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Cancer-testis antigens as biomarkers for Merkel cell carcinoma: Pitfalls and opportunities

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Abstract

Background: The prognosis and treatment options for metastatic Merkel cell carcinoma (MCC) are poor. The immune-privileged status of cancer-testis (CT) antigens imparts tumor specificity, making them ideal candidates for targeted immunotherapy. We investigate the usefulness of the CT antigens SPA17 (sperm protein-17 [SP-17]), IGF2BP3 (insulin-like growth factor-II mRNA-binding protein 3 [IMP-3]), and transmembrane protein with epidermal growth factor (EGF)-like and two follistatin-like domains 1 (TMEFF1) as potential MCC biomarkers and evaluate their possible utility in immunotherapy and molecularly targeted image-guided treatment.

Methods: The CT antigens SP-17, IMP-3, and TMEFF1 were selected using transcriptome profiling to identify CT antigens expressed in MCC tumors. Antibodies directed against these CT antigens were stained. Twelve normal skin tissue samples were used as a control. The average percentage of positive cells in each tumor was computed.

Results: Twelve of 14 (86%) MCC cases showed crisp nuclear staining for SP-17, with 2.06% of cells staining positive. IMP-3 showed crisp, perinuclear staining in all 14 MCC cases, with 52.93% MCC cells staining positive. TMEFF1 showed perinuclear staining in all 14 MCC cases, with 96.51% of tumor cells staining positive.

Conclusions: CT antigens were found to be expressed in both MCC and some control tissues. SP-17 was the most specific yet the least sensitive. IMP-3 and TMEFF1 were both sensitive but not specific. CT antigens may represent valuable treatment targets in MCC.

Keywords

immunohistochemistry; IMP-3; Merkel cell carcinoma; SP-17; TMEFF1

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1 | INTRODUCTION

Cancer-testis (CT) antigens are encoded by genes that, in homeostatic conditions, are primarily expressed in germ cells within the testis and fetal ovaries, along with trophoblasts of the placenta.[1] Epigenetic regulation, including both altered CT antigen promoter methylation states and histone modifications, play a primary role in activating CT antigen expression in these normal tissues, as well as the abnormal reactivation of expression seen in cancer cells.[1,2] The specific function of most reactivated CT antigens remains poorly defined. Nevertheless, recent evidence suggests that they have direct utility in the tumor cell regulatory environment[3] and support tumorigenesis[2,3] by altering cellular processes, such as chromosomal separation and cell signaling.[1] For example, functional analysis of the CT antigen PRAME can directly repress retinoic acid receptor signaling, inhibiting retinoic acid-driven growth arrest and apoptosis.[4] Emerging evidence also supports that some CT antigens can foster epithelial-mesenchymal transition and the proliferation of cancer stem-like cells, which also promotes tumorigenesis and metastasis.[5]

Some CT antigens, including NY-ESO-1, are immunogenic and elicit humoral and cellular immune responses.[1,2] Genome-wide expression analysis of the CT genes has permitted their classification into two expression profiles: testis-restricted and testis/brain-restricted.[6] The blood-brain barrier and blood-testis barrier, along with the absence of major histocompatibility complex (MHC) class I molecules on the surface of developing sperm, conveys an immune-privileged status in the brain and testis, thus giving CT antigens tumor specificity[2,3] and making them ideal candidates for targeted immunotherapy.[1,2,6] Indeed, a number of cancer vaccine clinical trials are ongoing.[7] Although some studies seem promising,[3,8] a number of possible limitations in using CT antigens in targeted immunotherapy are yet to be overcome.[2,9,10]

