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ORIGINAL ARTICLE

Hotspot mutations in polyomavirus positive and negative Merkel cell carcinomas

Tuukka Veija [a], Virinder Kaur Sarhadi [a], Virve Koljonen [b], Tom Bohling [a], Sakari Knuutila [a,*]

[a] *Department of Pathology, University of Helsinki, Helsinki, Finland;* [b] *Plastic Surgery, University of Helsinki and Helsinki University Hospital, Helsinki, Finland*

Merkel cell polyomavirus (MCV) infection underlies most Merkel cell carcinoma (MCC), a primary neuroendocrine carcinoma of the skin. While previous research has focused on MCV-positive MCC tumors, less is known about the oncogenesis in MCV-negative tumors.

In this study, we analyzed mutational status of 27 MCC tumors with known MCV status for hotspot regions of 50 cancer-related genes by targeted next-generation sequencing using the Ion AmpliSeq Cancer Hotspot Panel.

In addition to previously reported *TP53*, *KIT*, and *PIK3CA* gene mutations, we found somatic mutations in the tyrosine kinase domain of the *EGFR* gene in a small proportion of the cells in six tumor tissues. *RB1* mutations were seen only in virus negative tumors.

Hotspot mutations were more frequent in MCV-negative tumors, although the difference was not statistically significant. No clear hotspot mutation profile was observed. Novel *RB1* mutations were detected only in MCV-negative tumors.

Keywords Merkel cell carcinoma, next-generation sequencing, mutations

Introduction

Merkel cell carcinoma (MCC) is a rare yet highly aggressive neuroendocrine skin cancer. The most important etiological factors leading to MCC are exposure to UV-radiation, immunosuppression, and polyomavirus infection (1). The vast majority of MCC tumors carry a clonally integrated Merkel cell polyomavirus (MCV) infection (2). The viral oncogenic pathway requires clonal integration of the MCV DNA into the primary tumor cell genome before tumor proliferation, accompanied by mutations in the large T antigen (LT) (2,3).

For a while, all MCC tumors were considered to carry the MCV infection (4). However, accumulating evidence suggests two separate subgroups: MCV-positive and MCV-negative tumors. The subgroups differ profoundly from each other in histopathological, immunohistochemical, and oncogenic features (5–9) as well as in clinical characteristics (10). MCV-positive tumors are more frequent in females, located in the extremities and associated with a lower stage at presentation and a better outcome, including longer overall and disease-free survival (5,10).

Mutational status research on cancer-related genes in MCC has yielded relatively modest results. *PIK3CA* and *TP53* mutations are infrequent and occur mainly in MCV-negative tumors (11–13). Mutations of transmembrane receptor tyrosine kinases *KIT* and *PDGFRA* have been reported. However, these mutations are non-activating, and thus, not candidates for therapeutic targets (14,15). Further, various nonsense and missense mutations have recently been published (16).

In the majority of MCC tumors, however, no oncogenic driver mutations have been found (17). Based on the different pathobiological properties of MCV-positive and -negative tumors, we hypothesized that the mutation profiles might be different. In this study, we sought to identify mutations in 50 cancer-related genes in MCC tumors with known MCV status by targeted next-generation sequencing.

Materials and methods

The study protocol was approved by the Ethics Committee of Helsinki University Hospital. The Ministry of Health and Social Affairs granted permission to collect patient data and the

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* Corresponding author.
E-mail address: sakari.knuutila@helsinki.fi

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National Authority for Medicolegal Affairs to collect and analyze tissue samples.

From our pool of 270 MCC tumor samples, we chose 15 MCV-negative and 12 MCV-positive tumors based on the amount of tumor sample available and the known MCV status. MCC diagnoses were confirmed by morphology compatible with MCC in microscopy and by immunohistochemistry positive for CK-20 and negative for TTF-1.

Patients

The study comprised 27 patients (19 females, 8 males) with primary MCC tumors. Table 1 presents the demographic and tumor-related data of these patients. The mean age of patients at presentation was 79 years, and the mean tumor size was 32 mm. Tumors were commonly located in the head and neck region. Based on the MCV status, the study group was divided into two subgroups. The MCV-negative cohort included 15 and the MCV-positive cohort 12 patients. No statistical differences emerged between the two cohorts with respect to age or tumor location/size.

