 6. Clinical features of Merkel cell carcinoma in Korea.

Study	No. of cases	MCPyV (PCR)	MCPyV (CM2B4 stain)	Prognostic factor
Our data	34	-	-	bMF in progression-free survival
Woo et al[8]	7	7 (100%)	-	Tumor thickness
Chun et al[9]	7	6 (86%)	5 (71%)	

Lee et al[10]	7	-	-	Marginal clearance with surgery, SLNB

	No. of cases	Age, mean, years (range)	Sex	Primary tumor sites
Total	55	68.6 (22–90)	M : F = 19 : 36	Head and neck (n=29, 52.7%) / Lower extremities (n=11, 20%) / Upper extremties (n=10, 18.2%) / Trunk (n=4, 7.3%) / Ear canal (n=1, 1.8%)

MCPyV, Merkel cell polyomavirus; PCR, polymerase chain reaction; bMF, borderline microenvironment fibrosis; SLNB, sentinel lymph node biopsy

In other studies, there was an increased overall risk for second malignancy 1 year after the diagnosis of MCC, and melanoma was the most common coexisting malignancy[25]. In our study, other malignancies not including melanoma were observed in 5 patients. No characteristics have been shared in patients with other malignancies, but further studies are needed to clarify the association between MCC and other malignancies.

In histological reviews of epidermis covering MCC in the cohort, there were no specific epidermal changes but one case presented with a coexisting Bowen's disease on the abdomen. The epidermal change was reported in less than 10% of all MCC cases, but MCC is occasionally found to coexist with other epidermal diseases such as squamous cell carcinoma, basal cell carcinoma, actinic keratosis, and rarely Bowen's disease[26]. MCC concurrent with Bowen's disease or squamous cell carcinoma usually didn't involve MCPyV, and may develop through different teratogenic pathway such as ultraviolet radiation compared to pure MCC[27]. In our study, one case of MCC concurrent with Bowen's disease may occur together by common oncogenic factors as previously reported.

Thorough evaluation of MCC is critical because of their aggressive behavior and poor clinical outcomes[2]. Overall survival at 5 years for local disease, nodal disease, and distant disease was 51%, 35%, and 14%, respectively[28]. Many clinicopathological markers were known to be poor prognostic factors including advanced age, male sex, fair skin, tumor site (head and neck), tumor size of 2 cm, distant metastatic site at diagnosis, and chronic immunosuppressive state[28-34]. Parameters such as an infiltrative tumor growth pattern are not yet included in the stage system but influence the prognosis of patients with MCC[19,35]. Therefore, assessment of various parameters including not only known clinical prognostic factors or TNM stage, but also histopathological factors is important to evaluate the prognosis of MCC. In our study, male sex, older age at diagnosis, and presence of lung diseases were poor prognostic factors of overall survival on univariate analysis but not in multivariate analysis. Male sex and presence of lung diseases were also found as the poor prognostic factors of progression-free survival in the univariate analysis; interestingly, presence of lung diseases was found as an independent prognostic factor in the multivariate analysis. More detailed research is required because there was no previous finding of the association between MCC and lung diseases.

The tumor microenvironment balances tumor growth and suppression through cell-cell interactions and humoral factors[18]. The tumor microenvironment and its association with clinicopathological features are an area of interest and have been actively studied recently in other malignancies. The presence of tumor-infiltrating lymphocytes is known to be the most reliable prognostic and predictive marker in MCC[16]. The levels of intratumoral CD8-positive T cells act as a stage-independent predictor of MCC-specific survival in that the patients in the highest level of the cells showed 100% survival and those in a minimum or no level of those cells showed 60% survival[36,37]. As a part of the tumor microenvironment, desmoplasia refers to a status of highly fibrotic-rich cancer-associated fibroblasts

(CAFs) and the extracellular matrix around the tumor. It can cause immunosuppression through multiple mechanisms including presenting a physical barrier to infiltrating T lymphocytes, mechanical compression of tumor vessels, and production of several suppressive cytokines[38-40]. Desmoplasia has been used as a hallmark of treatment-nonresponsive tumors, including metastatic breast cancers and pancreatic ductal adenocarcinoma[15,41]. Accurately evaluating tumor-infiltrating lymphocytes and desmoplasia requires additional immunohistochemical staining which has to be analyzed with a specialty.