Merkel cell carcinoma (MCC) is a neuroendocrine tumor of the skin that behaves aggressively,[11] with a 5-year survival rate of only 18% in metastatic disease.[12] MCC is classically found in elderly Caucasians in the head and neck region, with a predilection for sun-exposed areas, although it can arise in non-sun-exposed areas. The incidence of MCC is greatly increased in immunocompromised patients, with a 10- to 13-fold increase in solid organ transplant patients and those infected with human immunodeficiency virus (HIV)[11]; nevertheless, the majority of patients with MCC lack fundamental immune dysfunction.[13] Merkel cell polyomavirus (MCPyV) is now supported as an etiologic agent in the pathogenesis of a sub-set of MCC patients and can be detected in up tumors.[14] Polyomavirus is postulated to play a role in the carcinogenesis process in association with other etiologic and promoting factors including ultraviolet radiation and genetic predisposition.[11] If MCC is found to be confined to the primary cutaneous site, it can be cured by wide surgical excision. However, the prognosis for metastatic disease is poor, with reported median survival time after metastatic development to be roughly 9.6 months.[13] Therefore, developing new therapies may help to improve the patient's prognosis.

In this study, transcriptome profiling was used to identify CT antigens that are expressed in MCC tumors, but not in normal skin samples. We then investigated the immunohistochemical staining patterns of antibodies directed against three CT antigens

whose microarray messenger RNA (mRNA) profile showed a relatively high expression in selected MCC cell lines and excised Merkel tumors compared with normal human skin tissue. These CT antigens include sperm protein-17 (SP-17), insulin-like growth factor-II mRNA binding protein 3 (IMP-3), and transmembrane protein with EGF-like and two follistatin-like domains 1 (TMEFF1). In doing so, we examined the usefulness of these CT antigens as potential MCC biomarkers and evaluated their possible utility in immunotherapy and molecularly targeted image-guided diagnosis and/or treatment.

2 | MATERIALS AND METHODS

2.1 | Candidate antigen selection

According to the microarray profile of Merkel cell lines, MCC excised tumors and normal human skin tissue, three CT antigens were selected: SPA17 (SP-17), IGF2BP3 (IMP-3), and TMEFF1. These candidates were selected based on commercial availability and their positive expression in the four Merkel cell lines, 23 excised MCC samples (MT01-MT24) as compared with little to no expression in 12 normal human skin tissue samples (Figure 1).

2.2 | Case Selection

Fourteen archived human MCC cases were obtained from our dermatopathology lab files. In addition to hematoxylin and eosin (H&E), the diagnosis of MCC was further confirmed by positive perinuclear dot-like staining for cytokeratin 20. These MCC cases were used to assess the expressivity of the above-mentioned CT antigens using immunohistochemical staining.

Normal human skin tissue was used as a control and to assess expressivity of these biomarkers on normal background skin. Furthermore, a minimum of 10 tissue samples of each of vital organs including lung, kidney, and colon were also examined to evaluate expression should these biomarkers be translated in vivo as potential diagnostic and/or therapeutic targets.

2.3 | Immunohistochemistry

Specimens were formalin-fixed and paraffin-embedded. Sections were cut at 4.5 μm and deparaffinized using standard protocols. Antigen retrieval for SP-17 was performed using a high pH (pH 9) Tris-EDTA buffer. Retrieval for insulin-like growth factor-II mRNA-binding protein 3 (IMP-3), and TMEFF1) was performed using a low pH (pH 6) citrate buffer (Dako Corporation, Carpinteria, California). Endogenous peroxidase was blocked with hydrogen peroxide.

After antigen retrieval, the sections were incubated with each of the primary antibodies at 1:100 dilutions. Incubation with monoclonal rabbit anti-SP17 antibody (product # ab181079; Abcam, Cambridge, Massachusetts), and mouse anti-IGF2BP3-IMP3 antibody (clone 69.1; product # M362629–2; Dako Corporation) each lasted 60 minutes. Incubation with anti-TMEFF1 antibody (Santa Cruz Biotechnology, Dallas, Texas) lasted 20 minutes. The antibody staining was then visualized using the Dako EnVision system (Dako Corporation).