DNA extraction

DNA was extracted from 15 MCV-negative and 12 MCV-positive formalin-fixed paraffin-embedded (FFPE) MCC tumor samples. The MCV status of the tumor samples was determined as described elsewhere (10). DNA extraction was carried out according to the manufacturer's instructions with a QIAamp DNA Mini Kit (250) (QIAGEN, Hilden, Germany). DNA concentration was measured with a Qubit® 2.0 fluorometer (Life Technologies, Carlsbad, CA, USA).

Targeted next generation sequencing

Mutation analysis for 27 MCC tumor samples was performed by amplicon-based next-generation sequencing (NGS) using Ion Torrent technology (Life Technologies). The Ion AmpliSeq™ Cancer Hotspot Panel v2 (Life Technologies) was used to amplify 207 amplicons in 50 cancer-related genes. (Table 2) The amplicons covered 2855 hotspot mutations recorded in the COSMIC database (http://cancer.sanger.ac.uk/cosmic). In contrast to hotspot mutations, mutation is considered "novel" when it is not recorded in either the dbSNP142 or the COSMIC database.

The Ion AmpliSeq™ Library Kit 2.0 (Life Technologies) was used to construct the libraries from 10 ng of DNA. Libraries were bar-coded with the Ion Xpress™ Barcode Adapter Kit (Life Technologies). Library concentrations were measured using the Qubit 2.0 Fluorometer (Life Technologies). Libraries were diluted, and eight libraries were pooled together.

Table 1 Demographic and tumor-related data

Number	MCV	Total variations	Hotspot variations	Sex/age	Tumor location	Sun-exposed	Tumor size, mm	Gene with hotspot mutation
1	Pos	121	46	F/80	Posterior thigh	No	85	
2	Pos	44	22	M/59	Thorax	No	70	
3	Neg	602	90	M/67	Left cheek	No	15	*APC*, *EGFR*, *KIT*, *TP53*
4	Neg	52	21	F/83	Right arm	No	50	
5	Neg	642	105	F/85	Left temple	Yes	15	*ATM*, *BRAF*, *CTNNB1*, *EGFR*, *ERBB4*, *KRAS*, *PIK3CA*, *PTEN*, *PTPN11*, *STK11*, *TP53*, *VHL*
6	Pos	16	3	F/90	Forehead	Yes	40	
7	Pos	39	20	M/71	Right buttock	No	34	*EGFR*
8	Pos	18	5	F/95	Left cheek	Yes	18	
9	Pos	24	12	F/87	Left shoulder	No	30	
10	Neg	135	49	F/77	Right cheek	Yes	20	*KIT*
11	Neg	656	111	F/79	Right breast	No	20	*APC*, *CDKN2A*, *EGFR*, *FLT3*, *KIT*, *PTPN11*, *SRC*
12	Neg	524	124	F/72	Calf	No	13	*CTNNB1*, *EGFR*, *KDR*, *KIT*, *KRAS*, *TP53*
13	Neg	61	29	M/78	Neck	Yes	25	
14	Pos	34	18	M/79	Left forearm	Yes	40	
15	Neg	38	20	F/81	Left upperback	No	—	
16	Pos	19	4	F/84	Right shoulder	No	24	
17	Neg	124	55	M/82	Neck	Yes	28	
18	Pos	484	91	M/85	Left arm	No	75	*EGFR*, *TP53*
19	Neg	16	3	F/84	Back	No	75	
20	Pos	108	41	F/87	Cheek	Yes	20	*TP53*
21	Neg	15	2	F/60	Left foot	No	10	
22	Neg	38	13	M/80	Right breast	No	23	*PIK3CA*
23	Neg	29	13	F/83	Front of left ear	Yes	18	*PIK3CA*
24	Pos	325	98	F/69	Side	No	20	*KIT*, *MPL*, *PIK3CA*, *TP53*
25	Neg	737	105	F/68	Upper stomach	No	25	*APC*, *CTNNB1*, *MET*, *PIK3CA*, *STK11*, *TP53*, *VHL*
26	Pos	14	1	F/79	Right buttock	No	30	
27	Neg	22	4	F/100	Right cheek	Yes	30	*SMO*