Oral Oncology 147 (2023) 106602

Contents lists available at ScienceDirect

Oral Oncology

journal homepage: www.elsevier.com/locate/oraloncology

A rare case of Merkel cell carcinoma on the craniofacial region and characterization of its aneuploid CD31$^-$ CTCs and CD31$^+$ CTECs expressing EpCAM or Ki-67

ARTICLE INFO

Keywords
Merkel cell carcinoma
MCC
Tumor in the head and neck
Circulating tumor cells

Dear editor,

Merkel cell carcinoma (MCC) is a rare epithelial origin of cutaneous neuroendocrine carcinoma [1] with asymptomatic erythematous/violaceous nodules or plaques on skin exposed to the sun [2]. Here, a comprehensive cellular and molecular diagnosis of a rare case of Merkel cell carcinoma is reported.

Report of a case

An 89-year-old woman presented with a 4-month history of a violaceous, painless, rapidly expanding cutaneous nodule on the right side of her face. The nodule was circular in shape and about 2x2.5cm in size, the mobility was acceptable (Fig. 1 A-a). This case of MCC presented as *intermediate type* histopathologically. As shown in Fig. 1, large nests composed of basophilic cells with little round nuclei were observed in the deep dermis, and mitoses were observed as well (Fig. 1A-b). Immunohistochemical staining revealed positive staining of CK20, characterized by paranuclear dot-like pattern (Fig. 1 A-c). Neuroendocrine markers Syn and CgA, neurofilament, and epithelial marker EMA are all positive (Fig. 1 A-d-f). Co-expression of cytokeratins and neurofilament is a unique characteristic of MCC [3]. The patient was diagnosed with MCC based on the clinical presentation, histological and immunohistochemical results.

Aneuploid CD31$^-$ circulating tumor cells (CTCs) and CD31$^+$ circulating tumor endothelial cells (CTECs) in this MCC patient were co-detected by subtraction enrichment integrated with immunostaining-fluorescence *in situ* hybridization (SE-iFISH) [4]. As illustrated in Fig. 1 B and C, 25 CTCs and 10 CTECs were detected in six ml of patient's blood. Among 25 CD31$^-$ CTCs, three were EpCAM$^+$ (3/25 = 12 %) and one was Ki-67$^+$ (1/25 = 4 %). Sixty percent of CTCs (15 out of 25) were multiploid (≥pentasomy 8) and 80 % of the detected CTCs (20 out of 25) were large cells (>5 μm). Similarly, only one CTEC exhibits positive expression of EpCAM (1/10 = 10 %), 70 % of CD31$^+$ CTECs were large cells (7 out of 10), and 80 % of the detected CTECs were multiploid (8 out of 10). The remaining cells, including 84 % CTCs (21 out of 25) and 90 % CTECs (9 out of 10), were null cells with neither EpCAM nor Ki-67 expressed.

Discussion

Merkel cell carcinoma (MCC) is a rare and aggressive malignant tumor of the skin, with steadily increasing incidence reports worldwide [5]. Highly aggressive MCC has very poor prognosis and extremely low survival rate, with the epidemiologic data showing that nearly 40 % mortality rate from new MCC cases of the European Union per year (1000/2500) [6].

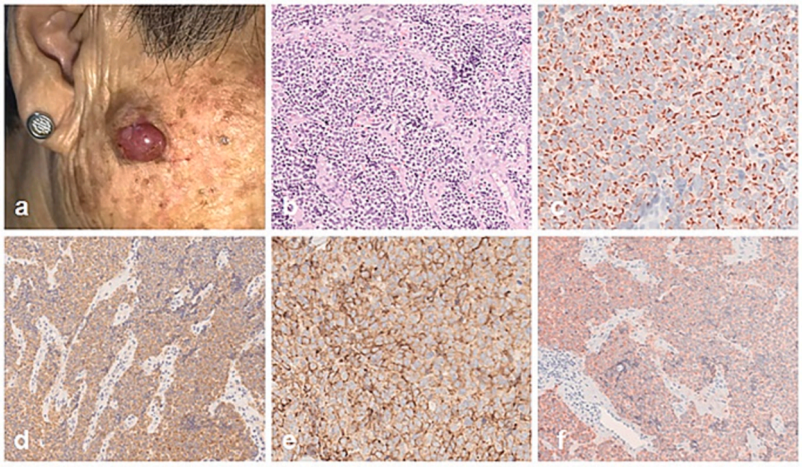
Circulating tumor cells (CTCs) are considered the real-time liquid biopsy for cancer patients [7]. The majority of endothelial cells in tumor vasculatures are tumor endothelial cells (TECs), some of which shed into peripheral blood to turn into circulating TECs (CTECs) [8]. CTCs and CTECs constitute a pair of circulating tumor biomarkers in cancer patients which may offer real-time insights into the course, prognosis, and effectiveness of cancer treatment [7,9].