2.4 | Case review

Sections were reviewed by two of the authors independently (DM and NN). Specimens were initially examined at low-power magnification for homogeneity of staining and to identify areas of necrosis that could skew interpretation. All sections displayed homogeneous staining without necrosis, and therefore the center of the tumor was chosen for consistency and reproducibility. Using a fixed grid at 40× magnification, each author counted the number of positive and negative cells at two different locations in the center of the tumors for each section. The percentage of positive cells for each location was calculated and an average case percentage computed. The results are recorded in Table 1.

3 | RESULTS

The microarray profile of four Merkel cell lines, 23 excised MCC samples (MT01-MT24), and 12 normal human skin tissue samples showed notable positive expression of three CT antigen as follows; SPA17 (SP-17), IGF2BP3 (IMP-3), TMEFF1, when compared to 12 normal human skin tissue samples, which served as control (Figure 1).

3.1 | SP-17

In total, 12 of 14 (86%) MCC cases showed some staining for SP-17 (Table 1). Cells stained with anti-SP17 antibody showed crisp nuclear staining. Surrounding epidermis for these cases remained negative. Examination of the 14 cases containing MCCs showed that, on average, 2.06% of cells stained positive (Figure 2A). In contrast, samples containing normal tissue of lung, kidney, inflammatory cells, colon, and normal epidermis were completely negative (Figure 2A,B). The mRNA expression of SP-17 showed similarly low levels of expressivity amongst MCC cell lines, MCC tissue samples, and normal skin (Figure 1).

3.2 | IMP-3

MCC tumors showed variable amount of staining with anti-IGF2BP3-IMP3 antibody in 100% of samples. Positive cells showed crisp perinuclear staining. On average, 52.93% (range 3.66%-96.44%) of MCC cells stained positive (Figure 3A). Similarly to SP-17, all samples containing normal tissues such as kidney, inflammatory cells, colon, and normal epidermis were negative (Figure 2B).

3.3 | TMEFF1

All (100%) MCC cases stained abundantly with anti-TMEFF1 antibody. Tumor cells showed perinuclear staining and there was substantial background staining in inflammatory cells as well. On average, 96.51% of MCC cells stained positive (Figure 4A). Strong positive staining was also present in kidney (94.13%). As shown in Figure 4A, and B, both lung and colon normal tissues were negative, again despite the presence of extensive background staining in inflammatory cells.

Although lung and colon do not show positive staining and therefore represent possible safe and therapeutic targets, the staining of TMEFF in normal kidney tissue suggest the need for further in vivo biodistribution and bioavailability studies to better assess potential adverse event of such treatment on healthy kidney tissue.

4 | DISCUSSION

The poor outcomes in patients with metastatic MCC calls for more efficacious treatment options in the new horizon of precision medicine. Wide local excision is the primary treatment for localized primary MCC requiring negative margins of 1 to 2 cm.[15] While there are mixed recommendations regarding postsurgical adjuvant radiation, this therapy remains the current guideline as the standard of care with several studies demonstrating reduced recurrence and improved survival.[15] In patients who are not surgical candidates, radiotherapy alone or combined with systemic therapy has shown to extend life to some degree. Systemic therapies are considered in metastatic disease when radiation is no longer applicable. More targeted strategies are being explored with variable outcomes.[13] To name a few, viral oncoprotein targeting, autologous T-cell immunotherapy, somatostatin analogues, signal transduction interference, and immune checkpoint inhibition including ipilimumab, [13,16] which is currently in Phase II clinical trials in patients with metastatic MCC.[17]

In this study, we have tested the expression of CT antigens in excised human MCC tumors to assess their potential application as targeting diagnostic or therapeutic biomarkers. Transcriptome profiling identified enhanced expression of CT antigens SP-17, IMP-3, and TMEFF1 in MCC tumor samples relative to normal skin. Using immunohistochemical staining, we assessed the expression of these CT antigens in a separate set of MCC tumors and a panel of other human tissues.

In our study, TMEFF1 had consistently high levels of expression in MCC, but also showed full expression in kidney and inflammatory cells surrounding the tumor. Therefore, follow-up in vivo studies using MCC animal models are warranted to better evaluate TMEFF1 as a potential theranostic biomarker and assess the possible effects of targeting such biomarkers on kidney and other healthy tissues. In theory, given the short half-life of inflammatory cells, we hope these would not be a limiting factor. Moreover, in vivo studies can provide biodistribution and toxicity information.

