Identification of biomarkers that predict responses to immunotherapy in Merkel cell carcinoma

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Abstract

Introduction: Merkel cell carcinoma (MCC) is an aggressive skin tumor with the highest case-by-case fatality rate among all skin cancers. The incidence of MCC is rising rapidly, with projections that the number of cases will increase by 30% from 2013 to 2025. The recent introduction of anti-PD1/PDL1 immunotherapy has revolutionized the treatment of MCC; however, ~50% of patients do not respond. Moreover, Merkel cell polyomavirus (MCPyV) positive and negative MCC have significantly different mutational burden, but both types respond to immunotherapy at a similar rate. Investigating the biomarkers and mechanisms of response and resistance for otherwise fatal, advanced MCCs is an important clinical need. **Methods:** We performed WES and bulk-RNA sequencing on 45 FFPE tumors that underwent anti-PD1/PDL1 immunotherapy. We identified the MHC class I haplotypes using four different typing pipelines. The MHC haplotypes were grouped by response and by viral status and association with response to anti-PD1/PDL1 immunotherapy was determined. We calculated the Grantham distance of exons 2 and 3 of the haplotypes to determine divergence. We used NetMHCPan4.1 to identify candidate antigens of length 8-12 from the MCPyV proteome and predicted their binding affinity to these HLA alleles. **Results:** We observed that C*04:01 is associated with response (5:3) in virus positive tumors. We observed the peptide sequence MFDEVSTKF from the MCPyV small T antigen has a 64.62 nM predicted binding affinity and a 0.99 score from NetMHCPan4.1. We also observed that B*08:01 (1:5) and B*44 (2:7) are correlated with non-response in virus negative tumors. We found HLA divergence score was not associated with response. **Conclusions:** Our data suggests that response to immunotherapy is associated with certain HLA alleles that may present MCPyV neoantigens or UV-associated neoantigens, which contribute to response. These results may aid in prognostication of patients with advanced MCCs and may serve as an aid in informed decision making for clinicians.

Background and Research Objectives

Merkel cell carcinoma (MCC) is among the most aggressive skin cancers on a case-by-case basis[1]. It is presumably derived from precursors of Merkel cells, the neuroendocrine cells that detect touch[2]. Despite being relatively uncommon, MCC is the second leading cause of skin cancer deaths due to its high mortality rate – it is twice as deadly as malignant melanoma[3]. The incidence of these cancers is rising rapidly with projections that the number of cases will increase by ~30% from 2013 to 2025 (from ~2500 cases a year in the US to ~3300 cases a year)[4]. Finding therapies for otherwise fatal, advanced MCCs is therefore an important clinical need.

Until recently, little was known about MCC pathophysiology. MCCs is an unusual cancer in that 80% of cases are caused by a virus, the Merkel cell polyomavirus (MCPyV), while the remaining 20% of cases have no evidence of viral integration[2]. Since cancer is fundamentally a genetic disease, the Choi lab performed the largest exome sequencing project in MCCs to date[5]. They studied 49 matched pairs of germline and tumor DNA to identify somatic mutations in the cancer[5]. Interestingly, they found that the MCPyV-positive and MCPyV-negative MCCs have dramatically distinct mutational spectra[5] (**Fig. 1**). MCPyV-positive MCCs have fewer somatic mutations than any epithelial cancer sequenced to date[5]. About 30% of cases did not have a detectable somatic mutation. In contrast, the MCPyV-negative MCCs have a higher burden of somatic mutations than any cancer sequenced by the Cancer Genome Atlas[5]. These mutations are caused by UV light and affect genes known to be mutated in cancers, such as *TP53* and *RB1*[5].

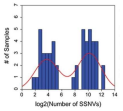


Figure 1. MCCs exhibit a bimodal distribution of mutational burden. MCPyV-infected cells tend to have fewer mutations than non-MCPyV-infected cells. UV radiation exposure is an etiology that results in a higher mutation burden and a more tumor neoantigens.

These findings have important and unexpected immunological implications. Somatic mutations in the DNA lead to expression of altered peptides. These peptides can be presented on the cell surface as tumor-specific neoantigens, which can be recognized by tumor-targeting T cells during immunoediting and immunoclearance[2]. The MCPyV-positive MCCs have almost no somatic mutations; thus, the tumor immunogenicity is driven by the expression of viral oncoproteins[5]. Since these proteins are not expressed normally in the human body, these viral oncoproteins (LT, ST) are intrinsically immunogenic, much as any microbial peptide[2,6]. MCPyV-negative MCCs are similarly immunogenic albeit by a different mechanism[2]. These samples have 100's to 1000's of tumor-specific neoantigens caused by UV-induced somatic mutations[5].

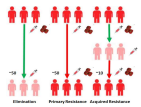


Figure 2. Three possible responses to immunotherapy occur: elimination, primary resistance, and acquired resistance. Patient blood were collected before and after treatment. Tumor and relapse samples were collected and stratified to these groups.