Blom et al. reported that CTCs conventionally detected by cytokeratin staining were closely related to MCC progression and patients' survival [10]. Compared to previous studies on MCC CTCs, we performed SE-iFISH to karyotypically and phenotypically co-detect CTCs as well as CTECs for the first time in the MCC patient. The obtained results demonstrated that the patient presented numerous aneuploid CD31$^-$ CTCs and CD31$^+$ CTECs. Furthermore, the expression of EpCAM and Ki-67 on CTCs and CTECs in this patient was also examined. EpCAM participates in epithelial-to-mesenchymal transition (EMT) and cancer metastasis [11,12]. EpCAM$^+$ aneuploid CTCs could be utilized to predict poor prognosis and tumor recurrence in malignancies, such as hepatocellular carcinoma and breast cancer [13,14]. Abundant expression of Ki-67 was found to be highly associated with cancer cell proliferation, growth, metastasis, and the tumor's clinical stage [15,16]. Co-examination of EpCAM and Ki-67 on CTCs and CTECs will be significant for investigating the clinical value of MCC CTCs and CTECs regarding tumor proliferation and metastasis. As shown in Fig. 1C, 12 % (3 out of 25) of detected CTCs and 10 % of CTECs (1 out of 10) in the presented case were EpCAM$^+$, while 4 % of CTCs (1 out of 25) were Ki-67$^+$. Obtained results suggested that the patient was likely exhibiting an active distant tumor metastasis and poor prognosis.

Longitudinal detection of diverse subtypes of CTCs and CTECs

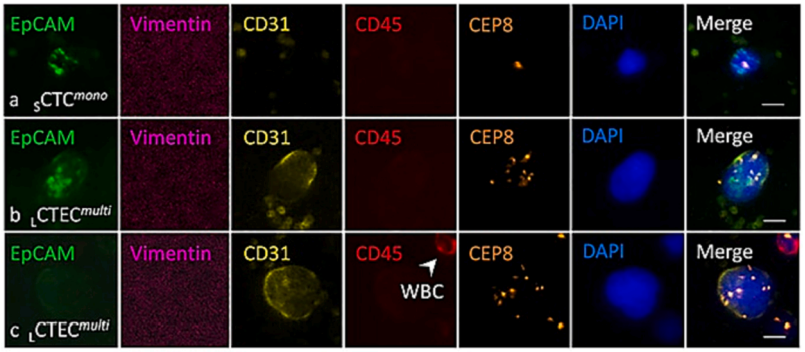
https://doi.org/10.1016/j.oraloncology.2023.106602
Received 17 October 2023; Accepted 19 October 2023