IMP-3 was positively expressed in all MCC samples according to the value color scale in Figure 1. Fifty-seven percent of samples showed over 50% expressivity. As seen in Figure 1, there was differential expression between MCC tissue samples and normal skin samples, which showed no detectable expression of IMP-3. Accordingly, all 12 Merkel cell lines and 21 out of 23 Merkel tissue samples showed various degrees of expression of IMP3 ranging from moderate to high levels of expression. Most importantly, there was no expression in normal healthy tissues, such as colon, kidney, inflammatory cells, and a background of normal epidermis, making it a promising potential therapeutic targeting biomarker.

Consistent with our findings, previous investigators have described a scattered, low-frequency expression of SP-17, with overall 12% positive expression across many solid tumor types and only 2.06% expression in MCC, also confirmed by our results.[18] Accordingly, SP-17 might be good in terms of specificity, but the low frequency of SP-17 positive cells in MCC and its nuclear position within cells may limit its diagnostic or therapeutic potential in vivo.

In conclusion, our study aimed to explore potential targets for a disease with poor prognosis and limited treatment options. In doing so, we aimed to identify biomarkers which would have low to absent expression in normal healthy tissues, yet high expression in MCC cell lines, to maximize targeted therapy and avoid collateral damage. Further studies are warranted to explore the utility of these markers in future in vivo application.

REFERENCES

1. Simpson AJ, Caballero OL, Jungbluth A, Chen YT, Old LJ. Cancer/ testis antigens, gametogenesis and cancer. Nat Rev Cancer. 2005;5(8): 615–625. [PubMed: 16034368]
2. Fratta E, Coral S, Covre A, et al. The biology of cancer testis antigens: putative function, regulation and therapeutic potential. Mol Oncol. 2011;5(2):164–182. [PubMed: 21376678]
3. Whitehurst AW. Cause and consequence of cancer/testis antigen activation in cancer. Annu Rev Pharmacol Toxicol. 2014;54: 251–272. [PubMed: 24160706]
4. Epping MT, Wang L, Edel MJ, Carlee L, Hernandez M, Bernards R. The human tumor antigen PRAME is a dominant repressor of retinoic acid receptor signaling. Cell. 2005;122(6):835–847. [PubMed: 16179254]
5. Yang P, Huo Z, Liao H, Zhou Q. Cancer/testis antigens trigger epithelial-mesenchymal transition and genesis of cancer stem-like cells. Curr Pharm Des. 2015;21(10):1292–1300. [PubMed: 25506890]
6. Hofmann O, Caballero OL, Stevenson BJ, et al. Genome-wide analysis of cancer/testis gene expression. Proc Natl Acad Sci USA. 2008;105 (51):20422–20427. [PubMed: 19088187]
7. Tagliamonte M, Petrizzo A, Tornesello ML, Buonaguro FM, Buonaguro L. Antigen-specific vaccines for cancer treatment. Hum Vaccin Immunother. 2014;10(11):3332–3346. [PubMed: 25483639]
8. Jager E, Gnjatic S, Nagata Y, et al. Induction of primary NY-ESO-1 immunity: CD8+ T lymphocyte and antibody responses in peptide-vaccinated patients with NY-ESO-1+ cancers. Proc Natl Acad Sci USA. 2000;97(22):12198–12203. [PubMed: 11027314]
9. Grizzi F, Mirandola L, Qehajaj D, Cobos E, Figueroa JA, Chiriva-Internati M. Cancer-testis antigens and immunotherapy in the light of cancer complexity. Int Rev Immunol. 2015;34(2): 143–153. [PubMed: 25901859]
10. Ghafouri-Fard S. Expression of cancer-testis antigens in stem cells: is it a potential drawback or an advantage in cancer immunotherapy. Asian Pac J Cancer Prev. 2015;16(7):3079–3081. [PubMed: 25854409]
11. Hughes MP, Hardee ME, Cornelius LA, Hutchins LF, Becker JC, Gao L. Merkel cell carcinoma: epidemiology, target, and therapy. Curr Dermatol Rep. 2014;3:46–53. [PubMed: 24587977]
12. Lemos BD, Storer BE, Iyer JG, et al. Pathologic nodal evaluation improves prognostic accuracy in Merkel cell carcinoma: analysis of 5823 cases as the basis of the first consensus staging system. J Am Acad Dermatol. 2010;63(5):751–761. [PubMed: 20646783]
13. Miller NJ, Bhatia S, Parvathaneni U, Iyer JG, Nghiem P. Emerging and mechanism-based therapies for recurrent or metastatic Merkel cell carcinoma. Curr Treat Options Oncol. 2013;14(2):249–263. [PubMed: 23436166]
14. Spurgeon ME, Lambert PF. Merkel cell polyomavirus: a newly discovered human virus with oncogenic potential. Virology. 2013;435(1):118–130. [PubMed: 23217622]
15. Miles BA, Goldenberg D. Merkel cell carcinoma: do you know your guidelines? Head Neck. 2016;38(5):647–652. [PubMed: 26716756]
16. Brummer GC, Bowen AR, Bowen GM. Merkel cell carcinoma: current issues regarding diagnosis, management, and emerging treatment strategies. Am J Clin Dermatol. 2016;17(1):49–62. [PubMed: 26596990]
17. Mauzo SH, Ferrarotto R, Bell D, et al. Molecular characteristics and potential therapeutic targets in Merkel cell carcinoma. J Clin Pathol. 2016;69(5):382–390. [PubMed: 26818033]
18. Gjerstorff MF, Ditzel HJ. Limited SP17 expression within tumors diminishes its therapeutic potential. Tissue Antigens. 2012;80(6): 523–527. [PubMed: 23137323]