Desmoplasia in MCC has not been studied yet, so we used a concept of bMF as a method to assess desmoplastic reaction in MCC. The concept of bMF was first suggested by the researchers of oral squamous cell carcinoma who thought that proximity between tumor cells and the tumor microenvironment was important for the biological function of the tumor microenvironment because the interaction between tumor cells and the tumor microenvironment depends on cell-cell interaction and various humoral factors[18]. bMF can be easily assessed on very narrow restricted areas of the tumor invasive margin via H&E staining and it can be a reliable method because it does not require additional staining or analysis. In the previous study, bMF was identified as a novel and independent poor prognostic marker of oral squamous cell carcinoma[18]. In this study, we identified bMF as a novel histomorphological prognostic marker of MCC in progression-free survival, but not in overall survival. In the relationship of other clinicopathological factors, bMF was associated with nodal involvement at last follow-up which is interpreted as that borderline fibrosis of tumor may be directly associated with the nodal invasion. However, further research is needed to clarify the prognostic significance of bMF in prognosis of MCC.

bMF can be an independent factor in the assessment of prognosis.

The definition of bMF is similar to that of desmoplasia which can be assessed by various immunohistochemical staining such as α-SMA. However, bMF may affect tumor growth through other mechanisms that are different from mechanisms already known in desmoplasia because bMF reflects only the proximity of tumor margin. We observed less tumor-infiltrating lymphocytes infiltration in the bMF-positive cases than in the bMF-negative cases, which was consistent with a previous study[18]. bMF may be associated with tumor invasion and suppression of immunity to the tumor in many ways such that borderline fibrosis acts as physical barriers to inhibit the lymphocytes spread. A previous study suggested that bMF, potentially composed of CAFs and collagen fibers, enhances tumor growth through direct secretion of growth factors or indirect suppression of tumor immunity[18]. Further basic research regarding molecular study will be needed to clarify such mechanisms.

Our data present the importance of desmoplasia evaluation by bMF in MCC for the first time. Our result suggests that evaluation bMF could be a reliable biomarker for MCC. bMF can be an easier assessment method of the tumor microenvironment which could be applied by physicians in clinics.

V. CONCLUSION

In conclusion, we established a Korean patient cohort with MCC through this study, found that MCC yielded high local recurrence and metastases rates among Koreans, and showed poor prognosis.

The tumor microenvironment including desmoplasia has not been studied well in MCC. We identified that bMF was a possible poor prognostic marker of progression-free survival in patients with MCC. Further studies are required to clarify bMF as a reliable independent prognostic factor of MCC.

Table S1. Details of 34 patients with Merkel cell carcinoma.

Patient no.	Gender /age	Tumor location	Stage at diagnosis	Progression (recurrence or metastasis)	Death	Treatment (primary tumor surgery / regional lymph node dissection / radiotherapy / chemotherapy / immunotherapy) *	bMF
1	F/72	Lt. Zygoma	IIIB	yes	no	+ / + / + / - / -	N/A
2	F/80	Rt. Shin	IA	yes	no	+ / + / + / + / -	+
3	F/68	Rt. Upper arm	IIA	yes	no	+ / + / + / + / -	N/A
4	M/76	Lt. Elbow mass	IIB	yes	no	+ / - / + / - / -	+
5	M/73	Abdomen	IB	yes	yes	+ / - / + / + / -	N/A
6	M/90	Rt. Upper eyelid	IB	yes	yes	+ / - / - / - / -	N/A
7	F/77	Lt. Ear lobe	IIA	yes	yes	+ / + / + / - / -	+
8	F/62	Rt. Eyebrow	IIIB	yes	yes	+ / + / + / + / +	-
9	F/78	Rt. Cheek	IA	yes	yes	+ / + / + / + / +	+
10	M/85	Lt. Forearm	IIIA	yes	yes	+ / - / + / - / -	N/A
11	F/67	Lt. buttock	IIA	yes	yes	+ / + / + / + / -	N/A
12	F/42	Lt. Thigh	IA	yes	no	+ / + / - / - / -	N/A
13	M/74	Lt. Lower abdomen	IIB	yes	yes	+ / - / + / + / -	+
14	M/71	Rt. Hip	IIIB	yes	yes	- / - / + / + / -	N/A
15	F/63	Lt. lower eyelid	IA	no	no	+ / + / + / - / -	N/A
16	F/77	Rt. Forearm	IA	no	no	+ / - / + / + / +	-
17	F/66	Lt. Zygomatic area	IB	no	no	+ / - / - / - / -	N/A
18	M/80	Lower lip	IIIA	yes	yes	+ / - / + / + / +	+
19	F/58	Rt. Posterior thigh	II	no	no	+ / - /- / - / -	N/A
20	F/73	Rt. Cheek	IIIB	no	no	+ / + / + / - / -	N/A
21	F/46	Rt. Zygomatic area	IA	no	no	+ / + / + / - / -	-
22	M/85	Lt. Mandibular area	IIIA	yes	no	+ / - / + / - / -	+
23	F/82	Rt. Cheek	IA	no	no	+ / + / + / - / -	-
24	F/71	Lt. Flank	IIIA	no	yes	+ / + / + / + / -	N/A
25	M/83	Rt. Lower back	IB	no	no	+ / - / - / - / -	N/A
26	F/32	Lt. External auditory canal	IB	no	no	+ / - / - / - / -	+