A Representative images of MCC

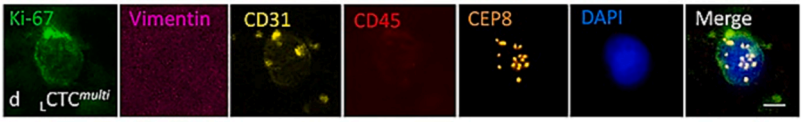


B Representative images of MCC aneuploid CTCs and CTECs

EpCAM/Vimentin-i•FISH



Ki-67/Vimentin-i•FISH



C Quantitative and molecular characterization of aneuploid CTCs and CTECs

Classification	Cell size	Tumor markers	Ploidy: Haploid	Near-diploid	Tri-ploid	Tetra-ploid	Multi-ploid	Sum2 (size)	% (Sum2/Tot)	Total	Sum: Ep+	Ki67+	Null
CD31⁻CTC	Large	EpCAM+	0	0	0	0	0						
		EpCAM-	0	0	1	2	7	20 (large)	80% (large)				
		Ki67+	0	0	0	0	1						
		Ki67-	0	0	1	1	7			25			
	Small	EpCAM+	1	2	0	0	0				3	1	21
		EpCAM-	0	0	0	1	0	5 (small)	20% (small)				
		Ki67+	0	0	0		0						
		Ki67-	0	0	0	1	0						
		Sum1	1	2	2	5	15						
		% (Sum1/Tot)	4%	8%	8%	20%	60%				12%	4%	84%
CD31⁺CTEC	Large	EpCAM+	0	0	0	0	1						
		EpCAM-	0	0	0	0	4	7 (large)	70% (large)				
		Ki67+	0	0	0	0	0						
		Ki67-	0	0	0	0	2			10			
	Small	EpCAM+	0	0	0	0	0				1	0	9
		EpCAM-	0	0	0	1	0	3 (small)	30% (small)				
		Ki67+	0	0	0	0	0						
		Ki67-	0	0	1	0	1						
		Sum1	0	0	1	1	8						
		% (Sum1/Tot)	0	0	10%	10%	80%				10%	0	90%

(caption on next page)

Fig. 1. Clinical and histopathological diagnosis of MCC and comprehensive detcction of MCC CTCs and CTECs. **(A)** Representative images of MCC. (A-a) An isolated violaceous-colored nodule, about 3 × 4 cm in size on the right side of the patient's face. (A-b) H&E staining shows little round cells with vesicular nuclei, and nuclear mitosis in the dermis. Pathological immunohistochemistry reveals that dot-like positive staining for CK20 (A-c), positive staining for Syn (A-d), CgA (A-e) and EMA (A-f). **(B)** Representative images of non-hematologic CTCs and CTECs respectively detected by EpCAM/Vimentin-iFISH and Ki-67/Vimentin-iFISH. (B-a) An $EpCAM^{+}/Vimentin^{-}/CD31^{-}$ haploid CTC in small cell size (≤5 mm, $_{S}CTC^{mono}$). (B-b) An $EpCAM^{+}/Vimentin^{-}/CD31^{+}$ multiploid CTEC in large cell size (>5 mm, $_{L}CTEC^{multi}$). (B-c) A large $EpCAM^{-}/Vimentin^{-}/CD31^{+}$ multiploid null CTEC ($_{L}CTEC^{multi}$). A $CD45^{+}$ white blood cell (WBC) is indicated by the white arrow. (B-d) A large $Ki\text{-}67^{+}/Vimentin^{-}/CD31^{-}$ multiploid CTC ($_{L}CTC^{multi}$). Bars, 5 mm. **(C)** Quantitative and molecular analyses. Among 25 detected CTCs, 20 of them are large cells ($_{L}$CTCs, 20/25 = 80 %), and the others are small cell sizes ($_{S}$CTCs). Degrees of ploidy in CTCs are monosomy 8 (1/25 = 4 %), near-disomy 8 (2/25 = 8 %), trisomy 8 (2/25 = 8 %), tetrasomy 8 (5/25 = 20 %), and multisomy 8 (15/25 = 60 %), respectively. Three CTCs are $EpCAM^{+}$ (3/25 = 12 %), and 1 CTC is $Ki\text{-}67^{+}$ (1/25 = 4 %), the remaining (21/25 = 84 %) are null cells without expression of either EpCAM or Ki-67. With respect to 10 CTECs, 7 are large cells (70 %), and the rest of the CTECs are small cells (30 %). Ploidies in CTECs are trisomy 8 (1/10 = 10 %), tetrasomy 8 (1/10 = 10 %), and multisomy 8 (8/10 = 80 %). One CTEC exhibits a positive expression of EpCAM (1/10 = 10 %), others are null cells.

expressing EpCAM and Ki-67 will be performed alongside therapy for this patient as soon as treatment begins, which will generate new insights into understanding the clinical utilities of MCC circulating rare cells detected by liquid biopsy.

Ethics statement

The study was conducted according to the Declaration of Helsinki Principles. An informed consent form, approved by the Ethics Review Committees (ERC) of the Dermatology Hospital of Southern Medical University, Guangzhou, China, was signed and obtained from the patient.

Funding

Natural Science Foundation of Guangdong Province of China 2020A1515010281.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

The authors sincerely thank the staffs in the Dermatology Hospital, Southern Medical University, Cytointelligen (China Medical City, Taizhou, Jiangsu, China) and Cytelligen (San Diego, CA, USA) for providing support to this study.

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