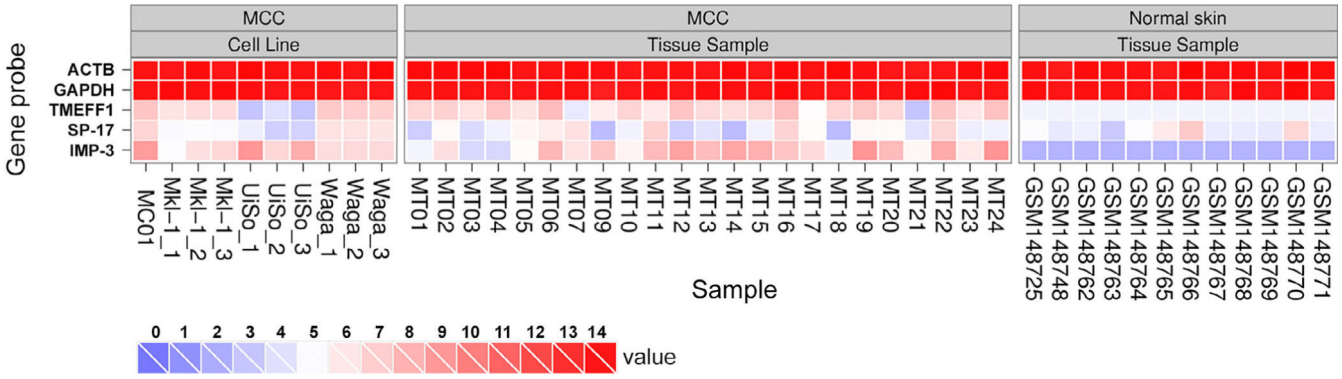

FIGURE 1.