Based on these data, it is reasonable that both MCPyV-positive and negative MCCs are responsive to immune checkpoint therapy. Indeed, refractory to all conventional therapies, roughly 50% of patients with metastatic MCPyV-positive and MCPyV-negative MCCs respond to either PD1 or PD-L1 inhibitors[7]. Despite the improvement in treatment of MCC, much work has yet to be done. ~50% of patients have primary resistance, i.e. they never respond to immune checkpoint blockade[7]. In addition, among the ~50% of patients who initially respond to therapy, ~14% go on to relapse (secondary resistance)[7]. ***Identifying the biomarkers and mechanisms, and thus the ways to combat primary and secondary resistance, is the most important question in this field.***

Methods

High throughput sequencing techniques, such as whole exome sequencing (WES), RNA-seq, etc. were performed on the tumor tissues from patients with stage IIIB and IV MCC prior to undergoing anti-PD1/PDL1 therapy. (**Fig. 2**). Tumor viral status was determined by immunohistochemistry, tumor mutation burden, and alignment to viral genome. The clinical treatment and response data was acquired and categorized into response (PR or CR) or non-response (SD or PD). The samples were grouped by MCPyV viral status and immunotherapy response in the subsequent analyses. MHC class I alleles of normal and tumor samples were determined using multiple public pipelines that align sequencing reads with all known MHC allele sequences to identify the most likely isotype: Polysolver[9] and Optitype[10] (WES), and arcasHLA[11] and Optitype (RNA-seq). A match at the two-field resolution by at least three of the four algorithms were considered accepted. We calculated the HLA divergence using the Grantham distance metric between exons 2 and 3 of the haplotypes. For MHC types associated with response, we used NetMHCPan4.1[12] on the viral proteins to determine likely neoantigens.

Determination of Viral Status from NGS Samples

Figure 4. Immunohistochemistry was available for 41 samples. We aligned the NGS sequences to known viral sequences sTAg, LTAg, VP1, and VP2. Counts data were determined by HtSeq. All of the samples that were virus positive by IHC were positive for sTAg expression. Virus negative samples by IHC had counts for all viral proteins that were significantly less than that of virus positive samples by IHC (p<0.001). We use this technique to determine virus status for samples without IHC.

IHC	LTAg	sTAg	VP1	VP2
VN	0	0	0	0
VN	0	0	0	0
VN	0	0	0	0
VN	1	0	0	0
VN	0	0	0	0
VN	2	0	0	0
VN	0	0	0	0
VN	0	0	0	0
VN	0	0	0	0
VN	0	0	0	0
VN	0	0	0	0
VN	0	0	0	0
VN	1	0	0	0
VN	0	0	0	0
VN	0	0	0	0
VN	1	0	0	0
VN	0	0	0	0
VN	0	0	0	0
VN	1	0	0	0
VN	0	0	0	0
VN	0	0	0	0
VN	0	0	0	0
VN	0	0	0	0
VN	0	0	0	0

IHC	LTAg	sTAg	VP1	VP2
VP	0	11	0	0
VP	200	174	400	206
VP	115	99	289	258
VP	0	37	0	0
VP	263	25	66	19
VP	1999	199	376	137
VP	3	530	0	307
VP	767	48	35	46
VP	37	77	0	1
VP	1425	176	63	42
VP	215	25	50	41
VP	461	176	279	80
VP	958	70	42	27
VP	244	337	194	10
VP	1055	94	52	50
VP	611	112	0	0
VP	587	310	1266	748
VP	529	106	113	76
VP	199	15	25	7

MHC Class I and Response Outcome

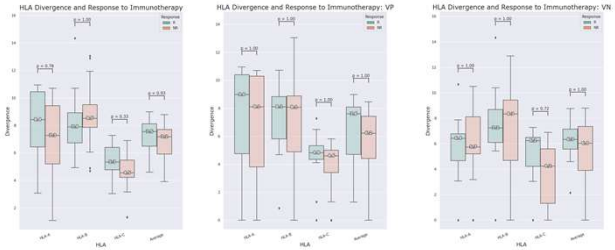
Figure 5. In virus positive MCC, C*04:01 was over-represented in the response group (5:3). In virus negative MCC, B*08:01 (1:5) and B*44:01 (2:7) are over-represented in the non-response group. We searched for candidate 8-12 amino acid peptide sequences from the viral proteome that bind to the MHC class I that are over-represented using NetMHCPan 4.1. The peptide sequences of the major viral proteins, the sTAg, LTAg, 57kT, ALTO, VP1, and VP2, were uploaded onto NetMHCPan4.1. The corresponding MHC class I allele over-represented with response (C*04:01) in virus positive MCC was used to predict the binding affinity and likelihood of a peptide being an MHC ligand (Score EL). The peptides with Score EL > 0.8 are displayed below.

HLA-C*04:01

Peptide	Viral Protein	Score EL	Affinity (nM)
MFDEVSTKF	MCPyV-sTAg	0.991134	64.62
SMFDEVSTKF	MCPyV-sTAg	0.850307	725.85

HLA Divergence Does Not Associate with Response

Figure 6. The MHC haplotypes were determined, and the peptide sequences were used to determine the HLA divergence. HLA divergence is defined as the average of the divergences of each of HLA-A, HLA-B, and HLA-C. Each divergence is determined by calculating the Grantham distance between exons 2 and 3 of the MHC allele. The overall HLA evolutionary divergence (HED) is determined by the average of the divergences for HLA-A, HLA-B, and HLA-C. In **a**, the HLA divergences was compared between response and non-response for both virus positive and virus negative MCC. For **b** and **c**, the virus positive and virus negative samples were isolated. Overall, there was not a statistically significant difference of the HLA divergence between response and non-response groups.



Conclusions

Successful immune response requires presentation of tumor-specific antigens on HLA alleles with appropriate affinity. We have observed associations of certain HLA alleles with response and non-response to immunotherapy. Specifically, we observed that C*04:01 is over-represented in response in virus positive MCC and predicted two peptides from the MCPyV genome that binds with high affinity. We found that B*44 and B*08:01 was under-represented in response in virus negative MCC. We also observed that HLA divergence did not associate with response to immunotherapy in both virus positive and virus negative MCC. These findings suggest that presence of specific HLA alleles, rather than HLA diversity, are associated with response to anti-PD1/PDL1 immunotherapy.

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Supported in part by Carolyn L. Kuckein Student Research Fellowship