27	F/60	Lt. Axilla	IIIB	no	no	+ / + / + / - / -	-
28	M/81	Lt. Lower eyelid(conjunctiva)	IIB	yes	no	+ / - / - / - / -	-
29	M/83	Lt. Upper eyelid	IB	no	no	+ / - / - / - / -	+
30	M/74	Lt. Upper eyelid	IB	no	no	+ / - / - / - / -	-
31	F/76	Lt. Cheek	IB	no	no	+ / - / + / - / -	-
32	M/76	Rt. Cheek	IIA	no	no	+ / - / - / - / -	-
33	F/58	Rt. Buttock	IA	yes	no	+ / - / - / - / -	+
34	F/71	Lt. Elbow	IV	no	no	+ / - / + / + / +	+

bMF, borderline microenvironment fibrosis; Rt, right; Lt, left
*Including treatment at the final stage of Merkel cell carcinoma

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ABSTRACT (IN KOREAN)

한국인 머켈세포암 환자의 코호트 구축 및 환자예후와 미세환경경계섬유화의 상관성 파악

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유대산

머켈세포암은 매우 드문 피부암 중 하나로, 피부색 또는 붉은색의 피하결절로 나타난다. 재발 및 전이가 흔하고 예후가 좋지 않으며, 발생빈도가 매우 낮아 지속적인 연구가 쉽지 않은 암종이다. 한국인에서 발생하는 머켈세포암은 아직 자료가 충분하지 않으며 잘 알려져 있지 않다. 종양미세환경은 종양주변의 섬유모세포, 섬유화, 면역세포, 혈관 그리고 세포외기질을 의미하며 최근 주목을 받고 있다. 하지만 종양미세환경의 정확한 평가를 위해서는 여러가지 특수염색을 시행해야하며 전문적인 해석이 필요하여 연구가 어렵고, 머켈세포암에서 아직 종양미세환경과 환자예후와의 상관성에 대해 연구가 부족하다.

본 연구는 한국인 머켈세포암의 코호트를 구축하고, 종양미세환경 중 하나인 미세환경경계섬유화를 조직학적 기본염색으로 평가하여 환자예후와의 상관성을 보고자 하였다.

본 연구는 2007 년부터 2020 년 사이에 신촌 및 강남세브란스병원에서 머켈세포암으로 치료받은 34 명의 환자를 대상으로 후향적 임상분석연구를 진행하였다. 총 34 명의 환자에 대한 코호트를 구축하였고, 조직학적 분석이 가능한 20 개의 조직슬라이드를 통하여 미세환경경계섬유화와 예후 사이에 상관성을 분석하고, 새로운 예후 인자로 사용될 수 있는지 평가하였다.

전체 34 명에서 나이의 중앙값은 74 세(32-97 세) 였으며, 남자 및 여자 환자는 각각 13 명, 21 명이었다. 머켈세포암의 호발부위는 두경부(52.9%)였으며, 이어서 하지(17.6%), 상지(14.7%) 그리고 몸통(11.8%)이 호발부위였다. 총 33 명의 원발암 병변을 수술받은 환자들 중 17 명에서 재발하였고(국소재발 13 명, 원발전이 4 명), 재발까지의 평균기간은 9.1 개월이었다. 총 34 명의 환자에서, 관찰기간의 중앙값은 18 개월이었으며(1.1-74.1 개월) 관찰기간 동안 11 명의 환자가 사망하였다.

예후인자를 파악하기 위한 단변량 분석에서, 남성, 진단시 나이, 그리고 폐질환 동반이 전체생존률에 영향을 미치는 예후인자로 확인되었고, 남성 그리고 폐질환 동반은 무진행생존률의 예후인자로도 확인되었다. 미세환경경계섬유화는 무진행생존률과 연관성이 있는 예후인자로서 관찰되었다(p=0.024).

한국인에서 발생한 머켈세포암은 이미 알려진 머켈세포암의 특성과 같이 공격적인 특성을 가지고 있는 것을 확인하였다. 종양미세환경 중 하나인 미세환경경계섬유화는 머켈세포암의 새로운 예후인자로서 사용될 가능성을 보였으며, 이에 미세환경경계섬유화를 머켈세포암의 재발 예측인자 및 예후인자로서 사용될 가능성을 확인하여 제시하는 바이다.

핵심되는 말: 머켈세포암, 한국인, 예후인자, 종양미세환경, 미세환경경계섬유화