Cancer-testis antigen messenger RNA expression profiles in excised Merkel cell carcinoma cell lines, Merkel cell carcinoma tumors, and normal human skin tissue

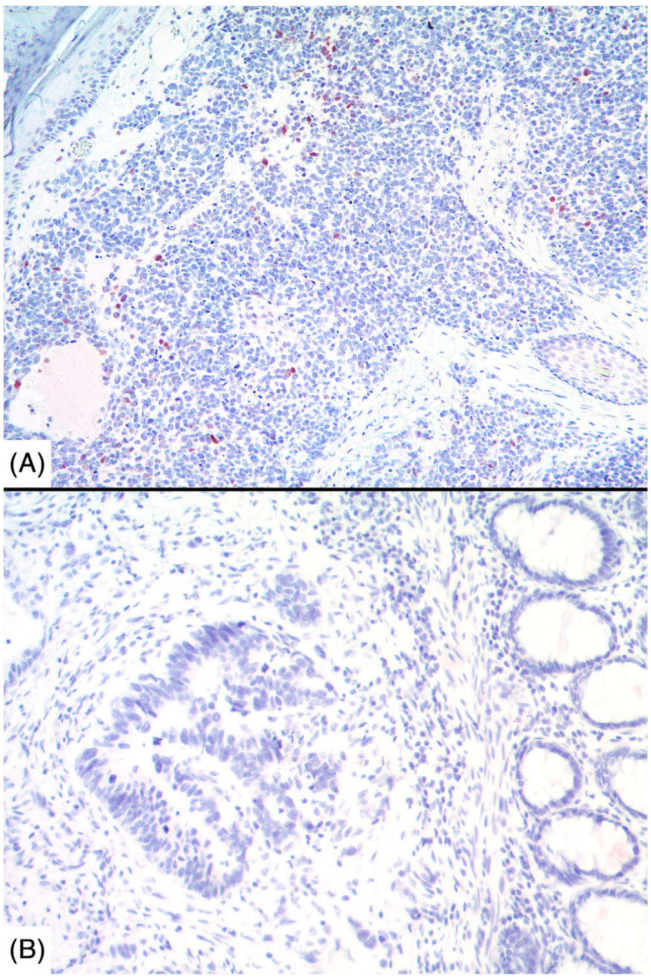

FIGURE 2.

Staining pattern for anti-SP-17 in (A) Merkel cell carcinoma (100×) and (B) normal tissue of the colon (right) and adenocarcinoma of the colon (left) (100×)

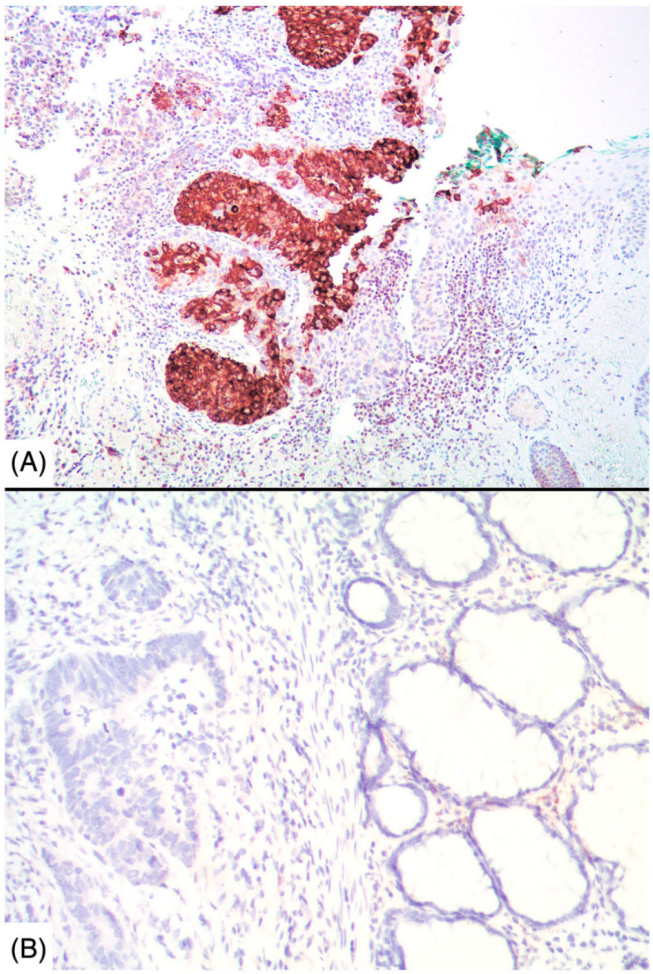

FIGURE 3.

Staining pattern for anti-IMP-3 in (A) Merkel cell carcinoma (100×) and (B) normal tissue of the colon (right) and adenocarcinoma of the colon (left) (100×)

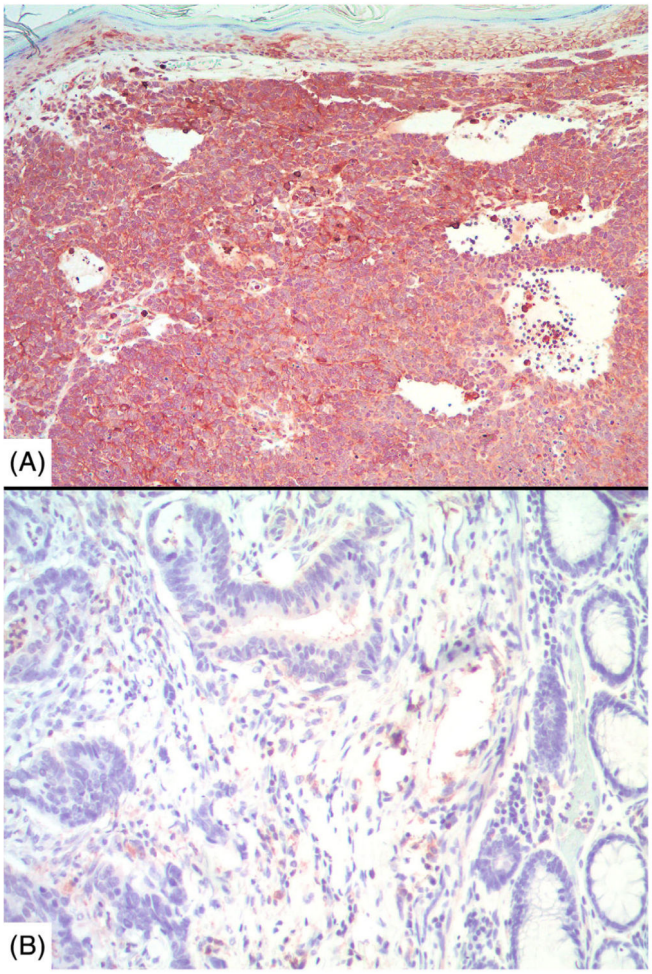

FIGURE 4.

Staining pattern for anti-transmembrane protein with EGF-like and two follistatin-like domains 1 (TMEFF1) in (A) Merkel cell carcinoma (100×) and (B) normal tissue of the colon (right) and adenocarcinoma of the colon (left) (100×)

TABLE 1

Immunohistochemical results

	Case #	SP-17 (%)	IMP-3 (%)	TMEFF1 (%)
Merkel cell carcinoma	1	1.31	83.06	96.58
	2	0.11	74.13	98.82
	3	3.70	92.64	98.42
	4	4.17	4.90	99.30
	5	0.20	96.44	97.39
	6	0.68	17.21	97.64
	7	0	10.25	93.75
	8	0.41	3.66	92.87
	9	3.95	4.45	98.59
	10	4.47	64.34	99.38
	11	0.24	92.92	94.60
	12	1.27	62.23	97.02
	13	N/A	86.78	93.78
	14	6.21	48.02	93.00
Colon		0	0	0
Epidermis		0	0	0
Inflammatory cells		0	0	100
Kidney		0	0	94.13
Lung		0	0	0

Abbreviations: IMP-3, insulin-like growth factor-II mRNA-binding protein 3; SP-17, sperm protein-17; TMEFF1 transmembrane protein with EGF-like and two follistatin-like domains 1.